The correlation of paraoxonase (PON1) activity with lipid and lipoprotein levels differs with vascular disease status

Laura S. Rozek,[*,†] Thomas S. Hatsukami,[††] Rebecca J. Richter,[†] Jane Ranchalis,[†] Karen Nakayama,[†] Laura A. McKinstry,[†] David A. Gortner,[§§] Edward Boyko,[††] Gerard D. Schellenberg,[§,††] Clement E. Furlong,[†,] and Gail P. Jarvik[1,†,**]**

Department of Internal Medicine,* University of Michigan, Ann Arbor, MI; Department of Medicine, Divisions of Medical Genetics† and Neurology,§ and Department of Genome Sciences,** University of Washington, Seattle, WA; Puget Sound Veterans Affairs Health Care Systems,†† Seattle, WA; and General Internal Medicine,§§ Virginia Mason Medical Center, Seattle, WA

Abstract Paraoxonase (PON1) is an HDL-associated enzyme. Low PON1 activity predicts vascular disease status and is a more reliable predictor of vascular disease than are functional PON1 genotypes. There is evidence that the relationship of PON1 to vascular disease is, in part, due to its antioxidant activity. However, the physical relationship of PON1 with HDL and the existence of cholesterol pathway regulatory elements at the *PON1* locus suggest a further relationship of PON1 with lipoproteins, which may contribute to its role in vascular disease. We investigated the relationship of PON1 activity and genotype to lipid-related traits in 91 Caucasian men with severe carotid artery disease and 184 without vascular disease who were not on lipid-lowering medications. Prior studies of PON1 relationship to lipids have not stratified by disease status. We found that PON1 activity was correlated with HDL traits in controls and with LDL- and VLDL-related traits in cases. We hypothesize differences in the joint regulation of PON1 and lipoproteins in cases and controls.—Rozek, L. S., T. S. Hatsukami, R. J. Richter, J. Ranchalis, K. Nakayama, L. A. McKinstry, D. A. Gortner, E. Boyko, G. D. Schellenberg, C. E. Furlong, and G. P. Jarvik. **The correlation of paraoxonase (PON1) activity with lipid and lipoprotein levels differs with vascular disease status. *J. Lipid Res.* 2005.** 46: **1888–1895.**

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There is a growing body of evidence that reduced activity of the HDL-associated (1–7) enzyme paraoxonase (PON1) is predictive of vascular disease in humans (8–11), including results from prospective studies (12, 13). A role of PON1 in vascular disease is also strongly supported by knockout and transgenic mouse studies. Knockout mice lacking serum PON1 have an increased susceptibility to atherosclerosis (14), with a 42% excess in stenosis (15). Conversely, human PON1 transgenic mice with 2- to 4-fold increased PON1 levels have a reduced susceptibility (16).

There is a 10- to 40-fold interindividual difference in serum PON1 paraoxonase activity (17–19) due, in part, to four common polymorphisms: functional promoter region polymorphisms include $PON1_{C\text{-}108T}$ and $PON1_{G\text{-}162A}$ (19), and the functional coding polymorphisms are $PON1_{Q192R}$ and $PON1_{L55M}$. Variations at these four common polymorphisms explain 44% of the phenylacetate and 88% of the paraoxon hydrolysis activity of PON1 in subjects without vascular disease (11) and 25% of the phenylacetate and 82% of the paraoxon hydrolysis activity in subjects with severe carotid artery disease, with $PON1_{192}$ largely accounting for the paraoxon hydrolysis variation. The promoter region polymorphisms affect protein level, best represented by the PON1 phenylacetate hydrolysis activity, which is reasonably independent of the $PON1_{192}$ polymorphism (20, 21).

Despite the large impact of PON1 genetic variation on PON1 activity, this variation is inconsistently associated with vascular disease status (11–13, 22), and meta-analyses have not detected a significant predictive effect for a limited set of PON1 genotypes (23, 24) that was not attributable to evident publication bias. Why PON1 activity affects vascular disease and its important genetic variability does not remains unexplained. Mechanisms by which PON1 activity may impact cardiovascular disease (CVD) risk that are independent of this genetic variation must be sought.

