Association between the Severity of Angiographic Coronary Artery Disease and Paraoxonase Gene Polymorphisms in the National Heart, Lung, and Blood Institute–Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study

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Paraoxonase (PON), a high-density lipoprotein–associated enzyme, is believed to protect against low-density lipoprotein oxidation and thus affects the risk of coronary artery disease (CAD). Three polymorphisms in the *PON1* (Leu55Met and Gln192Arg) and *PON2* (Ser311Cys) genes have been shown to be associated with the risk of CAD in several European or European-derived populations. In the present study, we examined the associations between these three markers and the severity of CAD as determined by the number of diseased coronary artery vessels in 711 subjects (589 whites and 122 blacks) from the Women's Ischemia Syndrome Evaluation (WISE) study. WISE is a National Heart, Lung, and Blood Institute–sponsored multicenter study designed to address issues related to ischemic-heart-disease recognition and diagnosis in women. Subjects were classified as having normal/minimal CAD (<20% stenosis), mild CAD (20%–49% stenosis), and significant CAD (≥50% stenosis). The women who had ≥50% stenosis were further classified into groups with one-, two-, or three-vessel disease if any of the three coronary arteries had diameter stenosis ≥50%. No significant association was found between the *PON* polymorphisms and stenosis severity in either white or black women. However, among white women, when data were stratified by the number of diseased vessels, the frequency of the *PON1* codon 192 Arg/Arg genotype was significantly higher in the group with three-vessel disease than in the other groups (those with one-vessel and two-vessel disease) combined (17.02% vs. 4.58%; $P = .0066$). Similarly, the frequency of the *PON2* codon 311 Cys/Cys genotype was significantly higher in the group with three-vessel disease than in the other groups combined (15.22% vs. 4.61%; $P = .018$). The adjusted odds ratios for the development of three-vessel disease were 2.80 (95% confidence interval 1.06–7.37; $P = .038$) for *PON1* codon 192 Arg/Arg and 3.68 (95% confidence interval 1.26–10.68; $P = .017$) for *PON2* codon 311 Cys/Cys. Our data indicate that the severity of CAD, in terms of the number of diseased vessels, may be affected by common genetic variation in the *PON* gene cluster, on chromosome 7.

Introduction

Coronary artery disease (CAD) is a major cause of mortality and morbidity in developed countries. The oxidative modification of low-density lipoprotein (LDL) is an important element in the development of atherosclerosis (Navab et al. 1996). Numerous studies suggest that oxidative modification of LDL initiates the development, in the arterial wall, of foam cell–laden fatty streaks, believed to cause atherosclerosis. Recent evidence suggests that high-density lipoprotein (HDL) inhibits the oxidation of LDL and thus may provide protection against the risk of CAD (M. I. Mackness and Durrington 1995; Navab et al. 2000). Serum paraoxonase (PON1), an HDL-associated enzyme, has been shown to be responsible for this antioxidative property of HDL (Watson et al. 1995). PON1 is a 44-kDa Ca^{2+}-dependent enzyme that remains associated with apolipoprotein (apo) A-I and apoJ on HDL (Blatter et al. 1993; Kelso et al. 1994). PON1 can hydrolyze a variety of substrates; is involved in the detoxification, in the P-450 system, of organophosphate insecticides such as parathion, chlorpyrifos, and diazinon; and protects against the nerve agents soman and sarin (M. I. Mack-

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ness et al. 1991, 1993; M. I. Mackness and Durrington 1995; Shih et al. 1998). *PON1* (MIM 168820)—along with two homologous genes, *PON2* (MIM 602447) and *PON3* (MIM 602720)—is located on chromosome 7q21.3-22.1 (Primo-Parmo et al. 1996). Recently, it has been shown that the rabbit (Draganov et al. 2000) and human (Reddy et al. 2001) PON3 enzymes are also associated with HDL and that human PON3 has biological activity similar to that of PON1 (Reddy et al. 2001). PON2 also has antioxidant properties and is capable of preventing the peroxidation of LDL, but, unlike PON1 and PON3, PON2 is not associated with HDL and may only exert its antioxidant function at the cellular level (Ng et al. 2001). Three genetic polymorphisms in the *PON1* (Leu55Met and Gln192Arg) and *PON2* (Ser311Cys) genes have been reported to be associated with the risk of CAD in many (Ruiz et al. 1995; Odawara et al. 1997; Sanghera et al. 1997, 1998*a*; Zama et al. 1997) but not all (Ko et al. 1998; Ombres et al. 1998; Aynacioglu and Kepekci 2000; Gardemann et al. 2000; Imai et al. 2000) studies.

