Paraoxonase1-192 Polymorphism Modulates the Nonfatal Myocardial Infarction Risk Associated With Decreased HDLs

Mariano Sentí, Marta Tomás, Jaume Marrugat, Roberto Elosua, for the REGICOR Investigators*

Abstract—Serum paraoxonase1 (PON1), a high density lipoprotein (HDL)-linked enzyme, appears to have a role in the protection of low density lipoproteins from oxidative stress. PON1 enzyme activity for paraoxon as a substrate is modulated, along with others at the PON1 locus, by the PON1-192 polymorphism, which contains low paraoxon–activity and high paraoxon–activity alleles (Q and R, respectively). The association of PON1 with HDL suggests that impaired serum concentrations of the lipoprotein could have consequences for the susceptibility to oxidative stress. Because PON1-192 polymorphism strongly influences PON1 activity toward paraoxon, we tested the hypothesis that this polymorphism may modulate the myocardial infarction (MI) risk associated with low HDL cholesterol concentrations. Two hundred eighty consecutive MI patients and 396 control subjects were studied. When considered as a whole, PON1-192 genetic polymorphism was not associated with higher MI risk. In the entire population, decreased HDL cholesterol concentration (<0.90 mmol/L in men and <1.11 mmol/L in women) conferred an MI risk of 2.56 ($P<0.0001$) compared with normal HDL levels. The risk increased to 4.51 ($P<0.0001$) in QQ homozygous HDL-deficient subjects relative to QQ homozygotes with normal HDL levels, decreased to 1.83 ($P=0.1046$) in QR heterozygote HDL-deficient subjects, and also decreased (to 1.41, $P=0.6243$) in RR homozygote HDL-deficient individuals compared with RR carriers with normal HDL cholesterol concentration. The effect of PON1-192 genotypes on the association of low HDL cholesterol levels and MI was related to gene dosage. A significantly decreased enzyme activity was found in HDL-deficient MI patients compared with HDL-deficient control subjects (median 208 U/L [interquartile range 136 to 298 U/L] versus median 235 U/L [interquartile range 163 to 350 U/L], respectively; $P=0.025$). QQ homozygous MI patients showed the greatest difference of PON1 activity levels between normal and HDL-deficiency state groups (14.9%, $P=0.002$). Our observations raise the question of whether the decrease in PON1 activity and the MI risk associated with HDL deficiency are more evident in the low paraoxon–activity QQ genotype. It can be argued that the low paraoxon–activity QQ genotype, which may be adequate to prevent lipid peroxidation in normolipidemic subjects, may be insufficient when an HDL-deficiency state and low PON1 activity reflecting oxidative stress coexist. The risk of nonfatal MI is increased in HDL-deficiency states, principally among subjects carrying the low paraoxon–activity QQ genotype. (Arterioscler Thromb Vasc Biol. 2001;21:415-420.)

Key Words: myocardial infarction ■ HDL ■ paraoxonase ■ PON1 genotypes

P paraoxonase1 (PON1) is a calcium-dependent esterase closely associated with HDL-containing apoA-I that has been reported to confer antioxidant properties on HDL by decreasing the accumulation of lipid peroxidation products.1 PON1 activity is under genetic and environmental regulation and appears to vary widely among individuals and populations. PON1 enzyme activity for paraoxon as a substrate is modulated by a number of polymorphisms at the PON1 locus in humans. One of them is the PON1-192 genetic polymorphism,2 which consists of PON1 Q, an isoform with low activity toward paraoxon hydrolysis that contains a glutamine at position 192, and PON1 R, an isoform with high activity toward paraoxon hydrolysis that contains an arginine at position 192.3 The report by Ruiz et al4 was the first in a series of studies on PON1-192 gene polymorphism and coronary heart disease. Reviewing these studies reveals discrepancies even in those conducted in the same ethnic population.5,6 In 1 study, carried out in the United States,7 the R allele was associated with coronary heart disease, but in Europe, 5 studies failed to show such an association.8–12 This variability in results suggests that gene-environment and/or gene-gene interactions might modulate the relationship between PON1-192 polymorphism and coronary heart disease.
Low concentrations of HDL increase susceptibility to atherosclerosis and, consequently, coronary heart disease. PON1 may be responsible for part of the antioxidant properties of this lipoprotein. Because the HDL particle is strongly associated with PON1, this lipoprotein emerges as a firm candidate to be analyzed in relation to PON1 activity and PON1-192 genotypes. The association of PON1 with HDL suggests that impaired serum concentrations of the lipoprotein could have consequences for susceptibility to oxidative stress. Because PON1-192 genetic polymorphism strongly influences PON1 activity toward paraoxon, we tested the hypothesis that this polymorphism may modulate the myocardial infarction (MI) risk associated with low HDL cholesterol concentrations.