The mechanisms by which PON1 activity influences risk of vascular disease continue to be evaluated. It is generally held that PON1 contributes to the antioxidant, thus, antiatherogenic properties of HDL. Virtually all of PON1's activity is associated with HDL-cholesterol (HDL-C) (25). PON1 appears to prevent LDL and HDL oxidation (26, 27). The $PON1_{192}$ polymorphism has been reported to af-

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[1] To whom correspondence should be addressed.
pair@u.washington.edu

fect the hydrolysis of oxidized LDL-C and HDL-C (28, 29). In accordance with this, HDL from PON1 knockout mice does not protect LDL from oxidation (30) and PON1 transgenic mice have improved protection of LDL (31).

The relationship between PON1 activity and vascular disease may be influenced by the relationship of PON1 activity or genotype to lipid and lipoprotein levels. The physical association of PON1 and the HDL subfraction 3 (HDL3) component of HDL in subjects with (5) and without vascular disease (5, 7, 25) and the presence of an important cholesterol transcriptional regulator, SREBP-2 (bp −104 to −95), in the proximal PON1 promoter region (32) suggest an important PON1-lipoprotein relationship. SREBP-2 was found to increase PON1 promoter activity in a dose-dependent manner (32), indicating an additional lipid-related mechanism of PON1 activation. As summarized in the discussion, previous reports of PON1-lipid relationships have been contradictory and do not consider a stratum of subjects affected by vascular disease. A further understanding of the relationship between the PON1 phenotypes and genotypes and lipids and lipoproteins may help to clarify the role of PON1 in vascular disease and, in particular, may address why PON1 activity level is more important than PON1 genetic variation.

The current study evaluated the effects of PON1 activities in the hydrolysis of paraoxon, diazoxon, and phenylacetate as well as the genotype at functional, common promoter region ($PON1_{-162}$ and $PON1_{-108}$) and coding ($PON1_{55}$ and $PON1_{192}$) polymorphisms on lipids and lipoproteins in subjects with and without carotid artery disease (CAAD). The aim was to determine which PON1 parameters are most correlated with these known cardiovascular risk factors, with the eventual goal of determining whether any role of PON1 in vascular disease may be attributable, in part, to PON1's relationship with lipids, lipoproteins, and apolipoproteins. The vascular disease subjects, having >80% carotid stenosis, and the nonvascular disease subjects, having <15% carotid stenosis bilaterally, represent tails of the vascular disease spectrum. The stratification by vascular disease status may shed light on differences in the PON1 relationships in these groups.

METHODS

Sample

This study included 275 Caucasian males who were not taking lipid-lowering medications—91 cases with and 184 controls without CAAD. Participants were identified through an Epidemiology Research and Information Center project at the Puget Sound Veterans Affairs Health Care System (PSVAHCS), Seattle, Washington and at Virginia Mason Medical Center (VMMC), Seattle, from September 1996 through April 2004. Of these subjects, 75 cases and 110 controls overlap with those described in more detail elsewhere (11), and ascertainment remains the same. Secondary analyses considered 110 CAAD and 67 controls who were part of the same study but who were taking lipid-lowering medications. Controls on lipid-lowering medications were generally being treated for hyperlipidemia. All CAAD cases had severe carotid artery disease: >80% internal carotid artery stenosis, unilaterally or bilaterally, on angiography using standardized guidelines. CAAD subjects who had carotid endarterectomy without prior angiogram were also included. Non-CAAD subjects were drawn from patients in a general internal medicine clinic database who did not have computerized codes for cardiovascular or peripheral vascular disease. Subject interview and medical record review confirmed the lack of any known vascular disease. All non-CAAD subjects had <15% internal carotid artery stenosis, bilaterally, on carotid ultrasound. Subjects with a fasting total serum cholesterol level >400 mg/dl and those with coagulopathies were excluded. A study physician or nurse performed chart and pharmacy record reviews for lipid-lowering medication use, which was then reconciled with the patient's own report of medications. Current smoking status was determined by self-report in a written survey. The study was approved by the Human Subjects review processes at the University of Washington, VMMC, and the PSVAHCS, Seattle. Written informed consent was obtained prior to participation.

