The Gln-Arg192 Polymorphism of Human Paraoxonase Gene Is Not Associated With Coronary Artery Disease in Italian Patients

Domenico Ombres, Gaetano Pannitteri, Anna Montali, Antonio Candeloro, Fulvia Seccareccia, Filomena Campagna, Renzo Cantini, Pietro Paolo Campa, Giorgio Ricci, Marcello Arca

Abstract—Serum paraoxonase (PON) is an HDL-bound enzyme protecting LDL from oxidation. A common polymorphism of the paraoxonase gene (PON1) involving a Gln-to-Arg interchange at position 192 has been demonstrated to modulate PON activity toward paraaxon, a nonphysiological substrate; Arg192 (allele B) is associated with higher activity than Gln192 (allele A). This polymorphism has been proposed as a genetic marker of risk for coronary artery disease (CAD). However, the relationships between codon 192 PON1 genotypes, coronary atherosclerosis, and the occurrence of myocardial infarction (MI) are still controversial. PON1 genotypes were determined in 472 consecutive subjects (>40 years old) who underwent coronary angiography. CAD (>50% stenosis) was detected in 310 subjects (CAD+); 162 subjects with <10% stenosis served as controls (CAD–). We also evaluated 204 randomly selected individuals as population controls. PON1 genotypes were determined by PCR and AlwI restriction enzyme digestion. Frequencies of alleles A and B were 0.70 and 0.30 in angiographically assessed subjects and 0.73 and 0.27 in population controls, respectively ($\chi^2=2.0; P<0.3$). Distribution of PON1 genotypes in CAD+ were not significantly different from those in CAD– ($\chi^2=2.10; P<0.3$). Similarly, no differences were observed in the subgroup of CAD+ with MI nor in that at higher oxidative risk (smokers and/or diabetics). After controlling for other coronary risk factors, no association was found between PON1 alleles and the presence of CAD. PON1 AA genotype was associated with reduced concentration of apolipoprotein B–containing triglyceride-rich lipoproteins. This study did not provide evidence of a significant association between codon 192 PON1 genotypes and coronary atherosclerosis in Italian patients. However, it did confirm that the PON1 low-activity allele is associated with a less atherogenic lipid profile. (Arterioscler Thromb Vasc Biol. 1998;18:1611-1616.)

Key Words: coronary artery disease ■ genetics ■ paraoxonase ■ myocardial infarction ■ LDL oxidation

Peroxidation of LDL is recognized to play a central role in atherogenesis.1,2 Therefore, gene variants influencing LDL oxidation are potential candidates for coronary artery disease (CAD). Paraoxonase (PON) is a Ca$^{2+}$-dependent ester hydrolase that is exclusively bound to HDL in human plasma.3,4 PON has been shown to protect LDL against oxidative modification in vitro by preventing accumulation of lipid peroxides.5,6 A polymorphism of the paraoxonase gene (PON1) provoking a glutamine (A allele)-to-arginine (allele B) interchange at amino acid 192 has been reported to determine differences in PON activity toward paraaxon, a nonphysiological substrate, which is hydrolyzed at a higher rate by the B allele than the A allele.7-9

Previous studies have investigated the association between codon 192 PON1 polymorphism and the risk of CAD. Two have reported evidence suggesting that the high-activity PON1 allele (B allele) is associated with increased risk of CAD.10,11 However, these observations have been challenged by Antikainen et al.,12 who did not find any association in a Finnish population. These discrepancies might be due to the fact that these studies have considered selected subgroups of CAD subjects. In fact, Serato and Marian10 studied subjects who underwent percutaneous transluminal coronary angioplasty, and Ruiz et al11 studied diabetic subjects; also, Antikainen et al12 included a selected group of survivors of coronary bypass surgery with low HDL cholesterol levels and without any other major dyslipidemia. Therefore, no conclusions can be drawn about a possible role of codon 192 PON1 polymorphism as a marker of CAD risk in the general population of coronary patients. On the basis of these considerations, the topic deserves further investigation. Accordingly, the present study was designed as a case-control study in a sample of Italian subjects undergoing diagnostic coronary angiography with the objective of addressing the ques-
tion of whether codon 192 PON1 polymorphism influences the risk of coronary atherosclerosis and its major complications such as myocardial infarction (MI).