Methods

Subjects
Two hundred eighty consecutive patients (253 men and 27 women, mean age 57.7 ± 9.6 years) with a first MI admitted to the only reference coronary unit in the catchment area were recruited between January 1996 and December 1998 in Gerona, Spain. Diagnosis of Q-wave MI was based on definite ECG (ie, new Q or QS waves) and at least one of the following: increased cardiac enzymes (at least twice the upper normal value) or typical pain (ie, located in the anterior chest wall) lasting ≥ 20 minutes for which no cause other than coronary heart disease was found. Non-Q-wave MI was diagnosed in 16% of patients who had persistent ST-segment elevation with progressive T-wave inversion or other ECG characteristics and in whom cardiac enzyme levels were at least twice the normal limit, together with typical chest pain lasting > 20 minutes. Eighty patients underwent coronary angiography for evaluation of coronary stenosis. Only 10 MI patients were on lipid-lowering drug therapy after leaving the hospital and were not excluded from the study.

Three hundred ninety-six control subjects (264 men and 132 women, mean age 53.6 ± 11.7 years) were randomly selected from a representative population sample composed of 1750 subjects who participated in a cross-sectional study designed to establish the prevalence of main cardiovascular risk factors in the province of Gerona, Spain. The reference population was composed of 6 women, mean age 53.6 years, who cardiac enzyme levels were at least twice the normal limit, together with typical chest pain lasting > 20 minutes. Eighty patients underwent coronary angiography for evaluation of coronary stenosis. Only 10 MI patients were on lipid-lowering drug therapy after leaving the hospital and were not excluded from the study.

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Written consent was obtained from all subjects, and the study was approved by the local ethics committee. Details on socioeconomic and demographic characteristics were obtained from all subjects by a standardized questionnaire, together with information on smoking, diabetes mellitus, and hypertension. In MI patients, blood samples for genetic analysis were obtained within 24 hours after admission to the coronary care unit. Samples for biochemical analysis were taken at least 3 months after the acute coronary event.

Laboratory Analyses

Analyses of Lipid and Lipoproteins
Blood samples were collected in controls and patients after an overnight fast. Serum cholesterol and triglyceride levels were determined enzymatically (Roche Diagnostica). LDL cholesterol was calculated by the Friedewald formula. HDL cholesterol was measured as cholesterol after precipitation of apoB-containing lipoproteins with phosphotungstic Mg2+ (Boehringer-Mannheim).

Analysis of PON1 Activity
PON1 activity was determined on frozen samples at −70°C, which were thawed just before the beginning of each assay. PON1 activity toward paraoxon was measured after the reaction of paraoxon hydrolysis into p-nitrophenol and diethyl phosphate catalyzed by the enzyme. PON1 activity was determined from the initial velocity of paraoxon hydrolysis (subtraction of spontaneous paraoxon hydrolysis) at 37°C and was recorded at 405 nm by an autoanalyzer Cobas-Mira Plus (Roche Diagnostica). Serum was added to a basal assay mixture to reach final concentrations of 5 mmol/L paraoxon, 1.9 mmol/L CaCl2, 90 mmol/L Tris-HCl (at pH 8.5), and 3.6 mmol/L NaCl. Two strategies were followed to avoid spontaneous hydrolysis of diluted paraoxon solutions. First, a blank determination of basal assay mixture without serum was made. Second, 5 mmol/L paraoxon basal assay mixture aliquots frozen at −40°C were used and thawed just before the beginning of each assay. Frozen aliquots of a serum pool, used as an internal control, were thawed just before the beginning of the assay. At least 1 aliquot of serum pool was measured in triplicate every 24 samples. The serum pool was used to correct for interassay variations. A PON1 activity of 1 U/L was defined as 1 μmol of p-nitrophenol formed per minute. The molar extinction coefficient of p-nitrophenol is 18 053 mol−1 cm−1 at pH 8.5. The intra-assay and interassay coefficients of variation were 0.78% and 1.69%, respectively.