PON1 genotype and activity measures

All laboratory measures were done blinded to clinical characteristics. DNA was prepared from buffy coat preparations by a modification of the procedure of Miller, Dykes, and Polesky (33). Genotyping was conducted by PCR amplification followed by polymorphism-specific restriction digestion and gel electrophoresis. The genotypes of the $PON1_{Q192R}$, $PON1_{L55M}$, $PON1_{C-108T}$, and $PON1_{A-162G}$ polymorphisms were determined as previously published (34–36). All of the 91 CAAD and 171 of the 184 non-CAAD subjects also had genotyping performed for the $PON1_{55}$ polymorphism by Illumina, Inc. There was a 1% difference in genotypes. Illumina genotypes were used when genotypes did not match. Four subjects were missing one or more genotypes. Genotype distributions did not significantly differ from Hardy-Weinberg equilibrium expectations.

The activity of PON1 in the hydrolysis of paraoxon, diazoxon, and phenylacetate (arylesterase activity) was measured by a continuous spectrophotometric assay, as described elsewhere (20, 36–38). These are termed POase, DZOase, and Arylase, respectively. Arylase was measured in triplicate. The plot of POase versus DZOase accurately predicts $PON1_{192}$ genotype in both cases and controls; this plot was used to fill in three missing genotypes (39).

Lipid-related measurements

Lipid-related measurements were performed by Northwest Lipid Research Laboratories, Seattle, WA. Subjects fasted for 12 h prior to sampling. HDL-C, HDL2, HDL3, apolipoprotein A-I (apoA-I), total cholesterol (TC), LDL-C, apoB, triglycerides (TGs), and VLDL-C were measured. Standard enzymatic methods were used to determine levels of TC, TG, VLDL-C, and HDL-C in whole plasma on an Abbott Spectrum analyzer (40–42). HDL2 and HDL3 were determined by precipitation of HDL2 from total HDL-C, and measurement of the HDL3 remaining in the supernate (HDL-C − HDL3 = HDL2). ApoA-I measurement used calibrators and quality control samples from in-house plasma pools with values assigned against World Health Organization international reference material (43). ApoB associated with LDL (LDL-B) was measured after pooling the LDL-containing density gradient ultracentrifugation (DGUC) fractions (44). LDL density was evaluated by nonequilibrium DGUC as described previously (45). LDL buoyancy was calculated as the peak LDL fraction divided by 38, the total number of fractions collected.

Statistical methods

Due to departures from normality, HDL-C, HDL2, HDL3, TC, TG, and VLDL-C were natural log-transformed prior to the analysis (lnHDL, lnHDL2, lnHDL3, lnTC, lnTG, and lnVLDL-C). Stu-

dent *t*-tests or chi-squared tests were used to evaluate differences in characteristics between CAAD and non-CAAD subjects. The relationship of PON1 activity and lipid measures was assessed separately for cases and controls. Bivariate Pearson correlations were calculated between POase, DZOase, and Arylase and lipid-related measures. This was done considering both raw PON1 activities and PON1 activities adjusted for the variation in $PON1_{-162}$, $PON1_{-108}$, $PON1_{55}$, and $PON1_{192}$ genotypes by dummy variable regression, which considers each genotype as two separate variables. The effects of the covariates age, body mass index (BMI), and smoking on the Arylase activity-lipid-related trait relationship were also tested by regression, with and without PON1 genotype dummy variables, using the lipid traits as dependents and including Arylase, BMI, age, and smoking status. The results with these additional covariates were the same as those found using the simple correlations, and they are not separately reported here. The simultaneous effects of PON1 polymorphisms on lipid measures were assessed by the same linear regressions, using genotype dummy variables and including current age, smoking, and BMI as covariates. No adjustment was done for multiple contrasts for the correlation analyses, because both multiple PON1 and multiple lipid-related measures are highly correlated. For the genotype effects, a very minimal adjustment for multiple contrasts was employed, using $P = 0.01$ as a criterion for statistical significance. All analyses were performed using SPSS 10.0 for Windows (46).

RESULTS

Sample descriptives, stratified by CAAD status, are shown in **Table 1**. Subjects on lipid-lowering medications at the time of sampling were excluded from the primary analysis by design. The sample was Caucasian and male. Because the study design matched age of CAAD onset (censored age) with current control subject age, the CAAD subjects were slightly older than the non-CAAD subjects. The CAAD subjects had a higher prevalence of smoking; lower HDL-C, HDL3, and apoA-I levels; and lower BMI than did non-CAAD subjects. As expected, PON1 activities were lower in CAAD than non-CAAD subjects. There were no significant differences in genotype distributions for any of the PON1 polymorphisms between CAAD subjects and non-CAAD subjects. Minor (rarer) allele frequencies for the PON1 polymorphisms are summarized in Table 1.

