Paraoxonase Gln-Arg(192) and Leu-Met(55) gene polymorphisms and enzyme activity in a population with a low rate of coronary heart disease

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Abstract

Objectives: To assess whether paraoxonase (PON1) polymorphisms at positions 55 and 192 and/or their phenotypic expressions influence the risk of myocardial infarction (MI) in Spanish population.

Design and methods: Two hundred and fifteen male survivors of a MI and their age-matched controls were included in the study. Lipids, apolipoproteins (apo) A-I and B, PON1 activity on paraoxon and phenylacetate and PON1 polymorphisms were determined.

Results: Genotype distribution was similar in patients and controls. Enzyme activities were lower in patients, but multiple logistic regression analysis did not show any independent association with a higher risk of MI.

Conclusion: None of the PON1 polymorphisms or their corresponding measured activities are independent risk factors for MI in our population.

Keywords: Lipids; Mediterranean diet; Myocardial infarction; Oxidation; Paraoxonase

1. Introduction

Oxidative stress in low-density lipoproteins (LDL) is considered to be a major contributor to the atherogenic process, the principal cause of mortality in developed countries [1]. High-density lipoproteins (HDL) are thought to protect LDL from oxidation [2,3] due to the presence of antioxidant enzymes that hydrolyze biologically active lipids in oxidized LDL (oxLDL), among which PON1 seems to be of major importance [4–7]. PON1 is a calcium-dependent esterase, closely associated to HDL in serum, which is known to catalyze the hydrolysis of organophosphates. Its physiologic role, however, has not been totally clarified.

PON1 has two aminoacid polymorphisms. The one at position 192 [glutamine (Q allele)/arginine (R allele)] modulates the hydrolytic activity of PON1 toward some exogenous substrates—such as paraoxon. The activity of the isoenzymes with glutamine at position 192 is lower while the activity of the isoenzymes with arginine at position 192 is increased by high concentrations of salt. However, this polymorphism does not affect the activity toward other substrates, like phenylacetate [8]. The other polymorphism, located at position 55, [leucine (L allele)/methionine (M allele)], does not affect the intrinsic activity of the enzyme so clearly, although it has been shown to modulate its concentration in plasma [9,10].

Some studies have associated the presence of the R allele or the homozygosity of the L allele with a greater susceptibility to coronary heart disease (CHD) [11–17] but this is still controversial [18–28]. More recently, the PON1 activity phenotype has been shown to be a better predictor of CHD than the PON1 (192) or PON1 (55) genotypes [29]. Our aim was to assess the relationship between PON1 activity and both of these gene polymorphisms in the Spanish population, which has one of the lowest incidences of MI in Western societies [30].
2. Methods

2.1. Subjects

The procedures complied with the ethical standards of the Hospital Universitari de Sant Joan and the Hospital Universitari Joan XXIII. As in a previous study [31], we located the medical records of patients registered as having had a MI and invited the surviving male patients who had a history such as that defined by the criteria of the World Health Organization to participate [32]. We made a baseline examination and assessment of the cardiovascular status of the first 215 patients who agreed to take part in the study and who had given informed consent. Samples were obtained 3 to 4 months after the last acute episode. The patients were matched for age (± 5 yr) and body-mass index (± 1.5) with 215 male controls from the same geographical area with no clinical evidence of coronary disease (they were all recruited at their place of work during a routine medical examination).

2.2. Laboratory measurements

DNA was obtained from leukocytes using standard procedures. To determine the polymorphisms at positions 55 and 192, DNA was amplified and analyzed by restriction isotyping as previously described [33]. Only serum drawn after overnight fasting was used for biochemical measurements. After separation the samples were frozen immediately at −70°C until they were analyzed. PON1 activities were measured using the discriminative paraoxon and the nondiscriminative phenylacetate substrates, as previously described [34,35]. Briefly, the baseline and the 1 M NaCl-stimulated rate of hydrolysis of paraoxon (paraoxonase activity) were measured by monitoring the increase in absorbance at 412 nm in an automated assay performed in an ILab 900 automatic analyzer (Instrumentation Laboratories, Milan, Italy). The rate of hydrolysis of phenylacetate (arylesterase activity) was measured by monitoring the absorbance at 270 nm in a spectrophotometer (UVIKON 922, Kontron Instruments, Zurich, Switzerland).

Serum levels for total cholesterol and triglyceride were measured by enzymatic methods (ITC Diagnostics, Barcelona, Spain). HDL cholesterol was measured with a homogeneous assay [36]. Apoprotein (apo) A-I and apo B levels were measured by immunoturbidimetry (BioKit, Barcelona, Spain).