PON1-192 Genotype Determination
Genomic DNA was isolated from white cells by the salting-out method. Polymerase chain reactions were performed by use of primer sequences derived from published data. The amplification cycle was performed on a Perkin-Elmer Cetus 2400 Thermal Cycler with initial denaturation for 4 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 1 minute at 61°C, and 1 minute at 72°C, and finally by 7 minutes of extension at 72°C. Polymerase chain reaction products were digested with AlwI for 4 hours at 37°C, and the samples were electrophoresed in 3% agarose gels for 75 minutes at 60 V.

Statistical Analysis
The χ2 test was used to analyze the association between categorical variables and the deviation of genotype frequencies from those predicted by the Hardy-Weinberg equilibrium. For comparisons of continuous variables between cases and controls, Student t test was performed, except for comparisons of PON1 activity and HDL cholesterol levels. For these comparisons, ANOVAs were performed on the combined sample of men and women, adjusting for age and sex. Spearman correlation coefficients were used to test the strength of the association between PON1 activity levels and HDL cholesterol concentrations. Because a number of cohort studies have revealed an HDL cholesterol <0.90 mmol/L to be a predictor of a high risk of coronary heart disease, this cutoff was selected to define men with low or normal HDL cholesterol. A cutoff of 1.11 mmol/L was defined in women according to the recommendations of the European Task Force of European and other Societies on coronary prevention. The odds ratios for the effect of low HDL cholesterol concentration and PON1-192 genotypes on MI risk were estimated by using logistic regression analyses adjusted for the effects of other cardiovascular risk factors. Logistic regression analysis was also used to test for interactions.

Results

Study Populations, Cardiovascular Risk Factors, and PON1-192 Genotypes in MI Patients and Control Subjects
Table 1 shows anthropometric and clinical characteristics, HDL cholesterol and other lipidic parameters, PON1 activity, and PON1-192 genotypes in MI patients and in the control group. MI patients were older and commonly had more cardiovascular risk factors than did control subjects. Serum HDL cholesterol levels were significantly lower and serum triglycerides were higher in MI patients compared with control subjects. Serum cholesterol and LDL cholesterol concentrations were significantly lower in patients than in control subjects, probably as a consequence of changes in...
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<table>
<thead>
<tr>
<th>TABLE 1. MI Risk Factors, PON1 Activity, and PON1-192 Genotypes in Control Subjects and MI Patients</th>
<th>Control Subjects</th>
<th>Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53.7±11.7</td>
<td>57.7±9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men/women, n/n</td>
<td>264/132</td>
<td>253/27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7±3.9</td>
<td>27.0±3.8</td>
<td>0.385</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>22.5</td>
<td>40.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>9.8</td>
<td>22.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>22.8</td>
<td>35.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.89±1.08</td>
<td>5.22±1.01</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.32±0.77</td>
<td>1.52±0.97</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.00±0.97</td>
<td>3.45±0.94</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.29±0.42</td>
<td>1.05±0.30</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Low HDL cholesterol, %</td>
<td>17.4</td>
<td>39.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PON1 activity, U/L</td>
<td>226 (159–351)</td>
<td>216 (146–298)</td>
<td>0.005†</td>
</tr>
<tr>
<td>LDL/PON1 activity ratio</td>
<td>0.019±0.001</td>
<td>0.018±0.009</td>
<td>0.430†</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean±SD, except for PON1 activity levels, which are given as median (interquartile range).

*Low HDL cholesterol was defined as <0.90 mmol/L in men and <1.11 mmol/L in women.

†Adjusted for the effects of sex.


dietary habits and drug therapy after the clinical event. The percentage of HDL-deficient MI patients was significantly higher than the percentage of HDL-deficient control subjects. Serum PON1 activity levels toward paraoxon were also significantly lower in MI patients than in control subjects. In both groups, PON1 activity and HDL cholesterol levels were not correlated (r=0.013 and P=0.800 in control subjects and r=0.080 and P=0.291 in MI patients).

The genotypic distribution of the PON1-192 polymorphism was in Hardy-Weinberg equilibrium in patient and control groups. There were no differences in the distribution of genotype frequencies of PON1-192 polymorphism between patients and control subjects.

