Tobacco Smoking Modifies Association Between Gln-Arg192 Polymorphism of Human Paraoxonase Gene and Risk of Myocardial Infarction

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Abstract—Paraoxonase, a high density lipoprotein–associated human serum enzyme, plays a role in atherosclerosis by protecting against lipid peroxidation. Its activity is modulated by 2 common amino acid polymorphisms at positions 192 (Gln→Arg) and 55 (Met→Leu) in the paraoxonase gene (*PON1*). We studied the association of *PON1* polymorphisms and myocardial infarction (MI) in a population-based study consisting of 492 cases and 518 controls matched for age, sex, and area of residence, all living in Costa Rica. The allele frequency of $PON1_{192Arg}$ was higher in cases (0.27) than in controls (0.24, $P=0.008$), whereas that of $PON1_{55Leu}$ was identical (0.26). Compared with $PON1_{192Gln\text{-}Gln}$, the $PON1_{192Arg}$ allele was associated with an increased risk of MI (odds ratio [OR] 1.36, CI 1.06 to 1.75), and this association was independent of the $PON1_{55}$ polymorphism, which was not associated with MI (OR 1.10, CI 0.82 to 1.48). Adjustment for lipid and nonlipid risk factors strengthened the association between $PON1_{192Arg}$ and the risk of MI (OR 1.51, CI 1.13 to 2.03). Interestingly, this association was evident only among nonsmokers (OR 1.90, CI 1.29 to 2.79): there was no evidence of an association in smokers (OR 0.95, CI 0.57 to 1.79). The interaction between $PON1_{192}$ and smoking status was statistically significant ($P=0.04$). Thus, the $PON1_{192}$ but not the $PON1_{55}$ gene polymorphism is associated with an increased risk of MI. This association is not evident among smokers. **(*Arterioscler Thromb Vasc Biol*. 2000;20:2120-2126.)**

Key Words: coronary heart disease ■ genetic epidemiology ■ paraoxonase ■ antioxidants ■ lipoproteins

Oxidative modification of LDLs is a major contributor to atherosclerosis.[1,2] By damaging endothelial cells, oxidized LDL provides a nidus for monocytes that can later become the lipid-laden foam cells prominent in the early stages of plaque formation.[1] HDLs can protect LDLs from oxidative damage.[3–5] The mechanism of this protective effect may involve paraoxonase, an HDL-associated enzyme capable of hydrolyzing lipid peroxides.[6–8]

Human serum paraoxonase is a 44-kDa Ca^{2+}-dependent glycoprotein. It remains exclusively associated with apoA-I on HDL through a hydrophobic region at its amino terminus.[9,10] Paraoxonase may lower the risk of vascular disease by destroying proinflammatory molecules formed by the oxidation of LDL.[6,11] For example, purified paraoxonase blocks the proinflammatory effect of oxidized LDL in a vascular cell culture system, probably by destroying oxidized arachidonic acid derivatives in the Sn-2 position of LDL phospholipids.[8] There is a 10- to 40-fold variability in the activity of the enzyme among individuals[9] that is influenced, in part, by differences in susceptibility to organophosphate poisoning.[12,13] This interindividual variability in activity has been attributed to 2 polymorphisms in the coding region of the paraoxonase gene (*PON1*)[14,15]: a Gln→Arg substitution at position 192 ($PON1_{192Arg}$) and a Met→Leu substitution at position 55 ($PON1_{55Leu}$).[9]

The $PON1_{192}$ and $PON1_{55}$ polymorphisms are common in white and Asian populations, which show frequencies of between 0.30 and 0.59 for the $PON1_{192Arg}$ allele[16–25] and between 0.27 and 0.91 for the $PON1_{55Leu}$ allele.[21,24,26] Paraoxonase 192Arg is associated with various levels of activity toward nonphysiological substrates.[9,27,28] Paraoxonase 192Arg hydrolyzes paraoxon faster, and diazoxon slower, than 192Gln does, yet the 2 alloenzymes show no difference in activity toward other substrates, such as phenylacetate. Most important, the ability of HDL to protect LDL from lipid peroxidation in vitro is significantly reduced in HDL particles containing paraoxonase 192Arg rather than 192Gln.[29,30] Carriers of the $PON1_{55}$ allele show an increased activity toward paraoxon that is independent of the $PON1_{192Arg}$ allele effect.[26,31]

