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Original Article

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The Association of the Cholesterol Efflux Capacity with the *Paraoxonase 1* Q192R Genotype and the Paraoxonase Activity

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Aims: Paraoxonase 1 (PON1) binds to high-density lipoprotein (HDL) and protects against atherosclerosis. However, the relationship between functional *PON1* Q192R polymorphism, which is associated with the hydrolysis of paraoxon (POXase activity) and atherosclerotic cardiovascular disease (ASCVD), remains controversial. As the effect of *PON1* Q192R polymorphism on the HDL function is unclear, we investigated the relationship between this polymorphism and the cholesterol efflux capacity (CEC), one of the biological functions of HDL, in association with the PON1 activity.

Methods: The relationship between *PON1* Q192R polymorphisms and CEC was investigated retrospectively in 150 subjects without ASCVD (50 with the *PON1* Q/Q genotype, 50 with the Q/R genotype, and 50 with the R/R genotype) who participated in a health screening program. The POXase and arylesterase (AREase: hydrolysis of aromatic esters) activities were used as measures of the PON1 activity.

Results: The AREase activity was positively correlated with CEC independent of the HDL cholesterol levels. When stratified by the *PON1* Q192R genotype, the POXase activity was also positively correlated with CEC independent of HDL cholesterol. *PON1* Q192R R/R genotype carriers had a lower CEC than Q/Q or Q/R genotype carriers, despite having a higher POXase activity. Moreover, in a multiple regression analysis, the *PON1* Q192R genotype was associated with the degree of CEC, independent of the HDL cholesterol and POXase activity.

Conclusions: The *PON1* Q192R R allele is associated with reduced CEC in Japanese people without ASCVD. Further studies on the impact of this association on the severity of atherosclerosis and ASCVD development are thus called for.

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Key words: Paraoxonase, High-density lipoprotein, Cholesterol efflux capacity, Arylesterase, Polymorphism

Introduction

It has been reported that the High-density lipoprotein cholesterol (HDL-C) levels are not causally related to the development of atherosclerotic cardiovascular disease (ASCVD)[1, 2]. Epidemiological studies have shown that extremely high HDL-C levels increase the risk of ASCVD and all-cause mortality[3-5], while Mendelian randomization studies have shown a negligible relationship between low HDL-C levels and the development of ASCVD[6, 7]. A meta-analysis of randomized controlled trials using niacin, fibrate, and cholesterol ester transfer protein (CETP) inhibitors under statin treatment showed that increasing HDL-C

levels did not improve the cardiovascular outcomes[8]. Therefore, in recent years, many studies have focused on HDL functionality rather than HDL-C to examine the beneficial effects of HDL on ASCVD[1, 2].

HDL shows various anti-atherogenic functions, including reverse cholesterol transport and antioxidant, anti-inflammatory, anti-apoptotic, and vasodilatory effects[2]. In particular, reverse cholesterol transport from peripheral cells to the liver is important, and cholesterol efflux from macrophages, the first step in this process, is thought to inhibit intracellular cholesterol accumulation and thereby contribute to the prevention of ASCVD[2]. A previous cohort study showed that the cholesterol efflux capacity (CEC), but not HDL-C, can predict ASCVD incidence; this relationship remained significant after adjusting for traditional cardiovascular risk factors and HDL-C[9]. Moreover, a meta-analysis of 20 clinical studies demonstrated an inverse relationship between CEC and the risk of adverse cardiovascular events or ASCVD[10].

Paraoxonase 1 (PON1) is carried on HDL and it is a multifunctional enzyme with paraoxonase (POXase: hydrolysis of paraoxon), arylesterase (AREase: hydrolysis of aromatic esters), lactonase and diazoxonase activities[11]. The anti-atherosclerotic effects of HDL, particularly its antioxidant, anti-inflammatory, and CEC activities, are largely attributable to PON1 [12]. A close relationship between *PON1* gene deficiency and an accelerated progression of arteriosclerosis has been found in animal models[13, 14], and a meta-analysis based on 43 clinical studies showed that decreased POXase and AREase activities are associated with an increased susceptibility to coronary heart disease[15]. Our recent human study speculated that the oxidative modification of HDL by aldehydes leads to decreased CEC[16]. Therefore, identifying the factors involved in the oxidative modification of HDL and subsequent reduction of CEC may allow us to identify new methods for the prevention or treatment of ASCV[2, 10, 17].

PON1 is a highly polymorphic gene with over 400 single nucleotide polymorphisms[18]. The most thoroughly studied polymorphism is Q192R (rs662), in which the R allele is associated with an increased POXase activity, but not AREase[18]. Previous epidemiological studies have shown that the *PON1* R allele with high POXase activity is associated with a decreased risk of ASCVD. Conversely, several studies have shown that this allele is associated with an increased risk of ASCVD[18-21]. It has been suggested that this discrepancy in the results may be influenced by differences in the subjects' background (*e.g.*, pathology, race, age, gender and lifestyle)[18-21]. No previous studies have shown an association between *PON1* Q192R polymorphism and CEC in Japanese people. Therefore, in this study, we examined the association between *PON1* Q192R polymorphism and CEC in Japanese subjects without ASCVD, matched for age and sex between the *PON1* Q192R genotypes, while taking into account the effects of the POXase and AREase activities.