When considering the relationship between PON1 activity levels and the age-adjusted lipid and lipoprotein levels, the strongest correlations (r) were found with the Arylase activities and the lipid measures (**Table 2**). This is not surprising because, of these, Arlyase activity is the best measure of the amount of PON1 protein present. In the non-CAAD subjects, the strongest correlations of Arylase activity were with HDL3 (0.330) and apoA-I (0.329). Several results were surprising. First, in CAAD cases, the PON1 activity correlation was much stronger with TC (0.389 with Arlyase vs. 0.156 for non-CAAD) and its components, LDL-C, LDL associated apoB (LDL-B), and VLDL-C than with HDL3 (0.113), despite that fact that the PON1 enzyme is physically associated with the HDL3 particle. Second, the correlation pattern was quite different for the

TABLE 1. Sample descriptives stratified by CAAD status

	No Lipid-Lowering Medications			Taking Lipid-Lowering Medications		
	CAAD (SD)	Non-CAAD (SD)	*P*	CAAD (SD)	Non-CAAD (SD)	*P*
N	91	184	NA	110	67	NA
Caucasian	100%	100%	NA	100%	100%	NA
Male	100%	100%	NA	100%	100%	NA
Censored age mean (range), years	67.0 (48–83)	66.7 (47–83)	0.87	65.3 (41–84)	63.5 (50–80)	0.17
Current age mean (range), years	70.4 (49–83)	66.7 (47–83)	0.002	69.6 (46–89)	63.5 (50–80)	<0.001
Lipid-lowering medications	0%	0%	NA	100%	100%	NA
Current smoker	42%	15%	<0.001	32%	9%	0.001
Ever smoker	92%	74%	<0.002	95%	68%	<0.001
Type 2 diabetes	15%	17%	0.73	22%	16%	0.44
BMI	26.6 (5.0)	29.1 (6.3)	0.001	28.4 (5.2)	30.2 (4.7)	0.020
HDL-C mg/dl	40.5 (14.0)	46.9 (16.1)	0.002	41.7 (14.2)	41.4 (9.9)	0.69
HDL2 mg/dl	8.1 (6.2)	8.2 (5.8)	0.45	7.0 (4.8)	6.3 (3.2)	0.36
HDL3 mg/dl	34.1 (13.7)	37.7 (11.4)	<0.001	34.7 (10.0)	35.2 (7.3)	0.40
ApoA-I	121.3 (22.0)	134.5 (24.5)	<0.001	120.5 (35.0)	128.9 (16.3)	0.11
TG mg/dl	159.4 (121.8)	145.0 (97.6)	0.13	180.9 (129.9)	206.8 (193.2)	0.40
VLDL-C mg/dl	31.9 (24.4)	29.3 (21.3)	0.14	36.1 (25.0)	36.9 (24.8)	0.68
TC mg/dl	197.1 (38.3)	190.7 (37.0)	0.12	186.6 (39.2)	192.7 (41.3)	0.30
LDL-B mg/dl	69.5 (17.4)	65.8 (18.4)	0.12	61.1 (16.2)	64.5 (17.7)	0.20
LDL-C mg/dl	106.4 (28.5)	103.5 (30.0)	0.38	89.2 (27.6)	96.7 (30.6)	0.10
LDL density (Rf) mg/dl	0.27 (0.03)	0.26 (0.03)	0.48	0.26 (0.03)	0.24 (0.03)	0.015
Arylase units/l	93.8 (42.4)	106.1 (42.1)	0.023	104.1 (41.8)	117.0 (41.8)	0.049
DZOase units/l	8,867.3 (3016.1)	9,684.4 (3297.7)	0.048	9,556.1 (3246.1)	10,326.8 (2818.5)	0.11
POase units/l	603.2 (424.1)	673.0 (429.1)	0.19	688.2 (484.6)	653.0 (418.8)	0.62
$PON1_{-162A}$ MAF	0.25	0.26	0.88	0.26	0.25	0.85
$PON1_{-108T}$ MAF	0.47	0.50	0.39	0.50	0.41	0.12
$PON1_{55M}$ MAF	0.35	0.38	0.41	0.35	0.41	0.40
$PON1_{192R}$ MAF	0.30	0.31	0.97	0.32	0.25	0.13

ApoA-I, apolipoprotein A-I; BMI, body mass index; CAAD, carotid artery disease; LDL-B, LDL associated apoB; LDL-C, LDL-cholesterol; MAF, minor allele frequency; NA, not applicable; TC, total cholesterol; TG, triglyceride. Although the lipid values in the table are untransformed for ease of interpretation, the statistical tests for these lipid values used ln transformations for the following variables: HDL-C, HDL2, HDL3, TGs, VLDL-C, and TC. *P* values reflect the test of the genotype distribution difference between groups, not allele frequency differences.