CAD is the leading cause of death in women in the United States. The Women's Ischemia Syndrome Evaluation (WISE) study is a National Heart, Lung, and Blood Institute (NHLBI)–sponsored, four-center study designed to explore mechanisms and genetic backgrounds for CAD among women and to improve the disease recognition, diagnosis, and treatment (Merz et al. 1999). As part of the WISE study, each subject underwent diagnostic quantitative coronary angiography, to assess CAD severity. The aim of the present study was to investigate the association between severity of the disease and variation in the *PON1* Leu55Met, *PON1* Gln192Arg, and *PON2* Ser311Cys polymorphisms.

Subjects and Methods

Study Sample Description

Women ⩾18 years of age were enrolled in the WISE study if they met all of the following criteria: (1) chest pain or other symptoms suggestive of myocardial ischemia, (2) clinically indicated coronary angiography, and (3) ability to give informed written consent. All patients underwent the clinical examination at one of four clinical centers (University of Alabama at Birmingham, Allegheny University of the Health Sciences at Pittsburgh, University of Florida at Gainesville, and University of Pittsburgh). Major exclusion criteria were as follows: pregnancy, cardiomyopathy, contraindications to provocative diagnostic testing, New York Heart Association class IV congestive heart failure, recent myocardial infarction, significant valvular or congenital heart disease, and recent coronary angioplasty or coronary bypass surgery. A total of 8,544 women were evaluated, of whom 1,903 (22%) were eligible for study entry. For the present study, DNA samples were available from 711 of the 941 (49% of eligible) women who were finally enrolled. In the present study, 589 were white (non-Hispanic) women, and 122 were black or African American women (not of Hispanic origin), with a mean age of 58 years. Homogeneity tests using genotype data showed no statistically significant difference between the four clinical sites from three geographic locations for whites (346 from Pennsylvania, 121 from Alabama, and 122 from Florida) and blacks (31 from Pennsylvania, 58 from Alabama, and 33 from Florida) separately. Thus, white and black subjects were pooled separately, and the data were analyzed by ethnic groups.

CAD was measured for all women by quantitative coronary angiography. In addition, atherosclerotic risk factors were assessed—including lipid, sex hormone, and homocysteine levels; brachial artery endothelial function; and demographic and psychosocial profiles (Merz et al. 1999).

Lipid samples were analyzed by the lipid core laboratory at the Cedars Sinai Medical Center, which is enrolled in the Centers for Disease Control and Prevention lipid-standardization program (Bittner et al. 2000). Fasting total plasma cholesterol, triglycerides, and HDL-cholesterol levels were determined by enzymatic assays, as described elsewhere (Lipid Research Clinics Program 1974). LDL cholesterol was calculated using the Friedewald equation (Friedewald et al. 1972). The coefficients of variation for total cholesterol, HDL cholesterol, and triglycerides were 1.80%, 1.23%, and 3.93%, respectively (Bittner et al. 2000).

Quantitative coronary angiography was performed using the WISE study protocol, and the data were analyzed at the angiography core laboratory at Brown University by investigators blinded to subject identifiers. Luminal diameter was measured at all stenoses and at nearby reference segments by using an electronic cine-projector–based "crosshair" technique (Vanguard Instrument) (Sharaf et al. 1997). Enrolled patients were classified into three groups: normal/minimal CAD (<20% stenosis), mild CAD (20%–49% stenosis), and significant CAD (⩾50% stenosis). Patients with significant CAD were further stratified into groups with one-, two-, or three-vessel disease, as determined by the number of arteries having ⩾50% stenosis.

Genetic Polymorphism Detection

Genomic DNA was isolated by the Puregene Systems DNA purification kit (Gentra). Standard PCR protocols, followed by restriction-enzyme digestions, were used to screen the three *PON* polymorphisms, as described elsewhere (Humbert et al. 1993; Sanghera et al. 1997, 1998*a*).

Size fractionations for all three polymorphisms were performed in 3% Nusieve (FMC) and 1% agarose gel.

