

Association Between Paraoxonase-1 and Paraoxonase-2 Polymorphisms and the Risk of Acute Myocardial Infarction

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Introduction and objectives. Two particular polymorphisms, namely *PON1-192* and *PON2-311*, in the genes encoding the antioxidant enzymes paraoxonase-1 (PON1) and paraoxonase-2 (PON2) have been associated with an increased risk of acute myocardial infarction (AMI). However, previous findings have been contradictory. The aim of this study was to investigate the association between the *PON1-192* and *PON2-311* polymorphisms and their interaction on AMI risk.

Methods. This case-control study involved 746 consecutive AMI patients and 1796 control subjects without cardiovascular disease, who were randomly selected from the same population from which the patients came. All participants were recruited between 1999 and 2000 from four Spanish autonomous regions. All were assessed for the presence of *PON1-192* and *PON2-311* and for classical cardiovascular risk factors. Multivariate analysis was carried out using logistic regression modeling.

Results. The odds ratios (OR) of AMI for patients with the *PON1-192* QQ and *PON2-311* SS genotypes (who comprised 50% and 66% of the population, respectively) were 1.26 (95% confidence interval [CI], 1.02-1.55) and 1.25 (95% CI, 1.04-1.50), respectively, compared with R and C allele carriers. Moreover, in patients with both QQ and SS genotypes, the adjusted OR of AMI increased to 1.41 (95% CI, 1.13-1.76).

Conclusions. Our results indicate that the *PON1-192* and *PON2-311* polymorphisms were independent risk factors of AMI in our population.

Key words: Coronary disease. Genetics. Antioxidants. Enzymes. High-density lipoprotein (HDL).

The full list of researchers participating in the IBERICA study is available at: http://www.regicor.org/inv_IBERICA_CC

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Asociación de los polimorfismos de la paraoxonasa 1 y la paraoxonasa 2 con el riesgo de infarto agudo de miocardio

Introducción y objetivos. La paraoxonasa 1 (PON1) y la paraoxonasa 2 (PON2) son enzimas antioxidantes cuyos polimorfismos *PON1-192* y *PON2-311* se han relacionado con el riesgo de infarto agudo de miocardio, con resultados discordantes. El objetivo de este estudio es determinar la asociación con el riesgo de infarto agudo de miocardio (IAM) de los polimorfismos *PON1-192* y *PON2-311* y su interacción.

Métodos. Se realizó un estudio de casos y controles en el que se reclutó a 746 pacientes consecutivos con IAM y 1.796 controles libres de enfermedad cardiovascular seleccionados al azar de la misma población de la que provenían los casos, en 4 comunidades autónomas españolas entre 1999 y 2000. Se determinaron los polimorfismos *PON1-192* y *PON2-311*, además de los factores clásicos de riesgo cardiovascular. Se estimaron mo-

delos de regresión logística para los análisis multivariados.

Resultados. Las *odds ratio* (OR) del genotipo QQ del polimorfismo *PON1-192* y el SS del *PON2-311* (presentes en el 50 y el 66% de la población, respectivamente) de presentar un IAM fueron 1,26 (intervalo de confianza [IC] del 95%, 1,02-1,55) y 1,25 (IC del 95%, 1,04-1,50), respectivamente, en comparación con los portadores de los alelos R y C. Además, para los sujetos que presentan ambos genotipos QQ y SS, la OR ajustada de tener un IAM se incrementó hasta 1,41 (IC del 95%, 1,13-1,76).

Conclusiones. Nuestros resultados indican que los polimorfismos *PON1-192* y *PON2-311* son factores de riesgo de IAM independientes en nuestra población.

Palabras clave: Enfermedad coronaria. Genética. Anti-oxidante. Enzimas. Lipoproteínas de alta densidad (HDL).

