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Indications that paraoxonase-1 contributes to plasma high density lipoprotein levels in familial hypercholesterolemia

Thomas M. van Himbergen,[1,*,†] Mark Roest,* Jacqueline de Graaf,† Eugène H. J. M. Jansen,§ Hiroaki Hattori, John J. P. Kastelein,†† Hieronymus A. M. Voorbij,* Anton F. H. Stalenhoef,† and Lambertus J. H. van Tits†**

Research Laboratory of the Department of Clinical Chemistry,* University Medical Center Utrecht, Utrecht, The Netherlands; Department of Medicine,† Division of General Internal Medicine, University Medical Center Nijmegen, Nijmegen, The Netherlands; Laboratory for Toxicology, Pathology, and Genetics,§ National Institute for Public Health and the Environment, Bilthoven, The Netherlands; Department of Advanced Medical Technology and Development,** BML, Inc., Saitama 350-1101, Japan; and Department of Vascular Medicine,†† Academic Medical Center, Amsterdam, The Netherlands

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Abstract HDL-associated paraoxonase type 1 (PON1) can protect LDL and HDL against oxidative modification in vitro and therefore may protect against cardiovascular disease. We investigated the effects of PON1 levels, activity, and genetic variation on high density lipoprotein-cholesterol (HDL-C) levels, circulating oxidized LDL (OxLDL), subclinical inflammation [high-sensitive C-reactive protein (Hs-CRP)], and carotid atherosclerosis. PON1 genotypes (L55M, Q192R, −107C/T, −162A/G, −824G/A, and −907G/C) were determined in 302 patients with familial hypercholesterolemia. PON1 activity was monitored by the hydrolysis rate of paraoxon, diazoxon, and phenyl acetate. PON1 levels, OxLDL, and Hs-CRP were determined using an immunoassay. The genetic variants of PON1 that were associated with high levels and activity of the enzyme were associated with higher HDL-C levels (*P* values for trend: 0.008, 0.020, 0.042, and 0.037 for L55M, Q192R, −107C/T, and −907G/C, respectively). In addition to the PON1 genotype, there was also a positive correlation between PON1 levels and activity and HDL-C (PON1 levels: $r = 0.37$, $P < 0.001$; paraoxonase activity: $r = 0.23$, $P = 0.01$; diazoxonase activity: $r = 0.29$, $P < 0.001$; arylesterase activity: $r = 0.19$, $P = 0.03$). Our observations support the hypothesis that both PON1 levels and activity preserve HDL-C in plasma.—van Himbergen, T. M., M. Roest, J. de Graaf, E. H. J. M. Jansen, H. Hattori, J. J. P. Kastelein, H. A. M. Voorbij, A. F. H. Stalenhoef, and L. J. H. van Tits. **Indications that paraoxonase-1 contributes to plasma high density lipoprotein levels in familial hypercholesterolemia.** ***J. Lipid Res.*** **2005.** 46: **445–451.**

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Serum paraoxonase type 1 (PON1) is a HDL-associated enzyme capable of hydrolyzing lipid peroxides in LDL (1). Because oxidized low density lipoprotein (OxLDL) has atherogenic and proinflammatory properties (2), PON1 may protect against atherosclerosis. This hypothesis is supported by observations in PON1-deficient mice, which are more prone to develop atherosclerosis than wild-type mice when fed a high-fat/high-cholesterol diet (3). An additional antiatherogenic property of PON1 is the inhibition of the oxidative modification of HDL and hence the preservation of HDL function (4). HDL protects against cardiovascular disease (CVD) by means of reverse cholesterol transport (5). Even though the physiological role of PON1 in vivo remains to be clarified, the inhibition of both LDL and HDL oxidation may contribute to protection against CVD.