Statistical Analyses

Among different patient groups, one-way analysis of variance was used to test for mean differences in lipid traits and continuous variables (e.g., age and BMI). For discrete variables, possible differences in proportions were compared among different groups with CAD by using Pearson χ^2 tests. These descriptive statistical analyses were performed with SPSS (version 10.0 for Windows).

All analyses of the relationships between *PON* genotypes and CAD-related measures were performed separately in white and black women. Allele frequencies were calculated by allele counting, and deviations of the observed genotype frequencies from Hardy-Weinberg equilibrium were identified by χ^2 test. Genotype-frequency differences among groups with different CAD severity were compared using a χ^2 test for a $2 \times k$ contingency table and a χ^2 test for trends (Newman 2001, pp. 115–117). Specifically, in the trend test, we assigned CAD-severity levels of 1, 2, and 5, to represent the groups with <20% stenosis, 20%–49% stenosis, and ⩾50% stenosis, respectively, and 1, 2, and 3, to represent the groups with one-, two-, and three-vessel disease, respectively. The differences in allele frequencies between two different CAD-severity diseased groups and between racial groups were estimated using a standard Z test. The relationships between three *PON* polymorphisms and the number of diseased vessels were further determined by logistic-regression analyses using R software (version 1.2.1) (Ihaka 1996). Adjusted odds ratios (ORs) and 95% CIs of the genotype-disease association were estimated after adjusting for known coronary risk factors, including age, BMI, smoking history, alcohol use, family history of CAD, history of hypertension, history of diabetes, menopausal status, lipid-lowering–drug (statins/other) intake, and lipid profile. Except for age, BMI, and lipid profile, which were continuous variables, all variables entered in the regression model were discrete variables.

We also investigated whether the *PON* gene haplotypes were associated with an increasing number of diseased vessels. Linkage disequilibrium among the three markers was estimated by the EH (estimate haplotype frequencies) program (version 1.2) (Xie and Ott 1993), which uses the expectation-maximization algorithm to obtain maximum-likelihood estimates of the haplotype frequencies. To test whether the estimated haplotype frequencies differed between the individuals with three-vessel disease and the individuals with no-vessel disease, we used a nonparametric T5 statistic, as implemented in the EH program; the T5 statistic is approximately distributed as a χ^2 (Zhao et al. 2000; Tsai et al. 2001).

Results

Distribution of Clinical Characteristics of the Study Subjects in Relation to the Severity of CAD

Table 1 shows the distributions of age, BMI, smoking history, alcohol use, menopausal status, and medical history, along with quantitative lipid levels, by the number of diseased vessels for white women. Age ($P < .001$), history of hypertension ($P = .024$), history of diabetes ($P < .001$), and lipid-lowering–drug (statins) intake ($P < 0.001$) were significantly associated with the number of diseased vessels, and plasma HDL-cholesterol levels ($P = .023$) were inversely related to the severity of CAD. Total plasma cholesterol and LDL-cholesterol levels did not show any significant differences among groups with different numbers of diseased vessels. Because of the small total number of black women, we did not have enough individuals in each category based on the number of diseased vessels (i.e., 72 had no-vessel disease, 21 had one-vessel disease, 10 had two-vessel disease, and 8 had three-vessel disease) to obtain meaningful results when using this characteristic.

PON *Polymorphisms among Whites and Blacks*

The allele and genotype distributions of three *PON* polymorphisms, stratified by race, are shown in table 2. The genotype distributions of the three polymorphisms were in Hardy-Weinberg equilibrium within all subgroups. The distributions of all three polymorphisms were significantly different between whites and blacks. The *PON1* Met*55* allele frequency was significantly higher in whites than in blacks (0.375 vs. 0.193; $P < .0001$), whereas frequencies of the *PON1* Arg192 (0.*295* vs. 0.631; $P < .00001$) and *PON2* Cys311 (0.239 vs. 0.311; $P = .0264$) alleles were lower in whites than in blacks.