ABBREVIATIONS

AMI: acute myocardial infarction
 CHD: coronary heart disease
 CI: confidence interval
 HDL: high-density lipoprotein
 LDL: low-density lipoprotein
 OR: odds ratio
 PON1: paraoxonase 1
 PON2: paraoxonase 2

INTRODUCTION

Coronary heart disease (CHD) continues to be the leading cause of mortality and morbidity in developed countries.¹ The paradigm of protective high-density lipoproteins (HDL) has been broadened due to their antioxidant properties, among others.^{2,3} The main antioxidant enzyme carried by HDL particles is paraoxonase 1 (PON1). PON1 is a member of the protein family that also includes PON2 and PON3.⁴ PON1 and PON3 form part of HDL particles, whereas PON2 is found in a large variety of tissue, such as endothelial cells, smooth muscle cells, and macrophages. The mechanism of action of the PON family is still unclear. PON1 has esterase activity to several substrates, whereas PON2 and PON3 show high lactonase activity.^{5,6} In addition, it has been suggested that PON enzymes have other biological activity, including phospholipase A2 activity, which hydrolyzes platelet activation factor, lipid oxidation, and a role in the hydrolysis and inactivation of the homocysteine thiolactone. Nevertheless, the main protective mechanism linked to HDL-transported PON1 seems to be the reverse transport of cholesterol and preventing low-density lipoprotein (LDL) oxidation.⁶ On the other hand, PON2 has shown antioxidant properties similar to those of PON1 in preventing LDL

oxidation.⁷ Several studies have focused on aminoacid substitution at position 311 (serine→cystein) in the *PON2* gene⁸ and at position 192 (glutamine→arginine) in the *PON1* gene,⁹ and their potential impact on the activity of these enzymes, and on individual susceptibility to CHD risk.

The relationship between *PON1-192* and *PON2-311* polymorphisms, and the risk of CHD is not well established. The results from previous studies have been contradictory.^{2,10,11} Although 3 metaanalyses concluded that the *PON1-192R* allele was weakly but significantly associated with an increased risk of CHD, other studies conducted in Europe,¹²⁻¹⁴ the USA,¹⁵ and Japan¹⁶ reported a trend towards increased CHD risk in individuals with the QQ genotype. Furthermore, few studies have assessed the relationship between the PON2 genetic variants and CHD. Some studies, although not all, have shown that *PON2-311 S* allele polymorphism is directly associated with CHD, vascular dementia and ischemic stroke, or to early microvascular complications in diabetic patients.^{17,18} Even fewer studies have simultaneously analyzed the risk of CHD attributed to PON1 and PON2 genetic variants.¹⁸⁻²²

The purpose of the present study was to assess the relationship between *PON1-192* and *PON2-311* polymorphisms, and their interaction with acute myocardial infarction (AMI).

METHODS

Study Design

This was a population-based case-control study including 4 Spanish regions: Castile-La Mancha, Girona, Mallorca, and the Basque Country. Identical methods were used in all locations to detect AMI events, record demographic and clinical characteristics, and perform laboratory tests which were conducted in an independent core laboratory. Informed consent was obtained from all subjects; the local ethical committee approved the study following the Declaration of Helsinki guidelines.

Case Identification

A total of 746 consecutive patients who had survived a first AMI were prospectively recruited (620 men and 126 women; mean age, 60.0 [10.3] years). They had been admitted to the reference coronary care units in the study areas between 1999 and 2000. Standard definitions and criteria for AMI diagnosis were employed.²³ All subjects were white and of European descent.

Selection of Control Subjects

Using data from the Spanish National Census, 1796 subjects (1508 men and 288 women; mean age, 59.3 [10.4] years) free of cardiovascular disease were randomly

selected as the control group. These subjects lived in the same region as the case subjects and were selected during the same period. The participation rate was higher than 70%. Angina and AMI was ruled out by medical history and electrocardiogram. Subjects with a history of stroke, noncardiovascular disease with poor short-term prognosis, mental disabilities, or drug abuse were also excluded from the study.

Sample Size and Statistical Power

The statistical power was 90% to detect an odds ratio (OR) ≥ 1.35 as statistically significant ($\alpha = .05$, 2-tailed test) and a dominant genetic model assumed, with a prevalence of 60% for the common homozygous genotype in the controls, and a 2.4 ratio between controls and cases.

Laboratory Test

Blood samples were collected from controls after overnight fasting. A blood sample to isolate DNA was obtained from the AMI patients a few hours after admission, and a second biological plasma, and serum sample was obtained from those surviving the acute phase 6 months after the acute coronary event.