<table>
<thead>
<tr>
<th>TABLE 2. Serum PON1 Activity and PON1-192 Genotype Frequencies in Control Subjects and MI Patients Stratified by Categorized HDL Cholesterol Concentration</th>
<th>Low HDL Cholesterol</th>
<th>Normal HDL Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.81±0.12</td>
<td>1.40±0.38</td>
</tr>
<tr>
<td>PON1 activity, U/L</td>
<td>235 (163–350)</td>
<td>227 (159–357)</td>
</tr>
<tr>
<td>Q</td>
<td>153 (122–180)</td>
<td>162 (130–190)</td>
</tr>
<tr>
<td>R</td>
<td>301 (214–375)</td>
<td>340 (290–402)</td>
</tr>
<tr>
<td>PON1-192 genotypes</td>
<td>511 (359–567)</td>
<td>543 (406–618)</td>
</tr>
</tbody>
</table>

| QQ, n (%) | 27 (39.1) | 166 (50.8) |
| QR, n (%) | 33 (47.8) | 132 (40.4) |
| RR, n (%) | 9 (13.0) | 29 (8.9) |

Values are mean±SD for HDL cholesterol and median (interquartile range) for PON1 activity levels.

*Adjusted for the effects of age and sex.

†P=0.002, which is significantly lower than PON1 activity of patients with normal HDL cholesterol concentration.

Serum PON1 Activity and PON1-192 Genotypes in Patients and Controls Stratified by Categorized HDL Cholesterol Concentration

As expected, in the whole group as well as in the HDL subgroups, the RR genotype was associated with a significantly increased PON1 activity compared with that of QR or QQ genotypes (P<0.0001).

As a next step, we compared PON1 activity levels between control subjects and patients stratified by HDL categories (Table 2). It is noteworthy that a significantly decreased enzyme activity was found in HDL-deficient MI patients compared with HDL-deficient control subjects. In contrast, PON1 activity values were similar in control subjects and patients with normal HDL cholesterol levels. PON1 activity levels were further stratified into PON1-192 genotypes. With the exception of QR subjects with normal HDL cholesterol concentrations, comparisons of PON1 activity between control subjects and patients were not significant. After HDL categories were compared by study groups, QQ homozygote MI patients showed the greatest difference of median PON1 activity between normal and HDL-deficiency state groups (14.9%, P=0.002). In MI R-carrier (QR or RR genotype) patients, as well as in control subjects of the 3 genotypes, differences in PON1 activity between HDL groups were substantially less marked and ranged from 5.8% to 11.5%.

Because diabetes mellitus is reported to be associated with low PON1 activity toward paraoxon, we reanalyzed the data by adjusting for diabetes in the ANOVA analyses. In the overall group of study subjects as well as in the HDL groups, the probability value for comparisons of PON1 between cases and controls remained essentially unchanged (data not shown). The percentage of diabetic MI patients in the low HDL category group was slightly higher than that in the normal HDL group (29% and 22%, respectively). However, when PON1 activity levels were compared between MI patients of the HDL groups with an adjustment for diabetes,
TABLE 3. Adjusted Odds Ratios and 95% CI for Effects of HDL-Deficiency Status on MI Risk

<table>
<thead>
<tr>
<th>Low HDL cholesterol concentration</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects (n=676)</td>
<td>2.56</td>
<td>1.62–4.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>QQ homozygotes (n=332)</td>
<td>4.51</td>
<td>2.28–8.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QR heterozygotes (n=274)</td>
<td>1.83</td>
<td>0.89–3.79</td>
<td>0.1046</td>
</tr>
<tr>
<td>RR homozygotes (n=70)</td>
<td>1.41</td>
<td>0.35–5.62</td>
<td>0.6243</td>
</tr>
<tr>
<td>Interaction of HDL (normal=0, low=1) with genotype (QR or RR=0, QQ=1) on MI risk</td>
<td>1.90</td>
<td>1.41–2.56</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Logistic regression analyses were adjusted for age, sex, diabetes mellitus, hypertension, and smoking.

The value was $P=0.434$, similar to that found without adjusting for diabetes ($P=0.482$).

PON1-192 genotype distribution in categorized HDL groups was also assessed. The genotypic distribution of the PON1-192 polymorphism was comparable between MI patients and control subjects with normal HDL cholesterol levels. However, although differences in genotype frequency distribution between cases and controls did not reach statistical significance, HDL-deficient MI patients had a higher frequency of the QQ genotype compared with HDL-deficient control subjects (50.9% versus 39.1%, $P=0.122$). Again, no significant correlations were observed between PON1 activity levels and HDL cholesterol concentration in patients and control subjects of each genotype group.