Association between PON *Polymorphisms and the Severity of CAD*

No significant association was observed between the three *PON* polymorphisms and stenosis severity in either white or black women (data not shown). However, when we stratified the patients with ⩾50% stenosis into groups with one-, two-, or three-vessel disease, significant associations were noted between the *PON1* codon 192 and *PON2* codon 311 polymorphisms and the number of diseased vessels in whites but not in blacks (table 3). The frequency of the Arg/Arg genotype of the *PON1* codon 192 polymorphism was significantly higher among the patients with ⩾50% stenosis in all three arteries (17.02%) compared with patients with one (7.69%) and two (0%) diseased vessels ($P = .0066$). Similarly, the frequency of the *PON2* codon 311 Cys/Cys genotype was significantly higher in the group with three-vessel disease (15.2%)

Table 1

Patient Characteristics According to the Number of Diseased Vessels in Whites

Characteristic	No. of Diseased Vessels				P
	None ($n=353$)	One ($n=78$)	Two ($n=53$)	Three ($n=47$)	
Age (years)	55.2 ± .6	63.2 ± 1.5	61.2 ± 1.5	64.9 ± 1.5	<.001
BMI (kg/m^2)	29.2 ± .4	28.5 ± .7	29.1 ± .9	29.1 ± .9	.826
Former smoker (%)	31.3	46.2	28.3	38.3	.247
Current smoker (%)	20.2	16.7	24.5	17	...
Alcohol use within past 6 mo (yes, %)	15.9	14.5	13.2	4.3	.214
Family history of CAD (yes, %)	68.7	64.5	76.9	61.7	.353
History of hypertension (yes, %)	49.4	57.7	65.4	67.4	.024
History of diabetes (yes, %)	11.7	25.6	35.8	46.8	<.001
Menopause (yes, %)	79.1	85.7	88.7	91.3	.072
Lipid-lowering–drug use:					
Statins (yes, %)	18.7	33.3	34.0	46.8	<.001
Others (yes, %)	4.2	9.0	1.9	4.3	.49
Total cholesterol (mg/dl)	198 ± 2.4	191 ± 4.8	198 ± 6.6	197 ± 6.6	.694
Triglycerides (mg/dl)	158 ± 7.3	169 ± 10.2	177 ± 11.7	169 ± 15.2	.677
HDL cholesterol (mg/dl)	55 ± .7	52 ± 1.4	50 ± 1.3	50 ± 1.9	.023
LDL cholesterol (mg/dl)	114 ± 2.1	106 ± 4.4	112 ± 6.0	116 ± 6.2	.367

compared with patients in the groups with one-vessel (3.9%) and two-vessel (5.7%) disease ($P = .018$). The χ^2 test for trends showed a significant association with an increasing number of diseased vessels for the *PON2* codon 311 polymorphism ($\chi^2 = 4.89$; $P = .027$) but not for *PON1* codon 192 ($\chi^2 = 2.26$; $P = .13$).

Given that age, BMI, smoking, alcohol use, menopausal status, medical history, lipid-lowering–drug use, and lipid profile are possible risk factors for CAD, logistic-regression models were used to evaluate the independence of the positive associations between CAD severity, in terms of the number of diseased vessels, and the *PON1* codon 192 and *PON2* codon 311 polymorphisms. The adjusted OR between the *PON1* codon 192 Arg/Arg genotype versus the *PON1* Gln192 allele carriers (Gln/Gln plus Gln/Arg genotypes) was 2.80 (95% CI 1.06–7.37; $P = .038$) for the presence of three-vessel disease. The adjusted OR for *PON2* codon 311 Cys/Cys genotype versus *PON2* Ser311 allele carriers (Ser/Ser plus Cys/Ser genotypes) was 3.68 (95% CI 1.26–10.68; $P = .017$) for the presence of three-vessel disease.

Haplotype Analyses

The pairwise measure of linkage disequilibrium, $|D'|$, is reported in table 4. A $|D'|$ value of 1 indicates complete linkage disequilibrium between two markers. Among white women, significant linkage disequilibrium was observed between *PON1* codon 55 and *PON1* codon 192, as well as between *PON1* codon 55 and *PON2* codon 311. In the sample of black women whom we studied, the *PON2* codon 311 polymorphism was in significant linkage disequilibrium with both the *PON1* codon 55 ($P = .03$) and *PON1* codon 192 ($P = .025$) polymorphisms.