TABLE 2. Correlations between lipid measures and PON1 activity measures with and without adjustment for PON1 genotypes in 91 CAAD/184 non-CAAD Caucasian subjects

	Arylase (adjusted Arylase)	DZOase (adjusted DZOase)	POase (adjusted POase)
CAAD/non-CAAD			
No Lipid-Lowering Medications			
Ln-HDL-C	−0.028/**0.290**[c] (−0.114/**0.310**[c])	**0.216**[a]/**0.207**[b] (0.060/**0.296**[c])	−0.177/**0.162**[a] (−0.026/**0.241**[b])
Ln-HDL2	−0.039/**0.184**[a] (−0.104/**0.219**[b])	−0.004/0.099 (−0.098/**0.191**[a])	−0.035/0.141 (−0.016/**0.169**[a])
Ln-HDL3	0.113/**0.330**[c] (0.048/**0.347**[c])	**0.209**[a]/**0.244**[b] (0.171/**0.342**[c])	0.107/**0.178**[a] (0.124/**0.269**[c])
ApoA-I	0.025/**0.329**[c] (0.040/**0.378**[c])	0.176/**0.219**[b] (0.196/**0.375**[c])	−0.046/**0.218**[b] (0.018/**0.294**[c])
Ln-TG	**0.301**[b]/−0.069 (**0.347**[b]/−0.033)	**0.240**[a]/−0.019 (**0.315**[b]/0.111)	0.185/0.070 (**0.328**[b]/0.064)
Ln-VLDL-C	**0.300**[b]/−0.066 (**0.347**[b]/−0.030)	**0.239**[a]/−0.019 (**0.315**[b]/0.111)	0.184/0.070 (**0.327**[b]/0.059)
Ln-cholesterol	**0.389**[c]/**0.156**[a] (**0.416**[c]/**0.204**[b])	**0.297**[b]/**0.157**[a] (**0.321**[b]/ **0.281**[c])	0.168/0.127 (**0.290**[b]/**0.292**[c])
LDL-B	**0.307**[b]/0.024 (**0.334**[b]/0.013)	0.149/0.082 (0.178/0.068)	0.132/0.045 (0.147/**0.185**[a])
LDL-C	**0.220**[a]/0.015 (**0.243**[a]/0.019)	0.062/0.079 (0.071/0.083)	0.075/0.038 (0.092/**0.181**[a])
LDL density (Rf)	**−0.262**[a]/0.008 (**−0.316**[b]/-0.017)	−0.092/0.042 (−0.202/-0.017)	**−0.237**[a]/−0.029 (**−0.251**[a]/0.003)
CAAD/non-CAAD On LLM			
Ln-HDL-C	−0.060/**0.248**[a] (−0.027/**0.286**[a])	0.033/0.161 (0.098/0.124)	0.147/0.149 (**0.220**[a]/0.164)
Ln-HDL2	−0.087/**0.271**[a] (−0.117/**0.339**[a])	−0.018/0.195 (−0.012/0.229)	0.076/0.083 (0.091/0.158)
Ln-HDL3	−0.049/0.213 (0.010/0.241)	0.046/0.123 (0.136/0.069)	0.171/0.152 (**0.259**[b]/0.150)
ApoA-I	−0.166/0.226 (−0.139/**0.285**[a])	−0.064/0.052 (−0.007/0.059)	0.011/0.216 (0.040/**0.337**[a])
Ln-TG	0.150/0.021 (**0.194**[a]/0.048)	0.114/0.162 (0.139/0.237)	0.023/−0.034 (0.002/0.178)
Ln-VLDL-C	0.152/0.004 (**0.195**[a]/0.001)	0.116/0.148 (0.141/0.199)	0.021/−0.072 (0.001/0.100)
Ln-cholesterol	0.125/−0.007 (**0.274**[b]/−0.069)	0.085/0.142 (**0.235**[a]/0.186)	0.156/**0.305**[a] (0.131/0.263)
LDL-B	0.113/−0.090 (**0.292**[b]/−0.163)	0.013/−0.019 (**0.206**[a]/0.038)	0.081/0.211 (0.042/0.053)
LDL-C	−0.016/−0.142 (0.181/−0.218)	−0.101/−0.034 (0.092/0.019)	0.023/0.192 (−0.005/−0.043)