PON1 expression is partly controlled by its molecular variation at the gene locus (6). Two polymorphic sites have been described in the coding region: a leucine (L)-to-methionine (M) transition at position 55 (L55M) and a glutamine (Q)-to-arginine (R) transition at position 192 (Q192R). The L55M polymorphism affects the enzyme concentration, whereas the Q192R polymorphism affects the catalytic efficiency, but not the concentration (7, 8). Four polymorphisms in the promoter sequence of the PON1 gene (−107C/T, −162A/G, −824G/A, −907G/C) also contribute to the variability in protein expression. During an earlier study, our group found a relation between the PON1 genotype combination LLQQ and increased intima-media thickness (IMT), a surrogate marker of CVD,

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Abbreviations: CCA-IMT, common carotid artery intima-media thickness; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HDL-C, high density lipoprotein-cholesterol; Hs-CRP, high-sensitive C-reactive protein; LDL-C, low density lipoprotein-cholesterol; OxLDL, oxidized low density lipoprotein; PON1, paraoxonase type 1.

[1] To whom correspondence should be addressed.
e-mail: t.m.vanhimbergen@lab.azu.nl

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in high-risk subjects with familial hypercholesterolemia (FH) (9). However, there is no consensus on the contribution of these genetic variants to the risk of CVD (10).

In addition to genetic influences, PON1 levels and activity could be modified by lifestyle determinants such as smoking (11, 12), vitamin C and E consumption (13), and alcohol intake (14). Therefore, studying PON1 levels and activity, in conjunction with variation at the gene level, gives a more complete view of the role of PON1 in the development of atherosclerosis.

The beneficial effects of PON1 on the inhibition of atherosclerosis might be more pronounced in a population that is prone to develop atherosclerosis than the general population. For this reason, we studied the role of PON1 in patients with FH. These patients are characterized by substantially increased serum low density lipoprotein-cholesterol (LDL-C) concentrations and sharply increased CVD risk. Our aim was to investigate the influence of PON1 genotypes and PON1 levels and activity on high density lipoprotein-cholesterol (HDL-C) levels, circulating OxLDL, inflammation markers [high-sensitive C-reactive protein (Hs-CRP)], and common carotid artery intima-media thickness (CCA-IMT) in patients with FH.

METHODS

Subjects

The study population consisted of 325 men and women with FH who participated in a prospective, randomized, double-blind, two-center trial as described elsewhere (15). Of the 325 participants, 23 were excluded because there was no DNA available for PON1 genotyping. After an 8 week placebo run-in (in which all lipid-lowering drugs were discontinued), baseline height, weight, blood pressure, and CCA-IMT were measured. DNA, plasma, and serum samples were stored at −80°C until analysis. Studies have shown that PON1 levels and activity can only be measured correctly in serum samples (16, 17). Because only one trial center collected additional serum for the determination of PON1 levels and activity, these measurements could be performed for only 134 of the 302 participants. All participants gave written informed consent, and the ethics committees of both trial centers approved the study.

Lipids and lipoproteins

Total cholesterol, LDL-C, HDL-C, and triglycerides were analyzed as described previously (18).

Ultrasound measurement of the carotid IMT

Ultrasound scanning of the common carotid arteries (CCAs) was performed using a Biosound Phase-Two real-time scanner (BiosoundEsaote, Indianapolis, IN) equipped with a 10 MHz transducer, as described in detail elsewhere (19). The IMT was measured in both the anterior and posterior walls of the distal 1.0 cm straight part of both CCAs. Images were analyzed using a semiautomatic software program (Eurequa; TSA Co., Meudon, France). A complete set of measurements was available for 288 participants. The CCA-IMT was expressed as the mean of both the anterior and posterior walls of the left and right CCAs.

PON1 genotype

The L55M and Q192R mutations were determined by means of the polymerase chain reaction followed by restriction fragment length polymorphism using primers and restriction enzymes as described by Humbert et al. (8). DNA fragments were separated on a 2% agarose gel and visualized with ethidium bromide. The 55L allele corresponded to the presence of a nondigested 170 bp fragment, the 55M allele to a 44 bp and a 126 bp fragment, the 192Q allele to a nondigested 99 bp fragment, and the 192R allele to a 33 bp and a 66 bp fragment. A PCR product was obtained successfully for 299 (L55M) and 298 (Q192R) DNA samples.