The estimated haplotype distribution for all three sites in white women is shown in table 5. As can be seen in table 6, the overall estimated haplotype frequencies of *PON1* codon 55, *PON1* codon 192, and *PON2* codon 311 were significantly different between case subjects (individuals with three-vessel disease) and control subjects (individuals with no-vessel disease) ($P = .012$). In particular, the estimated frequency of the Leu-Gln-Ser (15.4% vs. 24.4%; $P = .026$) haplotype was significantly higher in the control group than in the case (i.e., three-vessel disease) group. However, the frequency of

Table 2

***PON* Genotype and Allele Distribution in Whites and Blacks**

	Whites	Blacks	P
PON1 codon 55:			
Leu/Leu	227 (38.5%)	80 (65.6%)	
Leu/Met	282 (47.9%)	37 (30.3%)	
Met/Met	80 (13.6%)	5 (4.1%)	
Total	589	122	
Met allele	.375	.193	<.0001
PON1 codon 192:			
Gln/Gln	293 (49.7%)	19 (15.6%)	
Gln/Arg	245 (41.6%)	52 (42.6%)	
Arg/Arg	51 (8.7%)	51 (41.8%)	
Total	589	122	
Arg allele	.295	.631	<.00001
PON2 codon 311:			
Ser/Ser	343 (58.2%)	57 (47.9%)	
Ser/Cys	210 (35.7%)	50 (42.0%)	
Cys/Cys	36 (6.1%)	12 (10.1%)	
Total	589	119	
Cys allele	.239	.311	.0264

Table 3

***PON1* Codon 55, *PON1* Codon 192, and *PON2* Codon 311 Polymorphisms by Number of Diseased Vessels in Whites**

	No. of Diseased Vessels			Trend Test[a]
	One	Two	Three	
PON1 codon 55:				
Leu/Leu	34 (43.59%)	20 (37.74%)	13 (27.66%)	
Leu/Met	33 (42.31%)	25 (47.17%)	26 (55.32%)	
Met/Met	11 (14.10%)	8 (15.09%)	8 (17.02%)	
Total	78	53	47	$\chi^2 = .19$
Met allele	.353	.387	.447	$P = .67$
PON1 codon 192:				
Gln/Gln	37 (47.44%)	28 (52.83%)	20 (42.55%)	
Gln/Arg	35 (44.87%)	25 (47.17%)	19 (40.43%)	
Arg/Arg*	6 (7.69%)	0	8 (17.02%)	
Total	78	53	47	$\chi^2 = 2.26$
Arg allele	.301	.236	.372	$P = .13$
PON2 codon 311:				
Ser/Ser	48 (62.34%)	31 (58.49%)	26 (56.52%)	
Ser/Cys	26 (33.77%)	19 (35.85%)	13 (28.26%)	
Cys/Cys**	3 (3.90%)	3 (5.66%)	7 (15.22%)	
Total	77	53	46	$\chi^2 = 4.89$
Cys allele	.208	.236	.293	$P = .027$

[a] Performed among three groups with stenosis: *PON1* codon 55 (Met/Met vs. Leu/Leu plus Leu/Met), *PON1* codon 192 (Arg/Arg vs. Gln/Gln plus Gln/Arg), and *PON2* codon 311 (Cys/Cys vs. Ser/Ser plus Ser/Cys).

* $P = .0066$, between the group with three-vessel disease and the groups with one-vessel and two-vessel disease (χ^2 test).

** $P = .018$, between the group with three-vessel disease and the groups with one-vessel and two-vessel disease (χ^2 test).

the Met-Gln-Cys haplotype was significantly higher in case subjects (15.5%) than in control subjects (4.2%) ($P = .003$). Again, because of the small sample size, we did not perform the similar analyses on black women.

Gene-Environment Interaction

As a multifactorial disease, CAD is caused by interactions between environmental and genetic factors. Therefore, we performed a series of exploratory logistic-regression analyses, to test for possible interactions between the *PON* polymorphisms and other factors. Interaction between the *PON* genotype and one risk factor at a time was tested in the logistic-regression model. Two marginally significant interactions were found between *PON1* codon 192 polymorphism and BMI (OR 1.2; 95% CI 1.0–1.5; $P = .05$) and between *PON2* codon 311 and HDL-cholesterol level (OR 0.93; 95% CI 0.87–0.99; $P = .03$). However, considering that, in total, 27 comparisons were analyzed, these two findings are provisional and need to be explored and replicated.