Pearson correlations; 2-tailed tests; adjusted activities are adjusted for four PON1 genotype effects. Bolded values have $P \leq 0.05$.
[a] $P \leq 0.05$.
[b] $P \leq 0.01$.
[c] $P \leq 0.001$.

CAAD subjects and non-CAAD subjects. Non-CAAD subjects showed only the expected correlations of Arylase with HDL3 and apoA-I, as well as a weak correlation with TC that is not due to LDL-C and VLDL-C, but is probably related to the stronger correlation of PON1 activity with HDL-C.

DZOase activity correlations were in the same direction as those of Arlyase, although weaker, because of the larger role of the PON1 amino acid substitutions in DZOase activity. POase correlations were weaker still, reflecting the large impact of the *PON1*$_{Q192R}$ polymorphism on POase. These weaker correlations, and the lack of genotype distribution differences in cases and controls, indicate that the case-control differences in PON1 activity-lipid correlations are not due to the PON1 polymorphisms. Indeed, adjustment of the POase and DZOase for genotypes at the four functional PON1 polymorphisms using linear regression increased the similarity to the pattern of correlations seen with Arylase (Table 2).

Subjects with CAAD who are not on lipid-lowering medications represent a selected group of vascular disease patients. However, these medications affect the lipid-related trait levels, and statins increase PON1 activity (11, 47). Indeed, statins appear to increase PON1 transcription, possibly through an SREBP-2 mechanism (32). Nonetheless, we did examine these correlations in identically ascertained subjects with and without CAAD who were on lipid-lowering medications. Of 189 male Caucasian subjects on lipid-lowering medications, 158 (84%) were on statins. Descriptive statistics and correlations are seen in Tables 1 and 2. Correlations were low in 110 CAAD subjects on lipid-lowering medications. However, the correlations of genotype-adjusted Arylase with TC and LDL-B were significant, 0.274 and 0.292, respectively, as were weaker correlations with TGs (0.194) and VLDL-C (0.195). For 67 non-CAAD subjects on lipid-lowering medications, the Arylase activity relationship to HDL-related traits was seen, with significant correlations of genotype-adjusted Arylase with HDL-C, HDL2, and apoA-I levels of 0.285–0.339. Thus, the PON1 activity-lipoprotein relationships seen in both cases and controls on lipid medications show patterns similar to those not on medications, but with smaller magnitudes.

PON1 genotype effects were tested with and without pooling of the CAAD and non-CAAD groups. Genotype effects were sparse. No genotype predicted lipid levels, with a $P < 0.01$, for the combined groups or the non-CAAD subjects alone. For the CAAD cases, only *PON1*$_{192}$ genotype had a significant predictive effect on HDL-C level at the 0.01 level. The *PON1*$_{192}$ genotype QQ, QR, and RR groups had mean HDL-C levels of 40.4 (n = 43), 42.8 (n = 42), and 28.2 (n = 5) mg/dl, respectively, in CAAD cases, and 45.7 (n = 84), 46.1 (n = 87), and 46.4 (n = 13) mg/dl, respectively, in non-CAAD subjects. The range of HDL-C in *PON1*$_{192RR}$ genotype CAAD subjects was 7.0–33.0 mg/dl (23.0–33.0 with the 7.0 mg/dl value dropped) in CAAD cases and 29.0–67.0 mg/dl in controls. Deleting the 7.0 mg/dl HDL-C level outlier from the analyses did not remove the significant predictive effect of *PON1*$_{192RR}$ genotype on HDL-C level in CAAD cases.