PCR followed by hybridization with allele-specific oligonucleotides was used to analyze the promoter polymorphisms −107C/T, −162A/G, −824G/A, and −907G/C. Primers for the amplification of a 156 bp fragment coding for the polymorphisms at positions −107 and −162 were 5′-GAAAGTGCTGAGCTCCTGCG-3′ and 5′-CTAGGAGGCTCTGCTGCCTG-3′. Primers for the amplification of a 170 bp fragment coding for the polymorphisms at positions −824 and −907 were 5′-ACATGGAGCAAATCATTCA-CAG-3′ and 5′-ACACATAAAGCAAGAAAGGGGA-3′. The PCR products for polymorphisms −107 and −162 and for polymorphisms −824 and −907 were obtained successfully in 301 and 299 DNA samples, respectively. The fragments were transferred onto Hybond N^+ membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK) and hybridized with the allele-specific oligonucleotides. The allele-specific oligonucleotides for the −162 and −907 polymorphisms were as follows: 5′-GCAAGCCACGC-CTTCTGT-3′ (−162A), 5′-GCAAGCCGCGCCTTCTG-3′ (−162G), 5′-AGAGAAGAGAGACATGGTTG-3′ (−907G), and 5′-AGAGAA-GAGACACATGGTTG-3′ (−907C). For positions −107 and −824, we used the same allele-specific oligonucleotides as described by Leviev and James (20). All of the oligonucleotides were hybridized at 42°C, followed by two washes at room temperature and an allele-specific temperature wash.

Analysis of PON1 levels

PON1 levels were determined at the Department of Advanced Medical Technology and Development, BML, Inc. (Saitama, Japan) using an ELISA. This procedure has been described in detail elsewhere (21). PON1 levels were successfully measured in 131 serum samples. The intra-assay and interassay coefficients of variation were 5.5% and 5.1%, respectively.

Analysis of PON1 enzymatic activity

Paraoxonase and diazoxonase activities were analyzed spectrophotometrically at the Laboratory for Toxicology, Pathology, and Genetics of the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) using paraoxon and diazoxon as substrates in a Tris buffer (0.1 M, pH 8.5) containing 2 M NaCl and 2 mM $CaCl_2$ (22). After the addition of the serum sample (diluted 10-fold for paraoxonase and 20-fold for diazoxonase activity), the reaction was monitored on a microtiter plate for 5 min at 25°C. Paraoxonase and diazoxonase activities are expressed as units per liter of serum, where 1 unit equals 1 μmol of substrate hydrolyzed per minute.

Arylesterase activity was measured at the Research Laboratory of the Department of Clinical Chemistry, UMC Utrecht (Utrecht, The Netherlands) as the rate of hydrolysis of phenyl acetate into phenol. This process can be detected spectrophotometrically (23). Serum samples were prepared in sample buffer consisting of 20 mM Tris and 0.9 mM $CaCl_2$ (pH 8.0) in a 40-fold dilution. Five microliters of diluted serum was then added to 200 μl of freshly made substrate buffer containing 1 mM phenyl acetate, 20 mM Tris, and 0.9 mM $CaCl_2$ (pH 8.0). The reaction was monitored on a microtiter plate at 260 nm in a Fluostar microplate reader (BMG Labtech GmbH, Offenburg, Germany) at 37°C. The nonenzymatic hydrolysis of phenyl acetate, based on the hydrolysis rate in the absence of serum, was subtracted from the to-

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TABLE 1. Baseline characteristics of the FH population (n = 302)