Several studies have shown a positive association between the $PON1_{192Arg}$ allele and coronary disease,[16,20,22,24,32,33] and several other studies have shown no association,[17–19,23,25] including 1 study of the $PON1_{55}$ polymorphism.[21] Only 2 studies with a small sample size[21,34] have evaluated the $PON1_{192}$ and the $PON1_{55}$ polymorphisms simultaneously. In

Received January 10, 2000; revision accepted May 22, 2000.

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***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org**

diabetics, the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles have both been consistently associated with coronary disease,[26,35,36] although no association has been found with an increased risk of diabetes.[34] A lack of randomly selected control groups, small sample sizes, and differences in criteria for case definition may explain these inconsistent conclusions, although it is also possible that other genetic characteristics and the particular environmental conditions of a given population may amplify or attenuate the effect of the *PON1* gene on coronary disease.

The present study was designed to test whether the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles are associated with an increased risk of myocardial infarction (MI) in a population-based case-control study of Hispanics living in Costa Rica, Central America.

Methods

Study Population

The catchment area for this case-control study included 18 counties of Costa Rica served by the San Juan de Dios Hospital, the Rafael Angel Calderón Guardia Hospital, and the Mexico Hospital, all in San José. Most of the 1.092 million people in this area are ethnically mestizo and culturally Hispanic American.[37,38] Mestizo, from the Spanish word for mixed, connotes the admixture of whites predominantly from Spain, in the case of Costa Rica, and Amerindians. According to Costa Rican census information, admixture in Costa Rica started as early as the 16th century, and by 1801, mestizos were the predominant ethnic group (58% of the population), followed by mulattos (African and white admixture, 17%), Amerindians (16%), and whites (9%).[39] The Costa Rican population of today, considered to be 98% mestizo, is the result of 4 centuries of triracial admixture. The exact contribution of each primary race (Amerindian, African, and white) is unknown.[37–39]

All survivors of a first MI who were hospitalized between January 1994 and December 1997, who were aged <75 years old, and who had lived in the catchment area for at least 1 year before the event were recruited as cases (n=531, participation 97%). Ten participants were excluded after recruitment because they died after hospital discharge but before data collection was completed, and 29 were excluded because they did not have a blood sample. All cases met the World Health Organization criteria for MI, which require typical symptoms plus either elevations in cardiac enzyme levels or diagnostic changes in the ECG.[40] For consistency, one study cardiologist confirmed the diagnosis of first acute MI for all 3 hospitals before recruitment.

One free-living control subject for each case survivor, matched for age (±5 years), sex, and area of residence, was randomly selected from the general population by using information available at the National Census and Statistics Bureau of Costa Rica. Control subjects were considered ineligible if they had ever had an acute MI or were physically or mentally unable to answer the questionnaire. The participation rate for the controls was 90% (n=531). Thirteen subjects were not included because they did not have a blood sample. All study participants gave informed consent. Whenever possible, house visits were planned so that the interviews for case-control pairs were carried out by the same interviewer within 3 weeks of the pair patient's hospital discharge. The present study was approved by the Committee on the Use of Human Subjects in Research at the Harvard School of Public Health and by the Institute of Health Research at the University of Costa Rica.