Influence of PON1-192 Polymorphism on the Association of HDL-Deficiency Status and MI Risk

To determine whether the HDL-deficiency status was differentially associated with MI risk among PON1-192 genotypes, logistic regression analyses in the overall group of the study subjects and in each genotype were performed (Table 3). Models were adjusted for age, sex, diabetes mellitus, hypertension, and smoking. In the entire population, decreased HDL cholesterol concentration conferred an MI risk of 2.56 ($P=0.0001$) compared with normal HDL levels. The risk increased to 4.51 ($P<0.0001$) in QQ homozygous HDL-deficient subjects relative to QQ homozygotes with normal HDL levels, decreased to 1.83 ($P=0.1046$) in QR heterozygote HDL-deficient subjects, and also decreased (to 1.41, $P=0.6243$) in RR homozygote HDL-deficient individuals compared with RR carriers with normal HDL cholesterol concentrations. Therefore, the effect of PON1-192 genotypes on the association of low HDL cholesterol levels and MI was related to gene dosage. A statistically significant interaction between genotype and categorized HDL cholesterol levels was observed for MI risk ($P<0.0001$).

Separate regression logistic analyses were also performed in each HDL group. Although the odds ratios did not reach statistical significance, in the HDL-deficient group, the QQ genotype conferred MI risks of 1.38 (range 0.32 to 5.93, 95% CI) and 2.07 (range 0.29 to 14.75) compared with QR and RR genotypes, respectively. Conversely, the QQ genotype was found to be protective in the normal HDL group (0.81 [range 0.28 to 2.27] and 0.52 [range 0.13 to 2.05] compared with QR and RR genotypes, respectively). PON1 enzyme activity levels toward paraoxon were protective against MI risk in the overall group of study subjects (0.998 [range 0.996 to 0.999], $P=0.0196$) as well as in both HDL categorized groups (0.997 [range 0.994 to 0.999], $P=0.0412$ in the HDL-deficient group; 0.998 [range 0.996 to 1.000], $P=0.1216$ in the normal HDL group).

In the entire population, there were nonsignificant odds ratios for the effect of the R allele versus the QQ genotype on MI risk (1.19 [range 0.65 to 2.17]) or the QQ genotype versus the R allele on MI risk (0.87 [range 0.51 to 1.49]).

Discussion

Human and animal studies strongly support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis. Therefore, mechanisms preventing LDL oxidation appear to be antiatherogenic. HDL-associated PON1 may be, in this respect, a major defense against lipoperoxides from oxidized LDL, and the property of HDL to attenuate the oxidation of LDL seems to be largely attributable to PON1. Together with other polymorphic sites at the PON1 locus, the PON1-192 polymorphism is a major determinant of serum PON1 activity toward various organophosphates, such as paraoxon.

When considered as a whole, our results did not support the existence of a significant association between PON1-192 genetic polymorphism and MI risk. This finding is consistent with the results of previous studies carried out in the Mediterranean area but differs from other studies conducted in the United States and in a Japanese population. It is conceivable that PON1-192 polymorphism produces an effect on coronary heart disease risk only among particular subgroups of subjects in the presence or absence of additional factors. Until now, in those studies in which an association with coronary heart disease was observed, it was paradoxically the high paraoxon–activity R allele that was associated with it. However, we have recently reported that the risk of MI associated with a classic risk factor such as smoking may be increased in subjects homozygous for the low paraoxon–activity PON1 QQ genotype. Furthermore, it has also recently been shown that the QQ genotype may represent an additional risk factor for carotid atherosclerosis in subjects with familial hypercholesterolemia.

A finding of the present study confirms previous observations relating lower PON1 activity levels in MI patients than in controls. In addition to low PON1 activity in patients who had suffered from MI, a significant decrease in PON1 activity toward paraoxon hydrolysis has been shown in diseases with accelerated atherogenesis, such as familial hypercholesterolemia and diabetes mellitus.

In the present study, PON1 activity levels of HDL-deficient control subjects were similar to the levels in subjects with normal HDL cholesterol concentrations. Conversely, HDL-deficient MI patients showed the lowest PON1 activity. We found that the decrease of PON1 activity associated with HDL deficiency in patients was considerably more marked among those who were QQ homozygotes. Furthermore, the prevalence of the QQ genotype in HDL-deficient MI patients was 11.8% higher than in HDL-deficient control subjects. We also found that the effect of PON1-192 genotypes on the association of reduced HDL cholesterol levels and MI risk was related to gene dosage: the effect was highest in the QQ
These observations raise some interesting considerations. The QQ genotype frequency in MI patients with low HDL cholesterol levels was similar to that of MI patients with normal HDL cholesterol levels. Therefore, the low PON1 activity observed in QQ HDL-deficient MI patients cannot be attributable only to an overrepresentation of the QQ genotype in these patients. Neither was this low PON1 activity explained by an overrepresentation of MI diabetic patients in the HDL-deficient group. From a complementary point of view, the low frequency of the QQ genotype in HDL-deficient control subjects was not reflected in higher PON1 activity levels compared with those in control subjects with normal HDL levels. Therefore, it can be argued that the low PON1 activity observed in QQ HDL-deficient MI patients may be a consequence of some aspect of the disease or a combination of factors rather than the frequency of the QQ genotype alone. It has been reported that in genetically HDL-deficient states, PON1 activity is reduced but still present in appreciable quantities. This is logical when it is considered that PON1 is associated with a very small subfraction of HDL containing apoA-I. However, there is no doubt that a decrease of HDL compromises, at least in part, PON1 function.