2.3. Statistical analysis

The normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test. Since some measurements (paraoxonase and arylesterase activities and triglyceride and apo B concentrations) did not present with normal distributions, their values were log-transformed before the statistical analysis. One-way analysis of variance or the nonparametric Mann-Whitney U test was used. Allele frequencies were estimated by the gene-counting method and Hardy-Weinberg’s equilibrium was tested by the chi-square test. The strength of the association of the selected variables with the occurrence of myocardial infarction was estimated by calculating the odds ratios with Epi-Info (Centers for Disease Control and Prevention, Atlanta). Logistic regression was used to assess the ability to predict case status and to ascertain the independence of the cardiovascular risk factors. Statistical assessment was carried out using SPSS/PC + 10.0 (SPSS, Chicago, IL, USA). A significant difference was defined as a two-tailed \( p < 0.05 \).

3. Results

3.1. Study population and cardiovascular risk factors

Patients with MI were relatively young and the serum concentrations of HDL cholesterol and apo A-I were lower than in controls. The serum concentrations of cholesterol, triglycerides and apo B were higher in patients than in controls (Table 1). As expected, the prevalence of diabetes, hypertension and cigarette smoking was also higher in patients than in controls. Arylesterase activity was similar in patients and in controls but the paraoxonase activity (baseline and stimulated) was significantly lower in patients. Table 2 shows the odds ratios for the selected risk factors. The odds ratios for plasma triglycerides, apo A-I, apo B and the PON1 genotypes did not show any significant association with the presence of disease when compared with the age-matched controls. The risk factors associated with the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Main characteristics of the populations studied. Continuous variables are expressed as mean ± SD</th>
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<tbody>
<tr>
<td>Controls (n = 215)</td>
<td>Myocardial infarction (n = 215)</td>
</tr>
<tr>
<td>Age in years</td>
<td>62.1 ± 16.4</td>
</tr>
<tr>
<td>Body-mass index, kg/m²</td>
<td>26.2 ± 3.5</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>9 (4.2)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>8 (3.7)</td>
</tr>
<tr>
<td>Current or former smoker, n (%)</td>
<td>72 (33.4)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.27 ± 1.21</td>
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<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.23 ± 0.42</td>
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<tr>
<td>Triglyceride, mmol/L</td>
<td>1.52 ± 0.91</td>
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<tr>
<td>Apo A-I, g/L</td>
<td>1.38 ± 0.25</td>
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<tr>
<td>Apo B, g/L</td>
<td>1.10 ± 0.33</td>
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<tr>
<td>Paraoxonase activity (U/L), baseline</td>
<td>265 ± 54</td>
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<tr>
<td>Paraoxonase activity (U/L), stimulated</td>
<td>442 ± 146</td>
</tr>
<tr>
<td>Arylesterase activity (KU/L), baseline</td>
<td>92 ± 21</td>
</tr>
</tbody>
</table>

\[ ^{a} p < 0.05, \]

\[ ^{b} p < 0.005 \text { with respect to controls.} \]
highest estimated odds ratio were the presence of hypertension, smoking status and HDL cholesterol. The significances were confirmed with a multiple logistic-regression model that adjusted for the presence of other cardiovascular risk factors. However, 56% of the risk of developing MI in this population was already attributable to hypertension, smoking, plasma cholesterol and HDL cholesterol.

3.2. Genotypes do not predict myocardial infarction

Table 3 summarizes the genotyping data. These genotype distributions were in Hardy-Weinberg equilibrium. There were no statistical differences in the PON1 (55) and PON1 (192) genotype or the allele distribution between case patients and age-matched controls. Neither were there any differences in the frequencies of combined haplotypes (Table 3). A previously described linkage disequilibrium [8], which produced arginine at position 192 and leucine at position 55, was also observed in our sample (Table 3). The PON1 (55), PON1 (192) and combined haplotypes did not predict case status by use of logistic regression.

3.3. Influence of PON1 polymorphisms on enzyme activities

When the enzyme activities were plotted according to the polymorphisms studied, it was evident that PON1 (55) and PON1 (192) polymorphisms greatly influenced baseline and stimulated paraoxonase activities but arylesterase was less influenced. There were also some statistically significant differences in paraoxonase and arylesterase activities between patients and controls (Fig. 1) that disappeared when the haplotypes were considered. Table 4 shows the enzyme activities of the haplotypes for the whole group. The Met-Leu55 polymorphism influenced the hydrolysis of both substrates. The activity of isoenzymes with methionine at codon 55 was lower. Serum PON1 activity increased in the order MM < ML < LL in both the patient and control groups. There was also a good correlation between the baseline and stimulated paraoxonase activities within the PON1 (192) genotypes or combined haplotypes, and the activity increased in the order QQ < QR < RR. The linkage disequilibrium that associated L and R alleles, and which led to a greater proportion of R allele carriers among the L allele carriers than among the M allele carriers, may explain the different activity toward paraoxon.