Data Collection

The general questionnaire included closed-end questions regarding sociodemographic characteristics, smoking, physical activity, and medical history (including personal history of diabetes and hypertension). Self-reported diabetes and hypertension were validated by using the definitions recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[41] and the Third Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure,[42] ie, a fasting capillary whole blood glucose level ≥110 mg/dL (measured in the morning at the subject's home) or the ingestion of glucose control medications and a systolic blood pressure ≥140mm Hg, a diastolic blood pressure ≥90 mm Hg, or the ingestion of antihypertensive medications. The sensitivity, specificity, predictive value positive, and predictive value negative were 80%, 97%, 75%, and 98%, respectively, for self-reported diabetes and 52%, 96%, 93%, and 70%, respectively, for self-reported hypertension. Thus, the reliability of reports of diabetes and hypertension by subjects is high in the Costa Rican population. Physical activity was determined by asking subjects about the average frequency of several occupational and leisure-time activities during the past year (before MI for case subjects) and the amount of time spent on them. These activities were grouped into 6 categories (sleeping, sitting, and light, moderate, strenuous aerobic, and strenuous anaerobic activities) according to intensity or to METS, defined as the energy expenditure for sitting quietly, or ≈1 kcal · kg body $wt^{-1} \cdot h^{-1}$.[43] Energy expenditure was calculated as the product of frequency, time, and intensity and expressed as kilocalories per kilogram per hour. This aspect of the questionnaire was validated by checking its ability to predict fitness level (measured by the Harvard step test) in our previous studies of cardiovascular risk factors in residents of Puriscal, Costa Rica.[44]

Anthropometric measurements were collected in triplicate from subjects wearing light clothing and no shoes. Blood samples were obtained at the subject's home the morning after an overnight fast and were collected into tubes containing 0.1% EDTA. Samples were stored at 4°C in a cooler with ice packs and transported to the field workstation within 4 hours, where blood was centrifuged at 2500 rpm for 20 minutes at 4°C to separate the plasma from white and red blood cells. All samples were separated into aliquots and stored at −80°C, and within 6 months, they were transported on dry ice for analysis at the Harvard School of Public Health.

TABLE 1. Genotype and Allele Frequencies for $PON1_{192}$ and $PON1_{55}$ in Survivors of MI and Randomly Selected Controls

		Controls (n=518), % (n)	Cases (n=492), % (n)	*P*
$PON1_{192}$				
Gln-Gln		53.9 (279)	46.8 (230)	
Gln-Arg		43.6 (226)	52.2 (257)	
Arg-Arg		2.5 (13)	1.0 (5)	0.008
Allele frequency				
Gln		0.76	0.73	
Arg		0.24	0.27	
$PON1_{55}$				
Met-Met		55.6 (288)	54.3 (267)	
Met-Leu		36.3 (188)	39.6 (195)	
Leu-Leu		8.1 (42)	6.1 (30)	0.324
Allele frequency				
Met		0.74	0.74	
Leu		0.26	0.26	
$PON1_{192}$-$PON1_{55}$				
Gln-Gln	Met-Met	25.5 (131)	20.9 (103)	
Gln-Gln	Met-Leu	21.2 (110)	21.5 (106)	
Gln-Gln	Leu-Leu	7.3 (38)	4.3 (21)	
Gln-Arg	Met-Met	28.2 (146)	32.5 (160)	
Gln-Arg	Met-Leu	14.7 (76)	17.9 (88)	
Gln-Arg	Leu-Leu	0.8 (4)	1.83 (9)	
Arg-Arg	Met-Met	2.1 (11)	0.8 (4)	
Arg-Arg	Met-Leu	0.39 (2)	0.2 (1)	
Arg-Arg	Leu-Leu	0	0	0.04

TABLE 2. General Characteristics and Plasma Lipids in Survivors of MI and Randomly Selected Controls by *PON1* Genotype