All the analyses were performed by an independent core laboratory. Serum glucose, total cholesterol, and triglyceride concentrations were determined using enzyme kits (Roche Diagnostic, Basel, Switzerland) adapted to a Cobas Mira Plus analyzer (Hoffmann-La Roche, Basel, Switzerland). High-density lipoprotein cholesterol (HDL-C) was determined as the cholesterol remaining after phosphotungstate-Mg⁺⁺ precipitation of lipoprotein B-containing apolipoprotein (Boehringer, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) concentration was calculated by means of the Friedewald formula.²⁴

Genotyping

Genomic DNA was isolated from white blood cells using the salting-out method.²⁵ Polymerase chain reaction (PCR) was performed to genotype *PON1-192* (rs662 in dbSNP) polymorphism using primer sequences from published data.²⁶ The amplification cycle for *PON1-192* polymorphism genotyping was performed in a Perkin-Elmer Cetus 2400 thermocycler with an initial 4-min denaturation period at 94°C, followed by 35 cycles of 30 s at 94°C, 1 min at 61°C, and 1 min at 72°C, with a final extension of 7 min at 72°C. After amplification, PCR products were digested with AlwI (4 h at 37°C) and separated by electrophoresis in a 3% agar gel at 60 V for 75 min. Quantitative real time PCR using a TaqMan Assay-on-Demand probe (C-8952817-10, Applied Biosystems, Foster City, California, USA) was used to genotype *PON2-311* polymorphism (rs7493 in dbSNP).

Other Variables

Hypertension was determined according to the self-reported history of the patient. Diabetes was established by fasting glucose ≥ 126 mg/dL or by the use of insulin or a hypoglycemic agent. Subjects were classified as current smokers if they reported having smoked cigarettes during the previous year. Weight and height were measured on a scale with a stadiometer while the individual was barefoot and wearing minimal clothing. Body mass index was calculated as weight in kilograms divided by the height in meters squared. The use of dyslipidemic agents was also recorded. Daily energy expenditure due to physical activity during leisure time in the previous year was calculated using the Minnesota Leisure Time Physical Activity Questionnaire, and was administered during hospital stay. This instrument has been validated for use in the Spanish population.^{27,28}

Statistical Analysis

Due to the low prevalence of RR homozygotes (11%) and CC (5%) in *PON1-192* and *PON2-311* genotypes, respectively, a dominant genetic model was assumed. For continuous variables, differences between the 2 *PON1-192* genotype groups (ie, QQ vs R carriers) or between the 2 *PON2-311* genotype groups (ie, SS vs C carriers) and between AMI patients and the controls were analyzed with the Student *t* test or the non-parametric Mann-Whitney *U* test as appropriate, and the χ^2 test was used for discrete variables.

The odds ratio (OR) for AMI risk, adjusted for the *PON1-192* and *PON2-311* genotypes confounding variables, was estimated by means of a conditional logistic regression analysis.

The linear trend was analyzed using the χ^2 test for discrete variables, and ANOVA for continuous variables stratified by the *PON1-192* and *PON2-311* genotypes. The analysis of linkage disequilibrium between the 2 genetic variants was conducted with the *haplo.stats package* using R routines. Other analyses were performed using SAS version 8.2 (SAS Institute, Cary, N.C., USA). A *P* value less than .05 was considered significant.

RESULTS

The frequency of the *PON1-192* R allele was 0.32 in the control group and 0.29 in the case group, and the frequency of the *PON2-311* C allele was 0.21 in the control group and 0.19 in the case group. The proportion of diabetes, hypertension, and smoking was greater in the case group than in the control group, and energy expenditure due to physical activity during leisure time was less in the former (Table 1). Six months after the event, body mass index, total cholesterol, and

TABLE 1. Myocardial Infarction Risk Factors, *Paraoxonase1-192* and *Paraoxonase2-311* Genotypes in Myocardial Infarction Patients and Control Subjects

	AMI Patients	Controls	P
Baseline, n	746	1796	
QQ homozygote, %	52	47.2	.026
SS homozygote, %	67.6	62.6	.017
Female, %	16.9	16	.595
Age, mean (SD), y	60 (10.3)	59.3 (10.4)	.107
Diabetes, %	25.6	17.2	<.001
Hypertension, %	43.9	33.1	.003
Current smoker, %	45.9	22.1	<.001
EEPA, median (interquartile range), kcal/d	224 (106-456)	291 (162-503)	<.001
At 6 months, n	548	1780	
Total cholesterol, mean (SD), mmol/L	5.26 (0.88)	5.91 (1.11)	<.001
HDL-C, mean (SD), mmol/L	1.04 (0.28)	1.27 (0.36)	<.001
LDL-C, mean (SD), mmol/L	2.79 (0.66)	3.16 (0.78)	<.001
Triglycerides, mean (SD), mmol/L	1.36 (1.03-1.82)	1.27 (0.94-1.71)	.001
Glucose, mean (SD), mmol/L	6.27 (2.44)	6.11 (1.67)	.267
BMI, mean (SD)	27.2 (4.1)	28 (4)	<.001
Dyslipidemic treatment, %	70.2	13.1	<.001