3.4. The effect of PON1 activities is not independent of other risk factors

As expected, serum baseline and stimulated paraoxonase activity phenotypes significantly predicted CHD cases vs. controls (Baseline paraoxonase: $R^2 = 0.489$; B = 0.052; $p = 0.032$. Stimulated paraoxonase: $R^2 = 0.510$; B = 0.103; $p = 0.015$, respectively). Logistic regression was used with age as a covariate. However, when PON1 (192), PON1 (55) genotypes or combined haplotypes were added to paraoxonase in the prediction model, the effects were no longer significant. A similar effect was observed when the prediction model was adjusted for total cholesterol, HDL-cholesterol, triglycerides, apoA-I and apo B.

4. Discussion

There is increasing evidence showing that serum PON1 is related to the prevention of CHD [11–17]. According to
the oxidation hypothesis of atherosclerosis, this enzyme prevents lipoperoxides from accumulating in LDL particles [3–5]. Consequently, considerable research has been done to determine its physiological role and the functional differences between its isoenzymes. The Gln-Arg192 and Leu-Met55 polymorphisms are known to affect PON1 activity using paraoxon as a substrate and, therefore, both genotypes should be included in association studies although, to our knowledge, previous data are scarce [29].

In a Spanish sample, we found no significant differences in genotype and allele frequencies for PON1 polymorphisms at positions 55 and 192 between control subjects and patients with MI. The frequencies were similar to those described for other Caucasian populations [8,10,12,17,18], although the variability and discrepancies were considerable even in studies conducted in the same ethnic population. These discrepancies may be related to the effects caused by other genes as PON2 [37] or to posttranslational modifica-

![Fig. 1. Serum PON activities in patients (black bars) and controls (white bars) according to the 55 and 192 gene polymorphisms. *\(p < 0.05\) with respect to controls; \(p < 0.05\) with respect to the other two genotypes; \(p < 0.05\) with respect to QQ or MM, respectively.](image-url)
tions of the enzyme. However, we found that paraoxonase activity was significantly lower in survivors of MI. It is obvious, therefore, that if the genotype distribution in patients is not different from that in controls, these genetic polymorphisms or combined haplotypes cannot be the cause of the difference in PON1 activity between them.

In our sample, however, the two polymorphisms were associated with PON1 activity, which increased in the order of the QQ < QR < RR genotype in the PON1 (192) polymorphism and MM < ML < LL genotype in the PON1 (55) polymorphism. In our cohort, then, the genotype is seen to have an effect. The lower activity of PON1 using paraoxon as a substrate observed in our patients suggests that there is an association between paraoxonase and CHD, but the relationship seems to be weak (OR = 2.85) as compared with that of hypertension (OR = 5.12) or smoking status (OR = 3.99) (Table 2). Our patients, however, were survivors of myocardial infarction; a possible selection bias cannot be excluded. Patients who did not survive may have a different prevalence of this risk factor. In our patients, the association with well-known cardiovascular factors was stronger in terms of the estimated odds ratio and these conventional factors explain most of the attributable risk. Moreover, when we added these variables to the prediction model in a regression analysis, paraoxonase was no longer a statistically significant factor. Therefore, in accordance with previous results [19–26], our data suggest that differences in PON1 do not affect healthy individuals. Interestingly, however, most of the associations between PON1 polymorphisms and higher cardiovascular risk have been found in diabetics. The different protection of PON1 isoenzymes may therefore only be evident in individuals with certain additional cardiovascular risk factors or increased oxidative stress.

Although association studies into multifactorial diseases have played a key role in implicating some genes in MI, they do have certain limitations [38]. The PON1 genotypes do not seem to be the cause of the disease, and the positive association is probably dependent on the characteristics of the population studied [11–13]. However, these genotypes may still interact with other inherited traits to cause the disease process and some modifiers of paraoxonase activity not reflected by these two polymorphisms may play a role in atherogenesis. To illustrate this point it can be mentioned a recent finding by our group describing an interaction between apo E and PON1 (55) polymorphisms that affect PON1 activity [39].

Other factors should be considered in an attempt to shed some light on these confusing results. It has been suggested that some isoenzymes may protect LDL from oxidative modifications more effectively than others [40–43]. This suggestion, however, has raised considerable controversy. It may also be possible that the antioxidant effect of some PON1 isoenzymes prevents certain processes that are closely connected to the development of CHD and their effects are only detectable under stress conditions [44,45]. Diet should also be considered. One of the characteristics of the Mediterranean diet is that it is high in antioxidants and low in cooking-related oxidants. This decreases the waste of nutritional and endogenous antioxidants and may mask quantitative and functional changes in serum paraoxonase [46,47]. Similar effects have been observed with a moderate daily consumption of alcohol [48]. Moreover, a high intake of antioxidants can preserve the activity of the enzyme and its capacity to hydrolyze hydroperoxides [49–51], and the concentration of PON1 in populations with traditional Western-type diets is lower than in Mediterranean populations [39,52].

In summary, there is no association between the PON1 (192) and PON1 (55) genotypes and increased risk of myocardial infarction in our population but patients have lower plasma paraoxonase activity than controls. Further studies are needed to ascertain the role of antioxidants in preventing CHD.

Acknowledgements

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