Methods

Subjects

The study population consisted of 330 men and 142 women (>40 years old) who presented between January 1994 and January 1997 at the Department of Cardiosurgery of the University of Rome “La Sapienza” for coronary angiography, either because of symptoms relating to ischemic heart disease or because of unrelated conditions such as valvular disease. CAD was defined as the presence of 1 or more stenoses >50% in at least 1 major coronary artery. The classification was based on the visual assessment of 15 coronary segments following the criteria defined by an ad hoc committee of the American Heart Association.13 All angiograms were independently evaluated by 2 cardiologists. CAD was identified in 255 men and 55 women (CAD+); 75 men and 87 women defined as CAD-free (<10% stenosis) served as comparison group (CAD−). Among CAD+ subjects, 93 (30%) showed single-vessel disease, 99 (32%) double-vessel disease, and 108 (38%) triple-vessel disease. Ten subjects (3.2%), whose coronary anatomy was evaluated for valvular surgery, were identified to have significant coronary stenosis and therefore included in the CAD+ group. One hundred seventy-six CAD+ subjects (57%) provided clinical records allowing the accurate evaluation of previous ischemic complications; 80 of them had experienced MI that was clinically verified by ECG and other conventional criteria.14 The CAD− group included 128 subjects (79%) with valvular disease, 30 (19%) with other nonischemic diseases, and 4 (2%) suspected to have ischemic heart disease but in whom angiography was negative (<10% stenosis). The data collected included height and weight (to assess body mass index [BMI]; ie, weight in kilograms divided by height in meters squared), and systolic and diastolic blood pressures; a complete medical history (ie, weight in kilograms divided by height in meters squared), and systolic and diastolic blood pressures; a complete medical history was obtained by a questionnaire, which included questions about established CAD risk factors, cardiac history, and current medications. To determine the distribution of PON1 alleles in the general population, we also evaluated 204 unrelated individuals (aged 20 to 75 years) randomly selected from subjects participating in a community-based control program of CAD risk factors. In these subjects, the presence of CAD was excluded by the Rose questionnaire and ECGs (Minnesota coding),21 the use of which to classify CAD patients for population-based screenings has been well established.22 Fasting blood samples for routine laboratory examinations, lipoproteins, apolipoproteins, and isolation of DNA were obtained early in the morning after an overnight fast.

Determination of Codon 192 PON1 Genotypes

Blood was collected in 10 mL Na-EDTA tubes and kept frozen at −20°C. DNA was extracted by the salting-out method17 and stored in 10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0. Codon 192 PON1 genotypes were determined using the polymerase chain reaction (PCR) and restriction mapping with AlwI, as previously reported,23 with modifications. A set of primers was designed to amplify a 199-bp fragment encompassing the polymorphic region of PON1 gene: 5′-TATTGTGCTGTTGAGCCT- TGAG-3′ (upstream) and 5′-GACATCTGCGATCGGGTGA-3′ (downstream). The PCR contained 100 to 200 ng DNA template, 200 μmol/L dNTPs, 1.5 mmol/L MgCl2, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 0.1 μmol/L each primer, 1 U Taq polymerase, in a final volume of 20 μL. After initial denaturation at 96°C for 5 minutes, PCR was carried out for 30 cycles, each one comprised of denaturation at 94°C for 1 minute, annealing at 61°C for 1 minute, and extension at 72°C for 1 minute, with a final extension time of 10 minutes at 72°C. PCR products (10 μL) were digested with 5 U of AlwI (New England Biolabs) following the manufacturer’s instructions. Digested fragments were separated by electrophoresis on 3% agarose gel and identified by ethidium bromide staining. Allele A corresponds to a 199-bp fragment and allele B to 135- and 64-bp fragments. In each run, a homozygote for the B allele was included as control. In some AA and BB samples, genotyping was confirmed by direct sequencing of amplified DNA fragments.18 PON1 genotyping was performed without knowing angiographic data. Ambiguous samples were analyzed a second time.