Characteristics	Values
Sex (male/female)	121/181
Smoking (current/past/non)	98/87/117
Body mass index (kg/m^2)	25.7 ± 3.5
Systolic blood pressure (mm Hg)	130.7 ± 15.9
Diastolic blood pressure (mm Hg)	79.0 ± 7.9
Total cholesterol (mmol/l)	10.14 ± 1.99
HDL-C (mmol/l)	1.16 ± 0.31
LDL-C (mmol/l)	8.19 ± 1.95
Triglycerides (mmol/l)	1.86 ± 1.22

FH, familial hypercholesterolemia; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. Values are means ± SD, except for sex, which represents the number of males and females, and smoking, which represents the number of current smokers, past smokers, and nonsmokers.

tal rate of hydrolysis. The molar extinction coefficient used to calculate the rate of hydrolysis was 1,310 M^{-1} cm^{-1}. A pathlength correction was applied for the use of microtiter plates. Arylesterase is expressed as units per milliliter of serum, where 1 unit equals 1 μmol of phenyl acetate hydrolyzed per minute.

OxLDL ELISA

We determined the concentrations of OxLDL in EDTA-plasma samples that had been supplemented with saccharose and frozen at −80°C and not thawed before use. Samples from 110 patients fulfilled these criteria. We used a commercially available noncompetitive ELISA (Mercodia, Uppsala, Sweden). The intra-assay and interassay coefficients of variation were 6% and 7%, respectively.

Hs-CRP

Hs-CRP was measured by enzyme immunoassay according to the instructions from the manufacturer (Dako, Glastrup, Denmark). The coefficient of variation was 6%.

Statistical analysis

The relations between PON1 genotype, PON1 levels, PON1 activity, HDL-C levels, OxLDL, Hs-CRP, and CCA-IMT were tested in a linear regression model in which PON1 genotype served as the independent variable and the PON1 levels, PON1 activity, HDL-C levels, OxLDL, Hs-CRP, and CCA-IMT served as the dependent variables. The influence of age and gender on this relationship was investigated in a multivariate regression analysis. The Pearson correlation coefficient (r) was used to test the relationship between the variables displayed in Tables 4, 5, and 7. Hs-CRP had a skewed distribution, so the presentation in the tables and statistical analysis were based on log-transformed data. All of the analyses were performed using SPSS version 11.5.

RESULTS

The study population consisted of 121 men and 181 women with FH (**Table 1**). The mean age was 48 years; 98 of the participants were current smokers, 87 were past smokers, and 117 were nonsmokers. The subjects were slightly overweight but had normal blood pressure. Levels of triglycerides and HDL-C were normal. The average LDL-C level was higher than 8 mmol/l, which is characteristic for FH.

Table 2 shows the relationships between the PON1 genotypes (L55M, Q192R, −107C/T, −162A/G, −824G/A, and −907G/C) and PON1 levels and activities. All PON1 genotype distributions were in Hardy-Weinberg equilibrium. The 55L variant of the L55M polymorphism contributed to increased PON1 levels ($P < 0.001$) and increased paraoxonase, diazoxonase, and arylesterase activities ($P < 0.001$, $P = 0.018$, and $P = 0.029$, respectively). The 192R isoform of the Q192R polymorphism hydrolyzed paraoxon