Discussion

The oxidative damage to vital biological systems can lead to enhanced expression of inflammatory genes that ultimately contribute to the development of several chronic diseases, including CAD, cancer, and diabetes (Navab et al. 1996; Papas 1996), and that contribute to aging (Martin et al. 1996). The balance between oxidants and antioxidants basically affects all biological systems and, ultimately, the clinical course. The oxidation of LDL and its involvement in the development of foam cell–laden fatty streaks in the arterial wall are believed to initiate the atherosclerotic process (Steinberg et al. 1989; Witztum and Steinberg 1991). In vitro studies indicate that HDL-associated PON1 prevents LDL oxidation (Aviram et al. 1998) and can destroy biologically active lipids in

Table 4

Pairwise Measure of Linkage Disequilibrium, $|D'|$, in Whites and Blacks

Pairwise Comparison	Whites ($n = 588$)		Blacks ($n = 119$)	
	$\|D'\|$	P[a]	$\|D'\|$	P[a]
PON1 codon 55 vs. *PON1* codon 192	.594	.000	.168	.068
PON1 codon 55 vs. *PON2* codon 311	.303	.000	.429	.026
PON1 codon 192 vs. *PON2* codon 311	.002	.941	.293	.025

[a] Obtained by χ^2 tests.

Table 5

Estimated Percentage of Haplotype Distributions in White Women

Haplotype Distribution						
PON1 Codon 55	*PON1* Codon 192	*PON2* Codon 311	Total (n = 526)	Three-Vessel Disease (n = 47)	No-Vessel Disease (n = 349)	P[a]
Leu	Gln	Cys	8.8	5.5	9.6	.114
Leu	Gln	Ser	23.7	15.4	24.4	.026
Leu	Arg	Cys	9.6	8.8	9.8	.764
Leu	Arg	Ser	19.1	25.7	18.1	.110
Met	Gln	Cys	6.0	15.5	4.2	.003
Met	Gln	Ser	31.4	26.4	32.5	.211
Met	Arg	Cys	.4	.0	.5	.056
Met	Arg	Ser	1.2	2.8	.8	.263

[a] Obtained from Z tests, to compare haplotype frequencies between the groups with three-vessel and no-vessel disease.

mildly oxidized LDL (Watson et al. 1995), and PON1 may thus affect the process of atherosclerosis. Further evidence that PON is directly involved in the process of atherosclerosis comes from inbred strains of mice (Shih et al. 1996) and *PON1*-knockout mice (Shih et al. 1998). PON1 has also been localized in the normal human arterial wall, and its concentration increases significantly as atherosclerosis progresses, possibly as a result of oxidative stress that may overwhelm and retain HDL in the lesions and thus may increase PON concentration (M. I. Mackness et al. 1997). Recently, it has also been demonstrated that PON1 has the capacity to reduce oxidized lipids in human atherosclerotic lesions derived from either coronary artery or carotid specimens (Aviram et al. 2000). Because of the convincing in vitro and in vivo data that implicate PON in the prevention of LDL oxidation and because of the potentially important role that *PON* genetic variation plays in affecting the risk of CAD, Heinecke and Lusis (1998) have advocated adding the *PON* locus to a short list of genes that are involved in common forms of CAD. Numerous studies conducted in a case-control design have shown the association between the *PON1* Arg192 allele and the increased risk of CAD (Ruiz et al. 1995; Odawara et al. 1997; Sanghera et al. 1997; Zama et al. 1997), although the results have not been consistent across studies (Ko et al. 1998; Ombres et al. 1998; Aynacioglu and Kepekci 2000; Gardemann et al. 2000; Imai et al. 2000).

To our knowledge, no study has evaluated the role that *PON* polymorphisms play in the severity of CAD or whether the distributions of *PON* polymorphisms vary between whites and blacks. The present study was designed to address these two questions in women with chest pain or suspected myocardial ischemia. The results of the present study may improve the disease recognition, diagnosis, and treatment. On the basis of the severity of the disease, the subjects were divided into three groups: <20% stenosis, 20%–49% stenosis, and ⩾50% stenosis. Furthermore, the patients with significant disease (⩾50% stenosis) were stratified as having one-, two-, or three-vessel disease. Although this selected group of subjects is different from the general population, it is representative of patients evaluated for chest pain and, on the basis of its clinical data, also constitutes a good study population for studying the disease severity. The distribution of *PON* alleles in the group with no disease is very similar to those found in several other unaffected control groups (Garin et al. 1997; B. M. Mackness et al. 2001; Sentí et al. 2001; Topic et al. 2001), and the strength of our genetic association results is comparable to reported case-control studies for *PON*. These observations indicate that a possible bias caused by the inclusion of an angiographic case-control cohort (Fried and Pearson 1987; Pearson and Derby 1991; Reed and Yano 1991) may not have affected the outcome of the present study.