Other Laboratory Measurements

Cholesterol and triglyceride concentrations in total plasma and lipoprotein fractions were measured with a Technicon RA-1000 automated analyzer. HDL cholesterol was determined in the whole plasma after precipitation of apolipoprotein (apo) B–containing lipoproteins with phosphotungstic acid/MgCl2.19 Lipoprotein fractions were isolated by preparative ultracentrifugation as previously described.20 Plasma levels of total, VLD+IDL, and LDL apoB were measured as described elsewhere.21 Plasma concentrations of apoAI and apoAII were determined by immunoelectrophoresis using polyclonal antibodies.22 LpAI concentration was measured by double-antibody immunoelectrophoresis, and LpAI:AII was calculated from total apoAI according to a previously reported procedure.23

Statistical Analysis

Categorical variables were compared by the χ2 or Fisher’s exact test. Differences of continuous variables were evaluated by the Student’s t or Mann-Whitney U test, depending on the shape of the distribution curves. The frequencies of PON1 alleles and genotypes in CAD+ and CAD− subjects were obtained by direct count, and the departure from the Hardy-Weinberg equilibrium was evaluated by the χ2 test. Genotype distributions between the study groups were compared by construction of 2×2 and 2×3 contingency tables and χ2 analysis. The relations between PON1 genotypes and clinical and biochemical variables were evaluated by ANOVA. To estimate the relative risk of coronary stenosis associated with PON1 genotypes, odds ratios were calculated by the multiple logistic regression analysis, assuming the effects of the B alleles to be dominant (with score 0 for AA and score 1 for AB and BB combined) or recessive (with score 0 for AA and AB combined and score 1 for BB). Given the prevalence of PON1 B allele in the CAD− group of 0.30, we estimated that 620 alleles in the CAD+ group would suffice to detect an odds ratio (ie, odds of CAD+ given the presence of the PON1 B allele) of at least 1.4 (at 80% power and with 2-sided α error of 0.05).24 Age, sex, BMI, smoking, diabetes, hypertension, and plasma lipids were included in the logistic model as covariates. For each odds ratio we estimated 2-tailed probability values and 95% confidence intervals. P<0.05 was taken as statistically significant.

Results

Frequencies of Codon 192 PON1 Alleles and Genotypes

The frequencies of A and B PON1 alleles in the 472 angiographically assessed subjects were 0.70 and 0.30, respectively. These figures were not significantly different from those observed in the sample of 204 control subjects (0.73 and 0.27, respectively); the same holds for the frequency of each of the 3 genotypes (AA, 0.48 versus 0.52; AB, 0.43 versus 0.41; BB, 0.09 versus 0.07, respectively) (χ2=2.0; P<0.3). In both the CAD+ and CAD− groups, there was no significant deviation of PON1 genotype frequencies from those predicted by the Hardy-Weinberg equilibrium (χ2=0.64, P<0.7 for CAD+ patients and χ2=1.64, P<0.4 for CAD−, respectively). The distributions of PON1 genotypes in our population controls were virtually identical to those reported in other populations in Northern Europe,25 Canada,26 but different from those reported in a sample of general population from the United States.10 No differences were observed in the frequencies of PON1 alleles and genotypes according to age or sex.
bution of PON1 alleles and genotypes in CAD+ subjects with MI was found to be not significantly different from those in CAD− subjects or CAD+ without MI ($\chi^2=0.01, P<0.9$) (Table 2). Since serum PON is thought to exert its antiatherogenic effects by protecting LDL against oxidative modification, its role might become significant only when a higher oxidative risk is present. Several studies reported convincing evidence that both smoking and diabetes increase the susceptibility of LDL to in vitro oxidation. When we reevaluated PON1 genotypes in the subgroup of subjects who were current smokers and/or diabetics, no significant differences in the distribution of PON1 alleles and genotypes were observed between CAD+ and CAD− subjects (data not shown). Serum PON is exclusively bound to HDL; thus, HDL concentrations might affect the association between PON1 genotypes and the presence of CAD. However, when we repeated the analysis within strata defined by HDL cholesterol levels (using 1.03 mmol/L [40 mg/dL] as cut-off value), no differences were observed in the distribution of PON1 genotypes between CAD+ and CAD− subjects in either the low-HDL ($\chi^2=0.3, P<0.8$) or high-HDL subgroups ($\chi^2=3.6, P<0.2$) (data not shown). Finally, odds ratios were calculated to assess the degree of the association of PON1 genotypes with the risk of CAD (Table 3). After controlling for other coronary risk factors, no significant increase of CAD risk associated with the B allele was detected, regardless of whether the B allele was assumed to have a dominant or recessive effect. Sex ($P<0.001$), smoking ($P<0.01$), and history of hypertension ($P<0.01$) showed the strongest association with CAD. Also, age, diabetes, plasma total cholesterol, and triglycerides were found to be significantly associated with CAD, although at a lower degree of significance ($P<0.05$).