AMI indicates acute myocardial infarction; BMI, body mass index; EEPA, energy expenditure due to physical activity during leisure time; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation;

LDL-C, and HDL-C concentrations were significantly lower, and triglyceride concentrations higher in patients with AMI compared to the control group. The percentage of patients receiving dyslipidemic treatment was much higher in the AMI patients than in the control group. No linkage disequilibrium was detected between the 2 polymorphisms ($D' = 0.083$). The QQ and SS genotypes were observed more frequently in AMI patients (Table 1).

Among R-carriers, there was a smaller percentage of AMI cases, a greater percentage of women, higher HDL-C concentrations, and lower triglyceride concentrations than in QQ homozygotes (Table 2).

In *PON2-311* polymorphism, the C-carriers presented fewer cases of AMI.

The crude OR of undergoing AMI for the QQ genotype (*PON1-192*) was 1.21 (95% confidence interval [CI], 1.02-1.44). After adjusting for HDL-C and triglycerides, the OR was 1.26 (95% CI, 1.02-1.55). The crude OR of undergoing AMI for the SS genotype (*PON2-311*) was 1.25 (95% CI, 1.04-1.49). After adjusting for age and sex, OR was 1.25 (95% CI, 1.04-1.5). None of the first-order interactions between *PON1-192* or *PON2-311* polymorphisms and diabetes, hypertension, smoking, body mass index, HDL-C, and daily energy expenditure due to physical activity during leisure time on AMI risk were statistically significant. The crude OR of AMI for subjects who carried both QQ and SS genotypes was 1.38 (95% CI, 1.16-1.65). After adjusting for HDL-C and triglycerides, OR was 1.41 (95% CI, 1.13-1.76).

DISCUSSION

In this case-control study conducted in a large representative sample of the Spanish population, we found that *PON1-192* and *PON2-311* polymorphisms are independently associated with increased AMI risk in our population.

Other studies have already analyzed the role of *PON1-192* and *PON2-311* polymorphisms in CHD risk. Three recent metaanalyses concluded that the *PON1-192* R allele was weakly, but significantly, associated with increased CHD risk, although many of the studies included in these metaanalyses did not reach statistical significance.^{2,10,11} Moreover, some of the studies included in the metaanalyses showed that the QQ genotype had a tendency to be associated with CHD.¹²⁻¹⁶ The results from our study also suggest that the QQ genotype is associated with greater susceptibility to AMI. There are several reasons that may account for the differences between our study and the results of these metaanalyses. Metaanalyses usually suffer from some biases, such as positive-result publication bias and the heterogeneity of the studies themselves, which can often adversely affect the validity of the results. Furthermore, selection bias, population differences in ethnicity, and differences in the criteria used for defining phenotype between studies cannot be ruled out. Although there are also discrepancies concerning *PON2-311* polymorphism and an association with CHD, most studies have shown an increased CHD risk for S-carriers.^{17,18} The present study is based on a large sample, and is representative of several regions of an ethnically

TABLE 2. Myocardial Infarction Risk Factors in *Paraoxonase1-192* and *Paraoxonase2-311* Genotypes

	<i>PON1-192</i> Genotypes			<i>PON2-311</i> Genotypes		
	QQ Homozygotes	R-Carriers	P	SS Homozygotes	C-Carriers	P
Baseline, n	1235	1307		1628	914	
AMI patients, %	31.4	27.4	.026	31	26.5	.017
Female, %	14.6	17.9	.023	16	16.8	.565
Age, mean (SD), y	58.1 (10.5)	59.9 (10.2)	.051	59.4 (10.3)	60 (10.5)	.271
Diabetes, %	19	20	.622	19.5	19.6	.941
Hypertension, %	39.8	35.2	.191	37.9	36.8	.759
Current smoker, %	30	27	.117	29.2	27.4	.349
EEPA, median (interquartile range), kcal/d	260 (147-470)	291 (149-518)	.072	272 (150-491)	287 (146-497)	.818
At 6 months	1125	1204		1478	850	
Total cholesterol, mean (SD), mmol/L	5.72 (1.06)	5.78 (1.11)	.280	5.75 (1.09)	5.78 (1.11)	.671
HDL-C, mean (SD), mmol/L	1.19 (0.34)	1.24 (0.36)	.048	1.22 (0.36)	1.22 (0.34)	1.000
LDL-C, mean (SD), mmol/L	3.05 (0.74)	3.1 (0.8)	.200	3.1 (0.8)	3.05 (0.74)	.201
Triglycerides, median (interquartile range), mmol/L	1.29 (0.98-1.76)	1.28 (0.94-1.71)	.048	1.27 (0.96-1.69)	1.30 (0.96-1.77)	.157
Glucose, mean (SD), mmol/L	6.22 (1.94)	6.11 (1.67)	.147	6.11 (1.61)	6.22 (2.11)	.230
BMI, mean (SD)	27.9 (4.1)	27.7 (4)	.479	27.7 (4.1)	27.9 (4)	.356
Dyslipidemic treatment, %	31.8	28.4	.352	31.9	26.8	.172