Methods

Study Subjects

We retrospectively investigated 865 subjects who had participated in a health screening program at the Japanese Red Cross Kumamoto Health Care Center. Since *PON1* Q192R polymorphism affects POXase activity[18], 50 subjects with the *PON1* Q/Q genotype, 50 subjects with the Q/R genotype, and 50 subjects with the R/R genotype were randomly selected from the 865 subjects, each genotype being age- and sex-matched. The research protocol was approved by the Ethics Committee of the Faculty of Life Science, Kumamoto University, and the Japanese Red Cross Kumamoto Medical Centre, and the study complied with the principles of the Declaration of Helsinki. All participants provided their written informed consent prior to participation in the study. The sample size of the associations of CEC with *PON1* Q192R genotype at a significance (alpha) level of 0.05 (two-tailed) using the expected effect size based on the findings from a previous study[16]. A power analysis estimated that at least 144 subjects (*i.e.*, 48 subjects for each genotype) would be needed to detect any changes in high or low CEC due to differences in the genotypes, the power of which was 81.9%, thereby exceeding the required power limit (*i.e.*, 80%); therefore, we included 150 subjects in the present study.

Measurements of CEC

Plasma CEC was measured as previously reported[16, 22, 23]. To avoid the influence of other lipids, such as low-density lipoprotein (LDL) cholesterol, ApoB was removed by adding polyethylene glycol to the subjects' plasma. The ApoB-deficient plasma was

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then added to incubated J774 murine cells and 0.33 μCi of [1,2-H^3]-cholesterol. The efflux of radioactive cholesterol from the cells was quantified using liquid scintillation counting. To correct for plate-to-plate variation, pooled control plasma from 11 healthy volunteers was included in the measurement of CEC, and CEC in the study subjects was standardized against the CEC of the pooled control plasma (set to 100).

Measurements of POXase and AREase Activities

Paraoxon and phenyl acetate were used as substrates for the POXase and AREase activity, respectively. Human serum was diluted 20-fold and 670-fold with 50 mM Tris-buffered saline (pH 8.0) containing 1 mM $CaCl_2$ to measure POXase and AREase activities, respectively. Paroxon and phenyl acetate were dissolved in tris-buffered saline and methanol, respectively. The diluted serum was pre-incubated at 37℃ for 5 min, and the reaction was initiated by adding an equal volume of paraoxon solution (final concentration, 1 mM) or phenyl acetate dissolved in methanol (final concentration, 2.6 mM) at 37℃. The final concentration of methanol was maintained at 0.1%, which had no effect on the enzymatic reactions. The formation of *p*-nitrophenol and phenol was spectrophotometrically determined by the initial linear increase in absorbance at 405 and 275 nm, respectively (V-630; Jasco International Co. Ltd., Tokyo, Japan).

Genotyping

Genomic DNA was extracted from whole blood using a DNA purification kit (Flexi Gene DNA kit; QIAGEN, Hilden, Germany) and the *PON1* Q192R (rs662) genotype was detected using a real-time TaqMan allelic discrimination assay (assay no. C_2548962_20). For quality assurance, positive (samples with known genotypes) and negative (water) controls were included in the genotyping assay.

Statistical Analyses

Data are expressed as the mean ± standard deviation, median (range), or number (%) for categorical variables. One-way ANOVA or the Kruskal–Wallis test was used to compare continuous variables between the groups, the chi-squared test was used to compare categorical variables, and a Pearson correlation analysis was used to detect any correlations between two continuous variables. In the multivariable analysis of covariates related to CEC, adjusted standardized partial regression coefficients (β), unstandardized partial regression coefficients (B), and standard errors (SE) were calculated by a multiple regression analysis using a stepwise method. All statistical analyses were performed using the SPSS software package (version 28.0, IBM Japan Inc., Tokyo, Japan), and a P value <0.05 was defined as statistically significant.

Results

Subject Characteristics

The clinical characteristics of the study participants are presented in **Table 1**. CEC and POXase activity differed among *PON1* Q192R genotypes, but other clinical parameters and frequencies did not differ. Importantly, there were no differences in drinking and smoking habits between the genotypes, which have been reported to potentially affect CEC[16, 24].

Correlations of HDL-C with CEC, POXase and AREase Activities

The correlations between HDL-C and CEC, POXase, and AREase activities are shown in **Supplemental Fig. 1**. Overall, moderate correlations of HDL-C with CEC and AREase were observed, and stratified analyses by *PON1* Q192R genotype showed moderate correlations of HDL-C with CEC, POXase, and AREase activities in each genotype **(Supplemental Fig. 1)**.