TABLE 2. Relationships between PON1 genotypes and PON1 levels and activity

PON1 Genotype	PON1 Levels	n	P	Paraoxonase Activity	n	P	Diazoxonase Activity	n	P	Arylesterase Activity	n	P
	μg/ml			*U/l*			*U/l*			*U/ml*		
L55M												
LL	87.3 ± 22.4	58		792.6 ± 351.8	59		7,561.1 ± 2,173.2	59		76.4 ± 31.9	59	
LM	72.1 ± 18.6	57	<0.001	536.2 ± 282.8	57	<0.001	6,840.7 ± 1,630.9	57	0.018	69.6 ± 25.2	57	0.029
MM	61.9 ± 16.8	15		190.5 ± 44.4	17		6,563.9 ± 1,253.9	17		58.9 ± 32.8	17	
Q192R												
QQ	74.2 ± 23.7	50		246.5 ± 79.0	52		7,957.9 ± 1,881.1	52		70.5 ± 29.4	52	
QR	79.3 ± 21.1	66	0.130	764.5 ± 206.9	66	<0.001	6,902.1 ± 1,643.8	66	<0.001	72.3 ± 30.6	66	0.867
RR	82.9 ± 20.1	13		1,184.9 ± 245.1	14		5,083.4 ± 1,088.5	14		70.7 ± 28.6	14	
−107C/T												
CC	92.1 ± 21.6	38		771.7 ± 398.4	38		8,267.1 ± 1,854.1	38		85.9 ± 31.3	38	
CT	75.7 ± 17.1	61	<0.001	594.6 ± 320.9	63	<0.001	6,912.2 ± 1,588.2	63	<0.001	65.6 ± 25.0	63	0.001
TT	61.3 ± 14.5	31		421.2 ± 296.0	32		6,004.6 ± 1,404.1	32		63.5 ± 29.4	32	
−162A/G												
AA	101.8 ± 20.8	7		791.0 ± 483.4	7		8,657.3 ± 1,444.2	7		103.5 ± 29.7	7	
GA	84.7 ± 20.9	46	<0.001	595.8 ± 349.0	46	0.359	8,151.2 ± 1,736.4	46	<0.001	74.3 ± 31.3	46	0.002
GG	70.2 ± 18.1	77		591.4 ± 355.2	80		6,327.5 ± 1,481.2	80		66.1 ± 26.3	80	
−824G/A												
GG	68.9 ± 18.4	57		554.7 ± 367.9	60		6,256.0 ± 1,389.8	60		64.4 ± 28.5	60	
GA	81.1 ± 20.9	60	<0.001	626.5 ± 342.9	60	0.099	7,567.8 ± 1,926.1	60	<0.001	72.0 ± 28.4	60	0.001
AA	93.9 ± 19.6	13		722.1 ± 390.7	13		8,641.2 ± 1,206.6	13		95.9 ± 25.4	13	
−907G/C												
GG	91.6 ± 20.8	30		750.8 ± 398.0	30		8,072.7 ± 1,991.9	30		85.9 ± 29.5	30	
CG	77.3 ± 17.9	63	<0.001	605.3 ± 321.9	65	0.002	7,324.3 ± 1,695.8	65	<0.001	70.1 ± 29.5	65	<0.001
CC	64.9 ± 19.3	37		484.1 ± 356.3	38		5,881.6 ± 1,161.3	38		60.3 ± 28.5	38	

PON1, paraoxonase type 1. Values represent means ± SD, followed by the number of observations (n). P values are based on linear regression.

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more efficiently, whereas the 192Q isoform hydrolyzed diazoxon more efficiently ($P < 0.001$ for both relations). The ratio of paraoxon and diazoxon hydrolyzation segregates with the Q192R polymorphism: there was 99.2% concordance between the paraoxon/diazoxon ratio and the Q192R genotypes. The Q192R polymorphism was not associated with PON1 levels or with arylesterase activity.

The −107C, −162A, −824A, and −907G variants of the −107C/T, −162A/G, −824G/A, and −907G/C polymorphisms in the promoter sequence expressed higher PON1 levels, diazoxonase activity, and arylesterase activity than the −107T, −162G, −824G, and −907C variants ($P < 0.001$ for all relations except arylesterase activity; the latter has $P = 0.001$ for −107C/T and −824G/A and $P = 0.002$ for −162A/G). The hydrolysis rate of paraoxon was significantly higher for the −107CC and −907GG homozygotes than for the other genetic variants at positions −107 and −907 ($P < 0.001$ and $P = 0.002$, respectively). The ratio of paraoxonase/PON1 levels did not differ among the variants of these polymorphisms (data not shown). Furthermore, the −162A/G and −824G/A polymorphisms were not associated with paraoxonase activity. Finally, there was a strong correlation between PON1 levels and PON1 arylesterase ($r = 0.52$, $P < 0.001$) and PON1 diazoxonase ($r = 0.69$, $P < 0.001$) activities.