DISCUSSION

The reported association between paraoxonase genotype and CVD association is most commonly attributed to

the role of PON1 in the protection of LDL-C from oxidative modification, as discussed above. However, other mechanisms through which PON1 could affect vascular disease have not been well explored. The relationship of PON1 POase, DZOase, and Arylase activities, as well as $PON1_{192}$, $PON1_{55}$, $PON1_{-108}$, and $PON1_{-162}$ genotypes, with lipid-related measures has not been previously contrasted in a group of subjects with and without vascular disease.

In this study, the examination of non-CAAD male Caucasian subjects revealed only the expected weak correlation between PON1 activity and HDL3, as well as the HDL constituent, apoA-I. PON1 enzyme is physically associated with the HDL3 fraction. However, the additional evaluation of the CAAD case group provided a surprising result, showing strong correlations of PON1 activity with LDL-C and VLDL-C levels and the absence of the expected HDL3 and apoA-I associations. Of further interest, the correlation between PON1 activity and LDL-C and VLDL-C level is positive, whereas low PON1 activity and high LDL-C and VLDL-C levels are predictive of vascular disease. The four PON1 polymorphisms studied here do not appear to impact this joint regulation, inasmuch as adjustment of Arylase for all four PON1 polymorphisms did not reduce the correlations of PON1 activity and lipid-related measures. It is notable that this study, and most studies to date, have focused on only a fraction of the genetic variability in PON1 (11).

Although subjects not on lipid-lowering medications are a select subgroup of the population, it does not appear that limiting the sample to these subjects biased the results. The supplemental analyses of subjects on lipid-lowering medications showed the same trends. The CAAD cases here do represent severe disease, and the controls are disease free, not random with respect to disease, so these subjects represent the tails of the carotid artery disease spectrum. Subjects with intermediate disease would be of interest to study. Similarly, studies of females and non-Caucasians are required.

That the relationship of PON1 and lipid profile is different in CAAD cases versus controls in this report is consistent with prior reports of 44% of variance in Arylase cases and 25% in controls being attributable to these four PON1 functional polymorphisms in Caucasian men (11). That study also found that further adjustment of PON1 activities for age, statin use, current smoking, HDL, HDL2, and HDL3 only narrowed that gap to 47% versus 36%. Clearly, other factors influenced PON1 activity more in cases than in controls. One of these factors may be the coregulation of the PON1 activity with lipoprotein levels. It is possible that normal coregulation of PON1 and apoA-I is lost in the affected subjects.

Context-dependent transcriptional regulation of PON1 and lipoprotein components may be hypothesized to underlie the different correlations in cases and controls. PON1 is reported to be regulated by SREBP-2 (at bp −104 to −95) (32) and SP1 (−111 to −105) binding elements; SP1 includes the $PON1_{-108}$ polymorphism (32, 35, 48, 49). The $PON1_{-162}$ polymorphism is in a putative NF-1 binding site (35), which may result in the small $PON1_{-162}$ effect on HDL3 level observed here. HNF-4 and SP1 sites interact in the regulation of apoA-I (50). LDL-C levels have been shown to be predicted by two different polymorphism in SREBP-2 in hypercholesterolemic but not in normocholesterolemic subjects (51, 52), suggesting that SREBP-2 effects differ depending on LDL level. LDL has been shown to decrease SREBP-2 mRNA levels in cultured cells (53). The presence in the PON1 promoter of DNA elements that may bind SP1 and SREBPs may provide a mechanism linking plasma levels of HDL (apoA-I), LDL, and PON1 enzyme activity.

Alternatively, one could hypothesize that PON1 is associated with non-HDL particles more often in subjects with vascular disease. A model of PON1 secretion from cells to HDL has been proposed (54), and more recently, the ability of cells to secrete PON1 on VLDL as well as on HDL has been show in vitro (55). In this system, VLDL-associated PON1 was quickly transferred to HDL, if available (55). Low levels of PON1 activity were found to be identified with VLDL in human serum, although these were minor in comparison to activity associated with HDL (55). Interestingly, PON1 mass was positively correlated, whereas PON1 activity was negatively correlated with TG level (55). VLDL-associated PON1 differences in cases and controls would not fully explain our observations. First, while the trend was for higher VLDL in cases than in controls, this difference was not statistically significant. Indeed, the reverse nonsignificant trend was seen in the subjects on medications, with higher TG levels in the controls (mean TG level, 207 mg/dl) than in the cases (181 mg/dl), yet the higher VLD-PON1 activity correlation in cases was still seen. Additionally, stratification of both cases and controls not taking lipid-lowering medications into subjects with TGs less than, equal to, or greater than 150 mg/dl did not demonstrate differences in the PON1 activity correlations between TG groups (data not shown), as would be expected if VLDL-associated PON1 accounted for the case-control differences. Second, an increased excretion of PON1 to VLDL would probably not account for the higher PON1 correlation with LDL-C and LDL-B seen in cases, inasmuch as PON1 is rapidly transferred to HDL. Thus, differences in PON1 activity-lipoprotein correlations in cases and controls do not seem likely to be related to differential secretion to VLDL.

