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PRELIMINARY REPORT

Differential Effects of Smoking on Myocardial Infarction Risk According to the Gln/Arg 192 Variants of the Human Paraoxonase Gene

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Paraoxonase (PON1) seems to exert a major antioxidant effect by removing lipid peroxidation products. A common polymorphism of the PON1 gene modulates paraoxonase activity and has been related in some studies to coronary heart disease. PON1 genetic polymorphism includes PON1 Q, an isoform with a low activity toward paraoxon hydrolysis that has a glutamine at position 192, and PON1 R, the high-activity isoform with an arginine at position 192. In the present study, we investigated whether smoking, which is related to increased susceptibility to lipoprotein oxidation, has a differential effect by PON1-192 genotype on the risk of myocardial infarction (MI). One hundred fifty-six consecutive MI patients and 310 control subjects were studied. PON1 genotypes in the controls were distributed as follows: 154 (49.7%) QQ, 123 (39.7%) QR, and 33 (10.6%) RR. This distribution did not significantly differ from that of the MI patients: 84 (53.8%) QQ, 60 (38.5%) QR, and 12 (7.7%) RR. Subjects were classified into two groups, those who never smoked (n = 209) and those who were current smokers (n = 135) or ex-smokers (n = 122). In the latter, the variable "cigarette packs smoked per year" was defined as the number of packs smoked daily multiplied by the number of smoking years. As expected, smoking was significantly associated with an increased MI risk in the overall group. Subjects were then stratified by PON1 genotype. The packs smoked per year were significantly associated with an increased MI risk only in QQ homozygotes. This risk was higher among those in the higher tertile for cigarette packs smoked per year (odds ratio [OR] = 5.24, 95% confidence interval = 1.67 to 16.44, *P* for trend <.001). In contrast, the packs smoked per year were not significantly associated with MI risk in R-carrier subjects. We conclude that the risk of MI associated with smoking appears to be increased in subjects who are homozygous for the low-activity PON1 QQ genotype compared with R carriers, and this risk seems to be time- and dose-dependent.

PARAOXONASE (PON1), an enzyme closely associated with high-density lipoproteins, appears to exert an important antioxidant effect by removing lipid peroxidation products.[1] Serum levels of PON1 activity, based on the capacity of the enzyme to hydrolyze paraoxon, appear to vary among individuals and populations. The molecular basis of these variations is a polymorphism in the PON1 gene located on chromosome 7, which is clustered with at least two other related genes, PON2 and PON3. The PON1 gene polymorphism involves a Gln → Arg interchange at codon 192 defined by a low-activity isoform (Q allele) and a high-activity isoform (R allele) and has been related in some studies to coronary heart disease.[2,3] However, a variability in the results suggests that gene-environment and/or gene-gene interactions might modulate the relationship between PON1 polymorphism and coronary heart disease. Smokers seem to have great susceptibility to low-density lipoprotein oxidation, which probably contributes to their increased risk of atherosclerosis.[4] We investigated whether smoking, assessed by the number of cigarette packs smoked per year, has a differential effect related to the PON1-192 genotype on the risk of myocardial infarction (MI) in a case-control study.

SUBJECTS AND METHODS

One hundred fifty-six consecutive patients (140 men and 16 women; mean age, 57.1 ± 9.6 years) who had suffered a first MI were recruited. Three hundred ten control subjects (262 men and 48 women; mean age, 55.4 ± 11.2 years) were recruited by random sampling from the census of Gerona, Spain. The control subjects were judged to be free of angina or MI by history, clinical examination, electrocardiography, and routine

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laboratory data. Information on the history of dyslipidemia, blood pressure, and the family history of coronary heart disease and diabetes mellitus was obtained from all subjects. Details about the onset of smoking and the cessation of smoking in ex-smokers were recorded with a standardized questionnaire. For the purposes of this study, subjects were accordingly classified into two groups, those who never smoked (n = 209) and current smokers (n = 135) or ex-smokers (n = 122). In the latter, the variable "cigarette packs smoked per year" was defined as the number of cigarette packs smoked daily multiplied by the number of smoking years.

The PON1-192 polymorphism was identified by polymerase chain reaction using primer sequences derived from published data.[5] The amplification cycle was performed on a Perkin Elmer-Cetus (Norwalk, CT) 2400 Thermal Cycler with an 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 7 minutes of extension at 72°C. After amplification, polymerase chain reaction products were digested with *Alw*I for 4 hours at 37°C and the samples were electrophoresed in 3% agarose gels for 75 minutes at 60 V. According to the PON1-192 genotype, subjects were also classified into two groups, those who were homozygous for the Q allele (n = 238) and those with one or two R alleles (n = 228).

RESULTS

PON1 genotypes in controls were distributed as follows: 154 (49.7%) QQ, 123 (39.7%) QR, and 33 (10.6%) RR. This distribution did not significantly differ from that of the MI patients: 84 (53.8%) QQ, 60 (38.5%) QR, and 12 (7.7%) RR. The genotypic distribution of the PON1 polymorphism was in Hardy-Weinberg equilibrium in both controls and patients ($P = .260$ in controls and $P = .776$ in patients). Logistic regression analysis modeling with the variables PON1-192 genotype (assuming the R allele to have a dominant effect), family history of premature MI, history of hypertension, smoking, dyslipidemia, and diabetes mellitus showed that the R allele was not associated with an increased MI risk in the overall group of subjects (odds ratio [OR] = 1.05, 95% CI = 0.61 to 1.79). The cardiovascular risk factors associated with MI were diabetes (9.7% in controls *v* 19.2% in MI patients, $P = .001$), dyslipidemia (11.6% *v* 33.9%, $P < .001$), hypertension (26.4% *v* 32.7%, $P = .017$), smoking (24.8% *v* 37.1%, $P < .001$), and family history of premature MI (30.5% *v* 69.5%, $P < .001$).