The contribution of PON1 genotypes to HDL-C levels is presented in **Table 3**. The 55L, 192R, −107C, and −907G variants of the L55M, Q192R, −107C/T, and −907G/C polymorphisms predicted increased HDL-C levels ($P = 0.008$, $P = 0.020$, $P = 0.042$, and $P = 0.037$, respectively). After adjustment for age and gender, the relation between PON1 genotypes and HDL-C levels remained significant. The −162A/G and −824G/A polymorphisms were not associated with HDL-C levels.

Like the PON1 genotypes, PON1 levels and paraoxonase, diazoxonase, and arylesterase activities also contributed to increased HDL-C levels ($r = 0.37$, $P < 0.001$; $r = 0.23$, $P = 0.008$; $r = 0.29$, $P = 0.001$; and $r = 0.19$, $P = 0.031$, respectively; **Table 4**).

Thus, we have demonstrated an association of PON1 genotypes and intermediate phenotypes with plasma HDL cholesterol. Next, we investigated the relation with circulating OxLDL and with Hs-CRP. We observed no significant association of PON1 genotypes with OxLDL or with Hs-CRP (data not shown). As presented in **Table 5**, PON1 levels and activity also did not correlate with OxLDL levels. Furthermore, we observed only weak but significant correlations of diazoxonase and arylesterase activities with Hs-CRP (Table 5).

Hence, we found remarkable associations of PON1 genotypes, PON1 levels, and PON1 activity with HDL-C levels but not with circulating OxLDL and Hs-CRP. We also investigated the relation of PON1 genotypes and intermediate phenotypes with CCA-IMT. We found no significant relation between PON1 genotypes and CCA-IMT (**Table 6**) and no correlation of PON1 levels and PON1 activities with CCA-IMT (**Table 7**). Moreover, HDL-C, OxLDL, and Hs-CRP did not correlate with CCA-IMT.

TABLE 3. HDL-C levels according to PON1 genotypes

PON1 Genotype	HDL-C Levels	n	*P* Crude	*P* Adjusted
	mmol/l			
L55M				
LL	1.20 ± 0.31	137		
LM	1.15 ± 0.30	133	0.008	0.001
MM	1.03 ± 0.25	29		
Q192R				
QQ	1.11 ± 0.27	120		
QR	1.18 ± 0.31	149	0.020	0.033
RR	1.24 ± 0.38	29		
−107C/T				
CC	1.21 ± 0.31	88		
CT	1.15 ± 0.30	147	0.042	0.036
TT	1.11 ± 0.30	66		
−162A/G				
AA	1.11 ± 0.31	16		
AG	1.20 ± 0.30	101	0.482	0.354
GG	1.14 ± 0.31	184		
−824G/A				
GG	1.13 ± 0.31	144		
GA	1.19 ± 0.31	123	0.103	0.087
AA	1.19 ± 0.29	32		
−907G/C				
GG	1.19 ± 0.30	73		
GC	1.18 ± 0.30	142	0.037	0.024
CC	1.09 ± 0.31	84		

Values represent means ± SD, followed by the number of observations (n). Crude *P* values are based on linear regression. Adjusted *P* values are based on linear regression and are adjusted for age and gender.

TABLE 4. HDL-C levels with regard to PON1 levels and activity

Variable	HDL-C Levels		
	r	*P*	n
PON1 levels (μg/ml)	0.37	<0.001	131
Paraoxonase activity (U/l)	0.23	0.008	134
Diazoxonase activity (U/l)	0.29	0.001	134
Arylesterase activity (U/ml)	0.19	0.031	134

Values represent Pearson correlation coefficient (*r*), followed by the *P* value and the number of observations (n).