We examined two polymorphisms in the *PON1* gene (Leu55Met and Gln192Arg) and one polymorphism in the *PON2* gene (Ser311Cys). The distributions of all three polymorphisms were significantly different between white and black women. Whereas the frequency of the *PON1* Met55 allele was higher in whites than blacks (0.375 vs. 0.193; $P < .0001$), the reverse was true for the frequencies of the *PON1* Arg192 allele (0.631 vs. 0.295; $P < .0001$) and the *PON2* Cys311 allele (0.311 vs. 0.239; $P = .0264$). Previously, the lowest frequency of the *PON1* Met55 allele had been reported in Chinese (3.6%)

Table 6

Association Testing Based on the Haplotype Frequencies in White Women

Group	n	$\ln(L)$	χ^2	P[a]
Case (three-vessel disease)	47	−131.29	23.49	.012
Control (no-vessel disease)	349	−908.85	100.07	
Combination	396	−1,049.18	110.8	

[a] Calculated from the T5 statistic: $2[\ln(L)_{case} + \ln(L)_{control} - \ln(L)_{case+control}$; df = 7.

(Sanghera et al. 1998*b*), indicating a significant difference in the occurrence of this allele among three major racial groups. The relatively high frequency of the *PON1* Arg192 allele in blacks is similar to that reported previously in Chinese and Japanese, varying from 58% to 65% (Odawara et al. 1997; Sanghera et al. 1997; Zama et al. 1997; Ko et al. 1998; Imai et al. 2000).

The present sample of black women was very small; therefore, we did not perform analyses of CAD severity on the black sample, because such analyses would not be meaningful. Among white women, we did not observe any significant association between the three *PON* polymorphisms and stenosis severity; however, the *PON1* Gln192Arg and *PON2* Ser311Cys polymorphisms showed significant association with the severity of disease on the basis of the number of diseased vessels, as measured by quantitative coronary angiography. The frequencies of both the Arg192 allele (0.372 vs. 0.275; $P = .07$) and the Arg/Arg genotype (17% vs. 4.58%; $P = .0066$) of the *PON1* Gln192Arg polymorphism were higher in the group with three-vessel disease than in the groups with other numbers of diseased vessels. The adjusted OR associated with the Arg/Arg genotype was 2.80, similar to that reported in several other case-control studies, which also found positive association of the Arg192 allele with CAD risk (Ruiz et al. 1995; Odawara et al. 1997; Sanghera et al. 1997, 1998*a*; Zama et al. 1997; Imai et al. 2000). However, a number of other studies have reported no association between the Arg192 allele and CAD risk (Ko et al. 1998; Ombres et al. 1998; Aynacioglu and Kepekci 2000; Gardemann et al. 2000), a dilemma reflected in many association studies. A recent meta-analysis of 18 studies has confirmed the hypothesis that the *PON1* Arg192 allele is a risk factor for CAD (Durrington et al. 2001). Because association studies are subjected to several biases, further support of this hypothesis could be provided by the performance of functional studies. Because PON1 inhibits LDL oxidation, we expect that the Arg192 allele would be associated with less protection against the oxidative modification of LDL. Indeed, in vitro functional data show that the Arg192 allele is less efficient at protecting LDL from oxidation (Aviram et al. 1998; B. M. Mackness et al. 1998). The functional data, together with the association studies, strongly support the notion that the Arg192 allele of the *PON1* codon 192 polymorphism may be functional in vivo. Some recent studies have suggested that the PON activity or concentration, in addition to the *PON* genotype, should be included, to fully understand their joint contribution to the risk of CAD (Richter and Furlong 1999; Jarvik et al. 2000; B. M. Mackness et al. 2001). However, PON activity or concentration data were not available in the WISE cohort, so we were unable to explore this aspect.