As expected, smokers or ex-smokers in the higher tertiles for cigarette packs smoked per year showed a significantly increased MI risk (Table 1). The OR for the effect of smoking on MI risk was then calculated in subjects stratified by the two genotype groups. Whereas the number of packs smoked per year was not related to increased MI risk in R carriers, in QQ homozygotes it was significantly associated with an increased MI risk. This was higher among those in the higher tertile for cigarette packs smoked per year compared with those in the lower tertile (P for trend $<.001$). These findings remained unchanged after RR carriers were excluded from the analysis (results not shown). When we reevaluated the OR in the subgroup who were current smokers and in those who never smoked or who were ex-smokers, QQ homozygotes also showed an increased MI risk in each tertile for cigarette packs smoked per year. The OR was approximately 2.5 times higher in the highest tertile for smoking in QQ homozygotes versus current smoker R-carriers of the same tertile.

DISCUSSION

When considered as a whole, the results do not support a significant association of PON1-192 genetic polymorphism with MI risk. The lack of association between PON1-192 genetic variation and MI in the present study is consistent with the results of previous studies in the Mediterranean area.[6,7] Conversely, other studies in the United States and in a Asian Indian population have reported evidence suggesting that the R allele is associated with an increased risk of coronary heart disease.[2,3] A plausible explanation for these different findings is that PON1-192 polymorphism only produces an effect on the MI risk among particular subgroups of subjects in the presence of additional factors that may have different prevalence rates among populations. Therefore, the possible deleterious effect of PON1-192 polymorphism may be overexpressed when a particular genetic variant and a particular oxidative condition coexist.

It is also conceivable that discrepancies between association studies may be due to differences in linkage disequilibrium between populations of PON1-192 alleles with other functional alleles.[8] In this respect, the PON1-192 R allele could be in linkage disequilibrium with the L allele of the Met/Leu55 polymorphism, a common DNA polymorphism of the PON1 gene which has been shown to be associated with increased cardiovascular risk in diabetic patients.[9] On the other hand, it would be extremely useful to study whether the related genes PON2 and PON3 have any effect on the antioxidative properties of PON1.

PON1 is able to hydrolyze a number of substrates such as paraoxon and phenyl acetate; however, the physiological substrate of PON1 remains to be discovered. Uncertainties relative to whether PON1 activity, as measured by paraoxon hydrolysis, reflects the antioxidant capacity of the enzyme have recently been reported.[10] However, in addition to low PON1 activity in

Table 1. OR and 95% Confidence Interval for MI and Smoking in All Subjects and Subjects Stratified by PON1-192 Genotype

		Tertiles of Cigarette Packs Smoked per Year			
Group	Never Smoked	1 (<24)	2 (25-48)	3 (>48)	*P* for Trend
C/P (n)	155/54	56/30	50/36	49/36	
All subjects	1.00 Reference	1.35 (0.63-2.90)	2.65 (1.28-5.45)	2.39 (1.16-4.96)	—
QQ homozygotes	1.00 Reference	3.40 (1.13-10.29)	4.51 (1.46-13.88)	5.24 (1.67-16.44)	<.001
C/P (n)	77/30	31/17	23/16	23/21	
R carriers	1.00 Reference	0.57 (0.17-1.85)	1.80 (0.67-4.78)	1.36 (0.50-3.72)	—
C/P (n)	78/24	25/13	27/20	26/15	

NOTE. The 3 models were adjusted for family history of premature MI, history of hypertension, dyslipidemia, and diabetes mellitus.
Abbreviations: C, controls; P, MI patients.

patients who suffered a MI compared with a control group,[11] a significant decrease in PON1 activity has been shown in diseases with accelerated atherogenesis such as diabetes mellitus[12] and familial hypercholesterolemia.[13] Therefore, with a physiological substrate to be defined, the role of PON1 activity measured by paraoxon hydrolysis remains to be elucidated.

Among the factors that may be associated with increased oxidative risk, smoking emerges as a firm candidate. Remarkably, the observed effects in the present study appear to show a link between smoking, genotype QQ of the PON1-192 polymorphism, and an increased risk of MI. This appears to be in conflict with previous observations indicating a more protective effect of the allele Q against oxidation than the R allele.[14] However, in addition to the fact that the results on PON1-192 and coronary heart disease in case-control studies are controversial, it has also been reported that cigarette smoke extract inhibits paraoxonase activity.[15] Therefore, it is conceivable that oxidative stress may lead to a reduction in PON1 activity. Since PON1 is thought to exert an antioxidant effect and smoking predisposes to oxidative stress, the risk of MI associated with smoking may be increased in subjects who are homozygous for the low-activity PON1 QQ genotype. This increased MI risk appears to be time- and dose-dependent. This finding may provide a new perspective on the complex relationship among PON1, the PON1 gene-environment interaction, and atherosclerosis.

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