	$PON1_{192Gln}$		$PON1_{192Arg}$	
	Controls (n=279)	Cases (n=230)	Controls (n=239)	Cases (n=262)
Age, y	57±0.6	57±0.7	56±0.7	57±0.7
Sex, % female	23	25	28	24
Area, % living in urban area	56	53	55	57
Current smokers, % yes*	24	47§	32	43§
Diabetes, % yes†	12	24§	11	23§
Hypertension, % yes†	28	41§	25	43§
Angina, % yes‡	4	12§	6	11¶
Monthly income, US$	569±28	427±30§	546±30	501±29
Waist-to-hip ratio	0.93±0.004	0.95±0.004§	0.94±0.004	0.95±0.004§
Physical activity, $kcal \cdot kg^{-1} \cdot h^{-1}$	1.87±0.06	1.64±0.06‖	1.75±0.06	1.74±0.06
Triglyceride, mg/dL	205±100	243±107§	208±125	238±108‖
Total cholesterol, mg/dL	201±39	195±41	199±40	195±39
HDL cholesterol, mg/dL	42±12	35±11§	42±11	37±14§
	$PON1_{55Met}$		$PON1_{55Leu}$	
	Controls (n=288)	Cases (n=267)	Controls (n=230)	Cases (n=225)
Age, y	57±0.7	57±0.7	56±0.7	57±0.7
Sex, % female	27	24	23	25
Area, % living in urban area	59	55	52	55
Current smokers, % yes*	30	46§	25	43§
Diabetes, % yes†	11	23§	12	24§
Hypertension, % yes†	28	41‖	24	43§
Angina, % yes‡	4	9‖	5	14§
Monthly income, US$	550±27	491±28	569±30.4	437±31‖
Waist-to-hip ratio	0.93±0.003	0.94±0.004	0.93±0.004	0.96±0.004§
Physical activity, $kcal \cdot kg^{-1} \cdot h$	1.82±0.06	1.80±0.06	1.80±0.06	1.56±0.06‖
Triglyceride, mg/dL	210±122	241±108§	202±97	239±108‖
Total cholesterol, mg/dL	202±40	197±43	198±39	192±35
HDL cholesterol, mg/dL	43±12	36±12§	41±11	36±13§

Values are mean±SD. $PON1_{192Arg}$ includes the $PON1_{192Gln\text{-}Arg}$ and $PON1_{192Arg\text{-}Arg}$ genotypes. $PON1_{55Leu}$ includes the $PON1_{55Met\text{-}Leu}$ and $PON1_{55Leu\text{-}Leu}$ genotypes.

*Self-reported smoking ≥1 cigarette/d.

†Self-reported diabetes and hypertension.

‡Evaluated by Rose questionnaire (group comparison by χ^2 analysis).

§$P \leq 0.001$, ‖$P \leq 0.01$, and ¶$P \leq 0.05$ vs controls with same genotype.

Laboratory Analysis

DNA was extracted with a Qiagen blood kit at the average genomic DNA concentration of 250 μg/mL. Isolated DNA was genotyped by polymerase chain reaction, followed by restriction endonuclease digestion as described.[9] The $PON1_{192}$ and $PON1_{55}$ genotypes were identified by cleavage with *Alw*I and *Nla*III (New England Biolabs), respectively, at 37 C° for 4 hours. The products were then run on a 10% polyacrylamide gel (45 mA current per gel) and stained with ethidium bromide. Allele frequencies were estimated by the gene-counting method. Plasma triglyceride, cholesterol, and HDL cholesterol levels were assayed with enzymatic reagents (Boehringer-Mannheim). In our laboratory, cholesterol measurements are standardized according to the program specified by the Centers for Disease Control and the National Heart, Lung, and Blood Institute.

Statistical Analysis

The 492 cases and 518 controls for whom there was complete genotype information (93% and 98%, respectively, of the total study population) were included in the analysis, which was performed with software from Statistical Analysis Systems. After the data had been checked for errors, outliers, and distributions, crude means and frequencies for health characteristics and potential confounders were compared by using 2-sided *t* tests and the χ^2 test. Triglyceride values were normalized by $\log_e$ transformation, and data are presented as a geometric mean±approximate SD.

The presence or absence of the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles was used to define 2 groups for the gene effect, with the $PON1_{192Gln\text{-}Gln}$ and the $PON1_{55Met\text{-}Met}$ genotypes used as reference categories. Multiple nonconditional logistic regression was used to calculate odds ratios (ORs) with 95% CIs for case status. The

presence of the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles was compared with their absence. The distribution among controls of continuous variables (income, physical activity, waist-to-hip ratio, and triglyceride, HDL cholesterol, and total cholesterol levels) was used to compute quintile categories that were included in the multiple logistic regression models as covariates. The presence of diabetes, hypertension, and angina was compared with the absence of these diseases (reference). Subjects who smoked ≥1 cigarette per day were defined as current smokers and were compared with past smokers and those who had never smoked grouped together (referent category).