**Codon 192 PON1 Genotypes and Plasma Lipids**

Age-standardized plasma lipid and lipoprotein levels according to PON1 alleles in the whole group of angiographically assessed subjects are reported in Table 4. Homozygotes for the A allele showed significantly lower concentrations of total triglycerides and apoB ($P<0.05$) and higher concentrations of apoAII ($P<0.01$) than subjects carrying the B allele (AB and BB). No significant differences were observed in HDL cholesterol and apoAI concentrations between the two groups. As far as lipoprotein fractions are concerned, A allele carriers showed significantly lower concentrations of choles-

### Table 1. Levels of Coronary Risk Factors in Subjects With (CAD+) and Without (CAD−) Coronary Artery Disease

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAD+ (n=310)</th>
<th>CAD− (n=162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>255 (82.2)†</td>
<td>75 (46.3)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>55 (17.3)</td>
<td>87 (53.7)</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.2±0.5</td>
<td>59.1±0.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±0.2†</td>
<td>25.4±0.3</td>
</tr>
<tr>
<td>Menopause</td>
<td>51 (92.7)</td>
<td>71 (81.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>134 (43.3)†</td>
<td>37 (23.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>36 (11.5)*</td>
<td>7 (4.2)</td>
</tr>
<tr>
<td>Current cigarette smokers (%)</td>
<td>151 (48.7)†</td>
<td>43 (26.5)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.95±0.07†</td>
<td>5.33±0.08</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.12±0.02</td>
<td>1.16±0.03</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.21±0.08†</td>
<td>1.64±0.07</td>
</tr>
</tbody>
</table>

Data are reported as n (%) and mean±SEM. Clinical and biochemical variables are age and sex standardized. Percentages for menopause have been calculated on women’s samples. Statistical significance of differences has been evaluated using the unpaired Student’s t test (continuous variables) and z test (categorical variables).

*P<0.02; †P<0.001.

### Association Between Codon 192 PON1 Polymorphism and CAD

Table 1 shows the clinical characteristics of CAD+ and CAD− groups. The 2 groups were comparable for age and prevalence of menopause. However, the distribution of several other coronary risk factors reflected the expected differences between subjects with and without coronary artery disease.

Frequencies of PON1 genotypes and alleles in CAD+ and CAD− subjects are reported in Table 2. Compared with CAD−, we found no significant difference in the distribution of codon 192 PON1 genotypes in CAD+ subjects ($\chi^2=2.01, P<0.3$), and the frequencies of A and B alleles were similar in both groups (0.69 and 0.31 versus 0.70 and 0.30, respectively). PON1 genotypes did not exhibit any significant differences in patients with single-, double-, or triple-vessel disease (data not shown), indicating that PON1 polymorphism is not associated with the severity of coronary atherosclerosis. The subgroup of 176 CAD+ subjects ascertained for the presence of previous ischemic complications was used to test the association of PON1 polymorphism to the risk of MI, once substantial atherosclerosis has occurred. The distribution of PON1 alleles and genotypes in CAD+ subjects with MI was found to be not significantly different from those in CAD− subjects or CAD+ without MI ($\chi^2=0.01, P<0.9$) (Table 2). Since serum PON is thought to exert its antiatherogenic effects by protecting LDL against oxidative modification, its role might become significant only when a higher oxidative risk is present. Several studies reported convincing evidence that both smoking and diabetes increase the susceptibility of LDL to in vitro oxidation. When we reevaluated PON1 genotypes in the subgroup of subjects who were current smokers and/or diabetics, no significant differences in the distribution of PON1 alleles and genotypes were observed between CAD+ and CAD− subjects (data not shown). Serum PON is exclusively bound to HDL; thus, HDL concentrations might affect the association between PON1 genotypes and the presence of CAD. However, when we repeated the analysis within strata defined by HDL cholesterol levels (using 1.03 mmol/L [40 mg/dL] as cut-off value), no differences were observed in the distribution of PON1 genotypes between CAD+ and CAD− subjects in either the low-HDL ($\chi^2=0.3, P<0.8$) or high-HDL subgroups ($\chi^2=3.6, P<0.2$) (data not shown). Finally, odds ratios were calculated to assess the degree of the association of PON1 genotypes with the risk of CAD (Table 3). After controlling for other coronary risk factors, no significant increase of CAD risk associated with the B allele was detected, regardless of whether the B allele was assumed to have a dominant or recessive effect. Sex ($P<0.001$), smoking ($P<0.01$), and history of hypertension ($P<0.01$) showed the strongest association with CAD. Also, age, diabetes, plasma total cholesterol, and triglycerides were found to be significantly associated with CAD, although at a lower degree of significance ($P<0.05$).