In this cohort, the PON1 Arylase activity was the best predictor of the significant PON1 effects on TC, TG, VLDL-C, and LDL-C. This is notable because many studies of vascular risk only determine PON1 genotypes, and inclusion of PON1 activity (particularly DZOase and Arylase activities) is uncommon. With the possible exception of a $PON1_{192}$ effect on HDL-C level, $PON1_{192}$, $PON1_{55}$, $PON1_{-162}$, and $PON1_{-108}$ genotype effects on the lipid traits were not significant. Adjustment of Arylase for the effects of PON1 genotypes generally increased the magnitude of the significant lipid correlations for cases and controls, consistent with a relationship of PON1 activity to lipids that is unrelated to these genotypes. These results suggest that inclusion of Arylase as a measure of PON1 would contribute to our understanding of the role of PON1 in vascular dis-

ease. Direct measurement of PON1 protein levels in large cohorts is limited by cross-reactivity with PON2 and PON3 (unpublished observations).

Consistent with our activity results, a study of diabetics found significant correlations of POase activity with apoA-I in controls, but with apoB in both type 1 and type 2 diabetics with neuropathy (56). Similarly, Arylase but not POase activity was significantly correlated ($P < 0.05$) with HDL-C ($r = 0.31$) and apoA-I ($r = 0.43$) levels in healthy men and women (57). Within $PON1_{192QQ}$ subjects, both Arylase and POase significantly ($P < 0.05$) predicted HDL-C ($r = 0.6, 0.5$) and apoA-I ($r = 0.6, 0.5$) levels. In Chinese males, POase activity was positively correlated with HDL-C and negatively correlated with LDL-C (58).

In contrast to PON1 activity, studies of PON1 genotype effects on lipids do not suggest consistent effects. A study of Hutterites (n = 793) did detect a $PON1_{192}$ effect on HDL-C, LDL-C, apoB, and TG. However, this polymorphism accounted for roughly 1% of the variation in these traits (59). $PON1_{192}$ did not predict HDL-C or apoA-I levels in a smaller Caucasian sample (57). $PON1_{192}$ and $PON1_{55}$ polymorphisms were not consistently associated with lipid or lipoprotein levels in Turkish subjects with and without non-insulin-dependent diabetes mellitus (60). $PON1_{55}$ did not predict lipid levels in Scandinavian subjects (61). $PON1_{55}$ genotype did predict LDL-C variation in two Canadian aboriginal populations, although $PON1_{192}$ genotype did not predict lipid levels (62). $PON1_{192}$ genotype did predict LDL-C and apoB levels in Chinese subjects, although the genotype effects were in opposite directions (58). PON1 promoter region polymorphisms may not have been expected to have an effect on HDL-C levels, inasmuch as PON1 overexpression in mice did not significantly change HDL-C level or density (31); however, that study did not specifically look at the HDL3 level.

In this study, only $PON1_{192}$ genotype was predictive of a lipid trait (HDL-C) and then only in CAAD cases, where the $PON1_{192RR}$ genotype was associated with low HDL-C levels. Although this result is interesting, the rareness of this genotype results in sparse data and, thus, the result may be spurious. With only 6 of the 91 CAAD cases having the $PON1_{192RR}$ genotype, it is not surprising that the Arylase-HDL correlation was not impacted by adjustment for PON1 genotypes.

In conclusion, PON1 activity contributed to the prediction of the genetic architecture of lipid, lipoprotein, and apolipoprotein levels differentially in male Caucasian subjects with severe carotid artery disease and without vascular disease. This suggests that the joint regulation of PON1 with lipoproteins differs in these two groups, offering one explanation for the consistent finding that PON1 activity, but not coding genotypes, predicts vascular disease.

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