The first model included the polymorphisms of $PON1_{192}$ and $PON1_{55}$ and the covariates age, sex, and area of residence (urban, periurban, or rural). Two additional models also included smoking, income, physical activity, waist-to-hip ratio, diabetes, hypertension, angina, and triglyceride, cholesterol, and HDL cholesterol levels. Data for covariates are presented for the highest compared with the lowest quintile, for the presence compared with the absence of disease, and for smokers compared with nonsmokers. The cut points for the lowest versus highest quintiles among covariates were ≤$192 and ≥$871 for monthly income, ≤1.14 and ≥2.33 $kcal \cdot kg^{-1} \cdot h^{-1}$ for physical activity, ≤0.88 and ≥1.00 for waist-to-hip ratio, ≤128 and ≥263 mg/dL for triglyceride, ≤33 and ≥49 mg/dL for HDL cholesterol, and ≤128 and ≥263 mg/dL for total cholesterol. All covariates were also tested for their potential as effect modifiers. Because these analyses revealed a significant interaction between the $PON1_{192}$ polymorphism and smoking status, the effect of the $PON1_{192}$ polymorphism was investigated in additional analyses in smokers and nonsmokers separately. We also examined the $PON1_{192}$ polymorphism–smoking interaction in a model of 4 groups in which the referent category, nonsmokers with $PON1_{192Gln\text{-}Gln}$, was compared simultaneously with nonsmokers with $PON1_{192Arg}$, smokers with $PON1_{192Gln\text{-}Gln}$, and smokers with $PON1_{192Arg}$. Values of $P<0.05$ (2-sided) were the mark of statistically significant differences.

Results

Table 1 shows the genotype and allele frequencies for $PON1_{192}$ and $PON1_{55}$ in cases and controls. The presence of the $PON1_{192Arg}$ allele was more common in cases than in controls ($P=0.008$). No difference in genotype distribution was found for the $PON1_{55}$ polymorphism. When the $PON1_{192}$ and $PON1_{55}$ genotypes were analyzed together, the frequency of the PON_{192Arg} allele was higher in cases irrespective of their $PON1_{55}$ genotype ($P=0.04$).

General characteristics in MI cases and randomly selected controls by *PON1* genotype are presented in Table 2. Waist-to-hip ratio, smoking, and history of diabetes, hypertension, and angina were significantly higher in cases than in controls regardless of genotype. In both *PON1* genotypes, compared with controls, cases had higher triglyceride concentrations, lower HDL cholesterol concentrations, and similar total cholesterol concentrations. There was no significant difference between genotypes for any parameter within cases or controls.

Table 3 shows the ORs for the presence compared with the absence of the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles. The presence of $PON1_{192Arg}$ was associated with an increased risk of MI (OR 1.36, CI 1.06 to 1.75). This association did not change and remained statistically significant in a multivariate model that included nonlipid risk factors. The addition of an adjustment for lipid risk factors strengthened the association (OR 1.51, CI 1.13 to 2.03). In the same models, the $PON1_{55Leu}$ polymorphism was not associated with risk of MI (OR 1.12, CI 0.87 to 1.44). Among the covariates, higher income and HDL cholesterol were associated with lower risk of MI. Smoking, high plasma triglyceride levels, and history of diabetes, hypertension, and angina were associated with an increase in the risk of MI. Because of the potential for sex differences to confound the data analysis, we repeated the analysis in males only. The results for men were similar to those for the whole population, by univariate or multivariate analysis. For $PON1_{192Arg}$, the OR was 1.63, with the CI 1.17 to 2.27; for $PON1_{55Leu}$, the OR was 0.98, with the CI 0.70 to 1.37 (adjusting for covariates in model 3).

TABLE 3. ORs for MI Associated With Presence of $PON1_{192Arg}$ and $PON1_{55Leu}$ Alleles