**Codon 192 PON1 Genotypes and Plasma Lipids**

Age-standardized plasma lipid and lipoprotein levels according to PON1 alleles in the whole group of angiographically assessed subjects are reported in Table 4. Homozygotes for the A allele showed significantly lower concentrations of total triglycerides and apoB ($P<0.05$) and higher concentrations of apoAII ($P<0.01$) than subjects carrying the B allele (AB and BB). No significant differences were observed in HDL cholesterol and apoAI concentrations between the 2 groups. As far as lipoprotein fractions are concerned, A allele carriers showed significantly lower concentrations of choles-

### Table 2. Distribution of PON1 Genotypes and Alleles in the Different Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>PON1 Genotypes</th>
<th>PON1 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA, n (%)</td>
<td>AB, n (%)</td>
</tr>
<tr>
<td>CAD+ (n=310)</td>
<td>144 (46.4)</td>
<td>139 (44.8)</td>
</tr>
<tr>
<td>CAD+ with MI (n=80)</td>
<td>38 (47.5)</td>
<td>37 (46.2)</td>
</tr>
<tr>
<td>CAD+ without MI (n=96)</td>
<td>44 (45.8)</td>
<td>44 (45.8)</td>
</tr>
<tr>
<td>CAD− (n=162)</td>
<td>82 (50.6)</td>
<td>62 (38.3)</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; MI, myocardial infarction. Clinical information on ischemic complications of CAD were available for 176 (57%) CAD+ subjects. $\chi^2=2.01, P<0.3$ for comparison of all CAD+ vs control subjects; $\chi^2=0.01, P<0.9$ for comparison of CAD+ with MI vs CAD− without MI.
terol (P<0.05) and apoB (P<0.05) in the d<1.019-g/mL lipoprotein fraction (VLDL+IDL). Concentrations of HDL particles, LpAI, and LpAI:AII did not show any significant difference between the groups. The differences in the lipoprotein profile between A and B carriers were not attributable to differences in body weight, since mean BMI was comparable between the groups (26.1±0.2 versus 26.4±0.2 kg/m²).

**TABLE 3.** Logistic Regression Analysis of Determinants of Coronary Artery Disease in the Group of Angiographically Examined Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.03 (1.01–1.06)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>5.11 (3.05–8.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1.06 (0.99–1.13)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, Y/N</td>
<td>2.08 (1.25–3.46)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes, Y/N</td>
<td>2.84 (1.11–7.28)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hypertension, Y/N</td>
<td>2.25 (1.34–3.78)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.33 (1.05–1.70)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.60 (0.26–1.35)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.60 (1.10–2.33)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PON1 genotype dominant*</td>
<td>1.07 (0.65–1.76)</td>
<td>NS</td>
</tr>
<tr>
<td>PON1 genotype recessive*</td>
<td>0.54 (0.24–1.17)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**BMI** indicates body mass index; **PON1**, paraoxonase gene.

*Relative risk has been estimated vs AA PON1 genotype.

In the present study, by evaluating the distribution of codon 192 PON1 genotypes in angiographically defined CAD subjects, we found no indication that this polymorphism is significantly associated with the risk of coronary atherosclerosis.

Several points should be considered when interpreting these results. First, the study was case-control in design, and the subjects were not recruited prospectively. Therefore, a survival bias cannot be excluded. However, assuming early mortality from CAD in individuals carrying a particular PON1 allele, the latter would be overrepresented in the control group. But this was not the case. Moreover, the frequencies of PON1 genotypes in angiographically assessed subjects were comparable to those observed in the sample of general population, indicating no segregation of PON1 gene variants in the group of hospitalized individuals. This study was an all-ethnic association study, and it is known that several factors (number of subjects, admixture of genetically nonhomogeneous populations, lack of appropriate control groups, etc) may cause spurious results in such type of study. But such potential flaws have been taken into consideration. In fact, we recruited a large number of consecutive subjects with a well-defined coronary status. To avoid potential misclassification, only subjects without evidence of coronary atherosclerosis (<10% stenosis) were taken as control subjects (CAD−), and younger subjects (<40 years) were not considered. As a consequence of the study design, our CAD− group included individuals mainly suffering from valvular disease. Besides, the CAD− group was characterized by a low prevalence of atherogenic risk factors, and the clinical characteristics of CAD+ and CAD− subjects were almost identical to those observed in other large angiographically based, case-control studies. Finally, the size of our sample was adequate to reveal even a low risk associated to PON1 genotypes, if present. In fact, the smallest detectable odds ratio in our sample was 1.4, lower than those reported in previous studies showing a significant association between PON1 genotypes and the risk of CAD.