The HDL of transgenic mice overexpressing apoA-II has been described to have a decreased content of PON1. Recently, Ombre et al have reported that the QQ genotype is associated with significantly increased plasma levels of apoA-II. Therefore, the possibility that HDL-deficient MI patients may also have some qualitative changes in the protein content of the HDL particle that could cause a greater loss of PON1 activity cannot be ruled out. Possibly, this is an important point.

Another point of interest concerns the inactivation of PON1 in the presence of oxidative stress. PON1 activity has been shown to be reduced in the course of oxidative incubation with Cu²⁺-induced peroxidation of LDL. Oxidized LDL appears to inactivate PON1 through interactions between the enzyme-free sulfhydryl group and oxidized lipids, which are formed during LDL oxidation. There is evidence of an increase of lipid peroxidation products in patients with coronary heart disease. There are also some observations suggesting that a low PON1 activity toward paraoxon is likely to be present at the time of acute MI. In this respect, PON1 activity may be partially inactivated in the presence of oxidative stress, as probably occurs in patients with coronary heart disease or atherosclerosis. Nevertheless, as observed in the present study, the question that arises is why the decrease in PON1 activity and the MI risk associated with HDL deficiency are more evident in the low paraoxon–activity QQ genotype. Assuming that serum PON1 is inactivated in the presence of a high oxidative stress, as probably occurs in MI patients, it is possible that subjects with low HDL and the QQ genotype have a reduced ability to preserve PON1 activity. The QQ genotype, which is associated with low PON1 activity toward paraoxon, may be adequate to prevent lipid peroxidation in normolipidemic individuals. However, the low paraoxon–activity QQ genotype may be insufficient when an HDL-deficiency state (which compromises PON1 function) and an oxidative stress (which inactivates PON1 activity) coexist.

A second coding region in the human PON1 gene, the Met/Leu55 polymorphism, which is in strong linkage disequilibrium with the PON1-192 polymorphism, seems to be associated with differences in PON1 concentrations and activities. Remarkably, Levy and James have recently identified 3 polymorphisms in the promoter region of the human PON1 gene that appear to have a great impact on PON1 activity levels and PON1 concentrations. This finding contributes to the establishment of a genetic basis for variations in PON1 levels, with physiological importance on the genetic nature of the antioxidant properties of HDL. The relevance of these observations and the potential clinical impact of our results deserve additional research involving PON1 in HDL-deficiency status in relation to these polymorphisms.

Study Characteristics and Clinical Implications

Sample size required to detect gene-environment interaction is a function of the exposure frequency and the genotype prevalence. Our sample size was sufficient to detect a statistically significant interaction between genotype and categorized HDL. On the other hand, it is assumed that when the susceptible genotype is common, a modest sample size is adequate.

In genetic associations, several geographic differences may exist, with the most convincing evidence being reproducibility in different populations. Therefore, if confirmed, the results of the present study would appear to have a particular clinical relevance. Because the population prevalence of the QQ genotype seems to be very high (~50%), the impact of such a genetic characteristic in the population is considerable. Further studies are needed to determine whether applying more aggressive management of HDL-deficiency states could reduce the risk of MI in subjects carrying the QQ genotype.

Summary

Our findings lead to an interesting hypothesis: PON1-192 genetic polymorphism modulates the nonfatal MI risk associated with decreased HDL cholesterol levels. The risk of nonfatal MI is increased in HDL-deficiency states, principally among subjects carrying the low paraoxon–activity QQ PON1-192 genotype.

Appendix

The Registro Gironi del Cor (REGICOR) investigators involved in this research are as follows: R. Masía and J. Sala (Hospital Josep Trueta de Gerona) and M.I. Covas, R. Elouza, M. Gil, J. Marrugat, A. Pena, M. Sentí, and M. Tomás (Institut Municipal d’Investigació Mèdica).

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