	Model 1	Model 2	Model 3
$PON1_{192Arg}$	1.36‡	1.35‡	1.51‖
	(1.06–1.75)	(1.03–1.77)	(1.13–2.03)
$PON1_{55Leu}$	1.12	1.08	1.10
	(0.87–1.44)	(0.82–1.42)	(0.82–1.48)
Nonlipid risk factors			
Smoking (yes)*		2.40§	2.28§
		(1.79–3.23)	(1.66–3.13)
Income†		0.48‖	0.52‡
		(0.29–0.78)	(0.31–0.89)
Physical activity†		0.82	0.89
		(0.53–1.25)	(0.57–1.42)
Waist-to-hip ratio†		1.38	0.84
		(0.83–2.31)	(0.48–1.47)
Diabetes (yes)		2.21§	2.34§
		(1.50–3.26)	(1.54–3.55)
Hypertension (yes)		2.09§	1.87§
		(1.54–2.83)	(1.36–2.59)
Angina (yes)		2.37‖	2.50‖
		(1.40–4.01)	(1.44–4.34)
Lipid risk factors			
Triglyceride†			4.29§
			(2.35–7.82)
HDL cholesterol†			0.22§
			(0.13–0.37)
Total cholesterol†			1.02
			(0.34–3.00)

Values are ORs (with CIs in parentheses). The absence of the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles was used as the referent category. All models include the matching variables age, sex, and area of residence.

*Nonsmokers and past smokers were used as the referent category. †ORs are given for the highest quintile compared with the lowest.

‡$P<0.05$, §$P<0.0001$, and ‖$P<0.01$.

An association between $PON1_{192Arg}$ and MI (Table 4) was evident only among nonsmokers (OR 1.64, CI 1.19 to 2.26) compared with smokers (OR 0.89, CI 0.58 to 1.38), and it was strengthened by an adjustment for lipid and nonlipid risk factors. The interaction between $PON1_{192}$ and smoking status was statistically significant ($P=0.04$). No association between $PON1_{55Leu}$ and MI was detected in smokers or nonsmokers. Because stratification by smoking changed the sex distribution, we repeated the analysis in men only. The results for men were similar to those for the entire population: OR

TABLE 4. ORs for MI Associated With Presence of $PON1_{192Arg}$ and $PON1_{55Leu}$ Alleles by Smoking Status

	Nonsmokers	Smokers
Model 1		
$PON1_{192Arg}$	1.64*	0.89
	(1.19–2.26)	(0.58–1.38)
$PON1_{55Leu}$	1.13	1.15
	(0.82–1.57)	(0.74–1.79)
Model 2		
$PON1_{192Arg}$	1.70*	0.86
	(1.19–2.43)	(0.54–1.36)
$PON1_{55Leu}$	1.05	1.14
	(0.74–1.50)	(0.71–1.81)
Model 3		
$PON1_{192Arg}$	1.90†	0.95
	(1.29–2.79)	(0.57–1.79)
$PON1_{55Leu}$	1.09	1.07
	(0.74–1.59)	(0.64–1.79)

Values are ORs (with CIs in parentheses). The absence of the $PON1_{192Arg}$ and $PON1_{55Leu}$ alleles was used as the referent category. See Table 3 for covariates included in each model. The nonsmoker category includes past smokers. Value of *P* for $PON1_{192}$ genotype–smoking interaction is 0.04 and for $PON1_{55}$ genotype smoking interaction is 0.9.

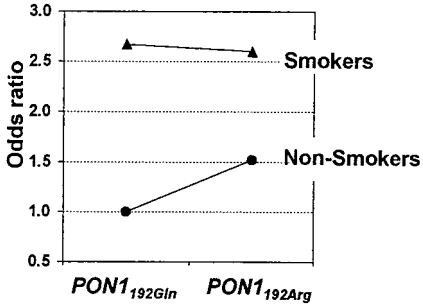
*$P<0.005$ and †$P<0.001$.

1.90 (CI 1.29 to 2.80) for $PON1_{192Arg}$ and OR 1.09 (CI 0.74 to 1.60) for $PON1_{55Leu}$.

The Figure shows the association between $PON1_{192Arg}$ and risk of MI when nonsmokers with the $PON1_{192Gln\text{-}Gln}$ genotype were used as the referent category. Smokers had an increased risk of MI regardless of their $PON1_{192}$ genotype: OR 2.66 (CI 1.75 to 4.05) for $PON1_{192Gln\text{-}Gln}$ and OR 2.60 (CI 1.72 to 3.9) for $PON1_{192Arg}$. The association between $PON1_{192Arg}$ and risk of MI was evident only among nonsmokers, although the magnitude of this effect, OR 1.52 (CI 1.08 to 2.15), was considerably smaller than the effect of smoking on risk of MI.