Previously, the *PON2* Ser311 allele has been shown to increase the risk of CAD in an Asian Indian sample (Sanghera et al. 1998*a*). However, in the present study, we found that the *PON2* Cys311 allele was associated with CAD risk. The frequencies of both the Cys allele (0.293 vs. 0.219; $P = .06$) and the Cys/Cys genotype (15.2% vs. 4.6%; $P = .018$) were higher in the group with three-vessel disease than in the groups with one-vessel or two-vessel disease. The adjusted OR for the *PON2* codon 311 Cys/Cys genotype was 3.68 (95% CI 1.26–10.68; $P = .017$) for the development of three-vessel disease. Additional functional studies with the *PON2* Ser311Cys allelic isoforms may help to determine the role that this polymorphism plays in the etiology of CAD. Like PON1 and PON3, PON2 also prevents LDL oxidation (Ng et al. 2001). However, unlike PON1 and PON3, PON2 is not associated with HDL and may exert its antioxidant function at the cellular level. The present study is the first study to show that the *PON1* codon 192 Arg/Arg and *PON2* codon 311 Cys/Cys genotypes are significantly associated with CAD severity in terms of the number of diseased vessels but not in terms of stenosis severity. We could hypothesize that PON1 and PON2 are not involved directly in the early stage of stenosis development but instead are involved in the late stage of multivessel-disease development. This hypothesis may also explain some of the inconsistent results in previously reported case-control studies, especially for the *PON1* polymorphisms. If this hypothesis is true, because the patients/case subjects included in each reported study may vary with regard to disease severity, those studies including patients with more severe CAD (i.e., having multiple vessels with $\geqslant 50\%$ stenosis) would detect a stronger association than those with fewer severely affected patients. Alternatively, LDL oxidation and HDL-associated PON protective action affect all stages of atherosclerosis, and the lack of association with the single-vessel and double-vessel disease in the present study may be due to the complex nature of atherosclerosis. However, detailed functional data on PON1 and PON2 would be essential, to verify whether these two genes are involved in the early or late stage of CAD development.

The results of the haplotype analyses were consistent with the single-site analyses and also showed significant association with CAD severity and *PON* haplotypes ($P = .012$). Since all three genes (*PON1, PON2,* and *PON3*) are linked on chromosome 7, a detailed characterization of the structure-function relationship of *PON* mutations would be helpful in elucidating their biological mechanisms in CAD.

The analyses of the interactions among the three genotypes and the genotype-biological factors might further support the hypothesis that *PON1* codon 192 and *PON2* codon 311 have functional effects on risk of CAD. For example, we found an interaction ($P = .05$)

between *PON1* codon 192 genotype and BMI in affecting the risk of CAD. This result suggests that the effect that the *PON1* codon 192 polymorphism has on the risk of CAD may differ among groups with high or low BMI levels. Because obesity (BMI > 30) is a significant risk factor for coronary disease (Al Suwaidi et al. 2001), this result is logical, although additional analyses will be needed to understand the mechanism of this possible interaction. We also observed an interaction between the *PON2* codon 311 polymorphism and HDL cholesterol. By itself, the *PON2* codon 311 polymorphism was associated with an increased risk of CAD (OR 4.26). However, by the inclusion of an interaction with HDL cholesterol, which is inversely associated with CAD risk (Gordon et al. 1989), the *PON2* codon 311 polymorphism was associated with decreased risk of CAD (OR 0.93). This result indicates that the association between the *PON2* codon 311 polymorphism and CAD risk may differ among specific population subgroups (e.g., individuals with low versus high HDL-cholesterol levels). However, because of the fairly large number of comparisons ($n = 27$) that we did in the interaction analyses, these two marginally significant interactions are provisional and will need to be replicated in additional studies.

In summary, results of our analyses of data in the WISE cohort suggest that common genetic variation in the *PON* gene cluster may affect the risk of CAD, especially the risk for development of severe CAD in women. Our finding that the *PON* polymorphisms may affect the severity of the disease may explain the inconsistent published reports in which the relationship between *PON* polymorphisms and CAD severity has not been examined.

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Electronic-Database Information

Accession numbers and the URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *PON1* [MIM 168820], *PON2* [MIM 602447], and *PON3* [MIM 602720])

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