The lack of association between variation at codon 192 of PON1 variation and CAD may indicate that this polymorphism is unrelated to the risk of coronary atherosclerosis. The mechanism by which this polymorphism could influence susceptibility to CAD is unknown. It gives rise to differences in enzyme activity, but this property has been presently defined by exogenous substrate (paraoxon) and is not valid for all substrates. On the other hand, the findings relating high-activity genotypes (AB and BB) to increased risk of CAD, 10,11 are opposite to those expected. Furthermore, despite the differences in genotypes between CAD+ and CAD− individuals, no differences in serum PON activity have been reported. Mackness et al have demonstrated that HDL isolated from homozygotes for the B allele (BB-HDL) are less capable of protecting LDL from Cu2+–induced oxidation than AA-HDL, but the mechanism is unknown. More recently, Garin et al postulated that a second, frequent polymorphism at codon 54 of the PON1 might exert an even stronger influence on PON function and thereby risk of CAD. The authors based their conclusions mainly on the observa-
tion that codon 54 shows a more consistent impact on concentrations and activity of serum PON than codon 192 polymorphism. However, this conclusion appears contradictory to previous findings demonstrating no effect of codon 54 polymorphism on enzyme activity toward paraoxon. On the other hand, it should be noted that in Garin’s study, position 54 appears to be discriminatory mainly when phenylacetate is used as substrate. Therefore, whether codon 54 polymorphism is more relevant with respect to functional differences or substrate specificity is unclear. Since in our study we did not screen for codon 54 PON1 variation, a possible significant effect of this polymorphism on CAD risk cannot be excluded. However, Garin et al. did not demonstrate that codon 54 is a better predictor of CAD risk than codon 192 polymorphism. At this stage, additional studies are needed to define putative independent effects of these 2 PON1 polymorphisms on CAD risk.

Previous studies have investigated the association between codon 192 PON1 polymorphism and blood lipids. Hegele et al. found a significant association of this polymorphism with plasma concentrations of HDL and LDL cholesterol, total triglycerides, and apoB in a genetically isolated North American population. The authors observed that homozygotes for the low activity allele (A) have a less atherogenic lipid profile than heterozygotes and homozygotes for the high-activity allele (B). Conflicting observations have been reported in non–insulin-dependent diabetic patients, in whom B allele carriers showed higher concentrations of HDL cholesterol and apoAl. Our data demonstrated that codon 192 PON1 polymorphism influences plasma lipids, confirming the A allele to be associated with less atherogenic lipid levels. It is interesting to note that the most significant differences between genotypes were distinctly related to triglyceride-rich lipoprotein fractions and apoAI. Convincing explanations for these findings are not available. One possible explanation might be that low serum PON activity alleles are associated with a decreased transfer of lipids between HDL and VLDL or LDL. However, additional investigations are needed to clarify this point. In any case, the implications of the effect of PON1 polymorphism on plasma lipids on CAD risk are difficult to evaluate. As a whole, our data seem to suggest a minor influence. Hegele et al. concluded that PON1 polymorphism accounted for only a 1% variation in total cholesterol and related lipoprotein traits, and the studies reporting a significant association between PON1 polymorphism and CAD failed to ascribe this effect to changes in plasma lipids.

A final comment should be made about the possibility that PON1 did not show any significant effect on CAD risk because the “oxidative risk” is low in the Italian population. Even though no experimental data are available, it is reasonable to assume that the common Italian diet rich in olive oil may grant a certain level of protection against oxidative stress. In this case, the potential role of any antioxidant enzyme would be limited. Unfortunately, accurate measurements of the oxidative risk were not performed in our subjects. Therefore, the question of whether genetic variations of PON1 weigh more in individuals at higher oxidative risk is still open.

In conclusion, our study did not provide evidence of a significant association between codon 192 PON1 polymorphism and the development of coronary atherosclerosis or its major ischemic complications. Although the present data cannot exclude a role of serum PON in atherogenesis, they indicate that at least this PON1 polymorphism seems to be of little usefulness as a genetic marker of CAD risk in the Italian population.

Acknowledgments
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References


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