Discussion

Our data show that the $PON1_{192Arg}$ allele was associated with a 36% increase in the risk of MI. This association was independent of the $PON1_{55}$ polymorphism, which was not associated with MI. An adjustment for lipid and nonlipid risk factors strengthened the association between $PON1_{192Arg}$ and the risk of MI. The association that we found between $PON1_{192Arg}$ and MI is consistent with the results of several studies[16,20,22,24,32,33,35,36] but not all.[17–19,23,25] These discrepancies could be due in part to the selection criteria of the control group, to differences in case definition, and to a lack of a population-based control group. These limitations apply to negative and positive studies, yet in the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) study, the largest population-based study (642 patients and 701 age-matched controls), no association between $PON1_{192Arg}$ and MI was found.[19] We hypothesize that environmental or behavioral factors could mask or induce the atherogenic potential of the $PON1_{192Arg}$



OR of MI associated with presence of $PON1_{192Arg}$ allele in smokers and nonsmokers, where nonsmokers with $PON1_{192Gln\text{-}Gln}$ are used as reference. Nonsmokers include past smokers.

allele. We believe that our findings are an example of a widespread phenomenon of modulation of genetic effects by environmental factors that vary among populations. Further studies must include and investigate as much information as possible on environmental factors that could modulate genetic effects.

Conflicting results are also found for the $PON1_{55}$ polymorphism. One study in French diabetic patients found a significant association between the $PON1_{55}$ polymorphism and coronary disease.[26] The $PON1_{55}$ polymorphism was not associated with risk of MI in the present study and in one previous report.[21]

The mechanism mediating the association between the $PON1_{192Arg}$ allele and an increased risk of MI is not known. In vitro studies show that HDL from carriers of the $PON1_{192Arg}$ allele is less effective in decreasing the accumulation of LDL lipid peroxides.[29,30] Perhaps this reduction in LDL protection (or in some other yet-to-be-identified activity conferred by *PON1*) explains the atherogenic effect of $PON1_{192Arg}$ observed in the present and other studies.[16,20,22,24,32,33]

HDL may play a significant role in the effect of *PON1* on coronary disease. HDL cholesterol levels and paraoxonase protein levels are significantly correlated.[45] In one study, the $PON1_{192Arg}$ allele was associated with lower HDL cholesterol levels,[46] but this was not confirmed in other studies.[35,45,47] We did not find an association between *PON1* polymorphisms and HDL cholesterol levels or a significant interaction between HDL cholesterol and *PON1* (data not shown).

A significant interaction between smoking status and genotype revealed that the presence of $PON1_{192Arg}$ was associated with a 64% increase in the risk of MI among nonsmokers in the present our study. There was no evidence of this association in smokers. It is possible that these results can be explained by differential survival among smokers. On the other hand, there is a biological basis for these results. Cigarette smoke extract decreases paraoxonase activity against nonphysiological substrates,[48] and it may also reduce *PON1* activities that are involved in cardioprotection. Thus, the deleterious effects of cigarette smoke may equalize or outweigh the differences in potentially positive enzyme activities conferred by the *PON1* genotype. However, because we did not measure *PON1* activity in the present study, we cannot determine whether this is the mechanism responsible for our results.

Further work is needed to clarify the mechanisms underlying the gene-environment interaction identified in the present study and to uncover other environmental factors that modify the effect of the *PON1* genotype on coronary disease.

Acknowledgments

This work was supported by research grants HL-49086 and HL-60692 from the National Institutes of Health. We are indebted to the participants for their commitment to the study; to the field workers of the Proyecto Salud Coronaria for their effort and dedication to the data collection; to the directors and staff of the emergency, cardiology, coronary, and intensive care units of the hospitals San Juan de Dios, Rafael Angel Calderón Guardia, and México for their efforts to assist in the recruitment of case subjects; and to the Centro Nacional de Estadística y Censos de Costa Rica for making the recruitment of controls possible.

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Arteriosclerosis, Thrombosis, and Vascular Biology

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Arterioscler Thromb Vasc Biol. 2000;20:2120-2126
doi: 10.1161/01.ATV.20.9.2120
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636