DISCUSSION

Previous investigations have demonstrated that high PON1 levels and high PON1 activity contribute to in-

TABLE 5. OxLDL levels and Hs-CRP with regard to PON1 levels and activities

Variable	OxLDL			Hs-CRP		
	r	*P*	n	*r*	*P*	n
PON1 levels (μg/ml)	0.002	0.98	107	0.16	0.06	129
Paraoxonase activity (U/l)	0.054	0.58	110	0.06	0.53	132
Diazoxonase activity (U/l)	−0.020	0.83	110	0.20	0.02	132
Arylesterase activity (U/ml)	−0.028	0.77	110	0.20	0.02	132

Hs-CRP, high-sensitive C-reactive protein; OxLDL, oxidized low density lipoprotein. Values represent Pearson correlation coefficient (*r*), followed by the *P* value and the number of observations (n).

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TABLE 6. CCA-IMT according to PON1 genotype

PON1 Genotype	CCA-IMT	n	P Trend
	mm		
L55M			
LL	0.86 ± 0.16	132	
LM	0.87 ± 0.17	126	0.38
MM	0.89 ± 0.23	28	
Q192R			
QQ	0.88 ± 0.19	110	
QR	0.85 ± 0.16	146	0.35
RR	0.90 ± 0.17	29	
−107C/T			
CC	0.85 ± 0.14	85	
CT	0.86 ± 0.18	139	0.13
TT	0.89 ± 0.18	63	
−162A/G			
AA	0.85 ± 0.14	15	
GA	0.87 ± 0.16	96	0.99
GG	0.87 ± 0.18	176	
−824G/A			
GG	0.88 ± 0.18	137	
GA	0.86 ± 0.16	118	0.44
AA	0.85 ± 0.17	30	
−907G/C			
GG	0.85 ± 0.15	70	
GC	0.86 ± 0.16	134	0.11
CC	0.90 ± 0.20	81	

CCA-IMT, common carotid artery intima-media thickness. Values represent means ± SD, followed by the number of observations (n). Trend *P* values are based on linear regression analysis and adjusted for age.

creased levels of HDL-C (17, 24, 25). The results of the present study reinforce and expand these findings by showing that not only high PON1 levels and high PON1 activity, but also the genotypes associated with high PON1 levels (55L, −107C, and −907C variants) and high PON1 activity (192R variants), were associated with the highest HDL-C levels. These findings support the hypothesis that PON1 is involved in the preservation of HDL (4). We speculate that HDL-associated LCAT may play an important role in this process. Recently, it was shown that in mice overexpressing PON1, LCAT activity is preserved (26). Activated LCAT prevents the catabolism of HDL and therefore may increase HDL-C (27, 28). Elucidating this mechanism is a subject for future studies.

In large cohort studies, it was established that increased HDL-C levels protect against CVD (29). Remarkably, several studies, including ours, did not observe a significant relation between HDL-C and CCA-IMT (30–33). Apparently, HDL-C has only minor effects on CCA-IMT. Similar to HDL-C, the contribution of paraoxonase to IMT is debatable. Some studies have reported a significant association between PON1 and IMT in FH patients (9), in middle-aged women (34), and in a subgroup of nonsmokers (35), but there are also a number of reports, including the current one, that did not find an association between PON1 and IMT (30, 36–38). These differences in outcome suggest that there is only a weak relation between PON1 and IMT that will not be observed in many small studies that lack the power to detect weak effects. Alternatively, PON1 does not play a role in early atherosclerosis (of which IMT is a measurement) but may play a role in a later stage of CVD. As suggested by Cao et al. (36), the lack of association of PON1 with IMT does not exclude the possibility of its involvement in the later phases of the atherothrombotic process. Therefore, we recommend that future studies on PON1 should be carried out in populations with clinical end points.