^aAMI indicates acute myocardial infarction; BMI, body mass index; EEPA, energy expenditure due to physical activity during leisure time; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *PON1-192*, *paraoxonase 1-192*; *PON2-311*, *paraoxonase 2-311*; SD, standard deviation.

homogenous country, with identical recruitment protocols in each region, and with a control group which was representative of the population from which the cases were selected.

In the present study, both *PON1* and *PON2* genotypes were analyzed in the same sample. Few studies to date have considered both genotypes together to estimate CHD risk.¹⁸⁻²² Two of them^{18,19} identified the *PON1-192* R allele as having the higher risk for CHD, together with *PON2-311* C or S alleles in a white or Asian-Indian population, respectively. Another study²⁰ found an interaction between the *PON2-311* C allele and smoking regarding AMI risk, whereas another²¹ did not reach statistical significance, probably due to the relatively small sample size. In the white population in the present study, homozygotes for both Q and S alleles in the same subject indicated an increased risk of AMI compared to homozygotes for only 1 of the polymorphisms. It has been suggested that the *PON2-311* S allele may act synergistically with *PON1* polymorphisms, independently of other classic risk factors.^{18,29}

The role of *PON1* as an antioxidant has been assessed in in vitro studies, which showed the capacity of *PON2* to prevent LDL oxidation, destroy lipoperoxides, and promote cholesterol efflux from macrophages, all of which are steps involved in the development of atherosclerosis.⁴ In vivo studies have shown increased development of atherosclerosis in *PON2* knock-out mice.³⁰ It is still under debate whether the *PON2-192* Q allele

in humans encodes for higher antioxidant capacity of the enzyme than the R allele.^{31,32}

The *PON2* enzyme, which is expressed in human endothelial cells and aortic smooth muscle cells among others, reduces LDL oxidation when the intracellular enzyme is overexpressed.⁶ In addition, macrophage *PON2* expression increases under oxidative stress.³³ Thus, it has been suggested that *PON2* may act as a selective antioxidant response at the cellular level and may play an antiatherogenic role by attenuating macrophage foam cell formation and reducing oxidative stress.⁷

It remains unknown which alleles in the *PON1* and *PON2* genes have greater activity for the physiological substrate in vivo. Determining this issue is relevant to understanding the mechanisms explaining the relationship between *PON1* and *PON2* polymorphisms and the risk of AMI.

Limitations

The study is limited by the fact that only AMI patients who arrived at hospital alive were recruited; therefore, a possible survival bias should be taken into account. Nevertheless, this limitation is inherent in all case-control studies. Another limitation is that serum values could only be determined for those AMI patients who survived for at least 6 months after the event. On the other hand, the other variables of interest were measured during hospital stay.

Although the associations reported in this study were statistically significant, they are lower than those

corresponding to classic cardiovascular risk factors, such as smoking, diabetes, hypertension, or cholesterol. This result was expected in the context of a complex disease such as myocardial infarction, where multiple genes and genetic variants could play a role.

On the other hand, this study may have some clinical implications: first, our study supports the role of paraoxonase in atherosclerosis pathogenesis; second, the association between the 2 *PON* polymorphisms as independent risk factors for AMI in our population was confirmed, and that these 2 markers could be used to identify those individuals at higher risk of myocardial infarction; and, finally, our results support a new potential therapeutic target. Further studies to identify the causal genetic variants of this association are warranted.

CONCLUSIONS

Our results indicate that the *PON1-192* and the *PON2-311* polymorphisms are independent risk factors for AMI in our population.

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