TABLE 7. CCA-IMT with regard to PON1 levels, PON1 activities, HDL-C, OxLDL, and Hs-CRP

Variable	CCA-IMT *r*	*P*	n
PON1 levels (μg/ml)	−0.04	0.67	130
Paraoxonase activity (U/l)	−0.03	0.75	129
Diazoxonase activity (U/l)	−0.06	0.49	129
Arylesterase activity (U/ml)	−0.10	0.28	129
HDL-C (mmol/l)	−0.09	0.11	288
OxLDL (U/l)	−0.03	0.79	110
Hs-CRP (mg/l)	0.04	0.62	152

Values represent Pearson correlation coefficient (*r*), followed by the *P* value and the number of observations (n).

Apart from preserving the function of HDL, PON1 may beneficially influence atherogenesis via inhibition of LDL oxidation (39–41), in that way preventing excessive cholesterol uptake by macrophages and inhibiting foam cell formation (42). Furthermore, inhibition of LDL oxidation may control inflammation in the arterial wall (43). Both LDL oxidation and inflammation are associated with an increased risk for CVD (44, 45). We studied the relation between PON1 and circulating OxLDL as a marker for LDL oxidation and Hs-CRP as a marker for inflammation and could not establish that PON1 is involved in the process of atherogenesis via the inhibition of LDL oxidation and later the induction of inflammation. Some caution is required, because the effect of PON1 on circulating OxLDL and Hs-CRP needs to be confirmed in different populations.

Most studies on the role of PON1 and CVD are restricted to PON1 genotypes. However, additional measurements of PON1 serum levels and activities give a more complete view of PON1 status because they reflect the combined genetic and environmental effects of PON1. In the present study, PON1 status was determined using different genotypes (L55M, Q192R, −107C/T, −162A/G, −824G/A, and −907G/C), PON1 levels, and activity toward three different substrates (paraoxon, diazoxon, and phenyl acetate). The PON1 genotypes were determined in all subjects, but PON1 serum level and activity measurements were restricted to 134 of the 302 subjects. Fortunately, the power was still sufficient to establish a significant relation between PON1 genotypes and PON1 levels and activity. The associations between the two coding and four promoter region polymorphisms and PON1 levels and activity are in agreement with previous reports: the high-expression alleles 55L, −107C, −162A, −824A, and −907G contributed to the highest PON1 levels (7, 20, 46). Furthermore, as described in the literature, the Q192R polymorphism was responsible for the difference in the hydrolysis rates toward paraoxon and diazoxon but not to-

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ward phenyl acetate (47). Both the activity measurements and the Q192R genotyping were reliable, because there was 99.2% agreement between PON1 phenotyping and the ratio between the hydrolysis rate of paraoxon and diazoxon and Q192R genotyping. However, the physiological substrate of PON1 is unknown, and measurements of PON1 activity by monitoring the rates of hydrolysis of phenyl acetate, paraoxon, and diazoxon may not quantify the degree of antiatherogenic potential of PON1 and should be regarded as surrogate markers of PON1 protective activity. In this study, PON1 levels and PON1 activities were measured independently at three different laboratories. There was still a strong correlation among the PON1 levels assessed directly with ELISA and the PON1 activity measurements. Therefore, PON1 activity measurements qualify as suitable surrogate markers for PON1 levels in serum.

We believe that the chance of finding effects of PON1 on CVD increased in a population at high risk for developing atherosclerosis and CVD. For this reason, we investigated the effects of PON1 in a population of patients with FH. These patients have increased lipid levels and present a lipid environment that is different from that of the population at large. Results obtained from such a population may not be extrapolated to the general population.

In summary, PON1 is involved in the preservation of HDL-C in plasma and therefore may protect against CVD. The genetic variants associated with high PON1 levels and activity and the high plasma levels and increased activity of PON1 predicted the highest levels of HDL-C. Neither PON1 nor HDL-C was associated with CCA-IMT. Further studies are required to assess the relevance of the HDL-C-preserving activity of PON1 in trials with clinical end points.

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