Correlation between the POXase and AREase Activities

The correlation between the POXase and AREase activities is shown in **Supplemental Fig. 2**. A weak overall correlation between the POXase and AREase activities was observed; however, a stratified analysis according to the *PON1* Q192R genotype showed strong correlations between the POXase and AREase activities in each individual genotype **(Supplemental Fig. 2)**.

Correlations of CEC with POXase and AREase Activities

The correlations between CEC and the POXase and AREase activities are shown in **Fig. 1 and 2**, respectively. Although there was no overall correlation between CEC and the POXase activity **(Fig. 1)**, a stratified analysis revealed a positive correlation between CEC and the POXase activity in each individual *PON1* Q192R genotype **(Fig. 1)**. In contrast, the overall AREase activity was correlated with CEC, and similar correlations were found in a stratified analysis with the individual *PON1* Q192R genotypes **(Fig. 2)**. Moreover, we analyzed the correlation between CEC and the POXase/AREase

Table 1. Clinical characteristics of the subjects

	All subjects ($n=150$)	*PON1* Q192R genotype			
		Q/Q ($n=50$)	Q/R ($n=50$)	R/R ($n=50$)	*P*
Age (years)	56.5 ± 7.3	56.5 ± 9.0	56.3 ± 6.2	56.7 ± 6.5	0.973
Female (%)	78 (52.0)	26 (52.0)	26 (52.0)	26 (52.0)	0.999 [a]
CEC	87 (49-125)	89 (52-117)	89 (57-111)	78.5 (49-125)	0.020 [b]
POXase activity (nmol/mL/min)	206.6 (31.4-584.5)	96.0 (31.4-143.4)	214.3 (92.0-348.9)	346.3 (95.1-584.5)	< 0.001 [b]
AREase activity (nmol/mL/min)	136.8 (32.5-224.0)	139.2 (42.4-224.0)	135.7 (54.3-192.4)	129.3 (32.5-193.3)	0.450 [b]
BMI (kg/m^2)	22.9 ± 2.6	22.8 ± 2.6	23.1 ± 2.5	23.1 ± 2.8	0.831
HDL-C (mg/dL)	68.4 ± 16.7	71.1 ± 19.0	67.5 ± 13.4	66.7 ± 17.2	0.379
LDL-C (mg/dL)	125.1 ± 25.4	121.1 ± 29.5	123.5 ± 25.8	130.7 ± 19.6	0.146
TGs (mg/dL)	96 (33-520)	92 (33-520)	95 (33-393)	100 (38-202)	0.835 [b]
FBG (mg/dL)	94 (68-244)	95 (77-244)	93.5 (77-118)	93 (68-121)	0.412 [b]
AST (U/L)	24.1 ± 6.7	24.5 ± 8.2	23.4 ± 5.5	24.3 ± 6.0	0.682
ALT (U/L)	23.4 ± 11.4	25.9 ± 15.1	21.9 ± 8.1	22.5 ± 9.7	0.169
GGT (U/L)	26 (8-205)	25.5 (11-205)	23 (8-189)	28.5 (9-168)	0.931 [b]
Albumin (g/dL)	4.58 ± 0.20	4.62 ± 0.21	4.55 ± 0.18	4.56 ± 1.76	0.165
hs-CRP (mg/L)	0.05 (0.00-0.70)	0.065 (0.00-0.57)	0.045 (0.01-0.30)	0.04 (0.00-0.70)	0.549 [b]
UA (mg/dL)	5.30 ± 1.52	5.01 ± 1.45	5.32 ± 1.60	5.56 ± 1.49	0.185
eGFR (ml/min/1.73m^2)	74.1 ± 12.4	76.3 ± 11.9	72.8 ± 11.5	73.2 ± 13.6	0.298
Alcohol intake (g/day)	14.3 ± 21.9	17.2 ± 21.7	12.7 ± 21.1	12.9 ± 23.1	0.516
Smoking status					
Never (%)	98 (65.3)	33 (66.0)	31 (62.0)	34 (68.0)	0.828
Ex (%)	33 (22.0)	9 (18.0)	13 (26.0)	11 (22.0)	
Current (%)	19 (12.7)	8 (16.0)	6 (12.0)	5 (10.0)	

Values are the means ± standard deviation, median (range) or number of subjects (%).
[a] Chi-squared test. [b] Kruskal-Wallis test (otherwise, One-way ANOVA was used).
PON1, paraoxonase 1 (gene); CEC, cholesterol efflux capacity; POXase, paraoxonase (activity); AREase, arylesterase; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol: TGs, triglycerides; FBG, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; hs-CRP, high-sensitive C-reactive protein; UA, uric acid; eGFR, estimated glomerular filtration rate.

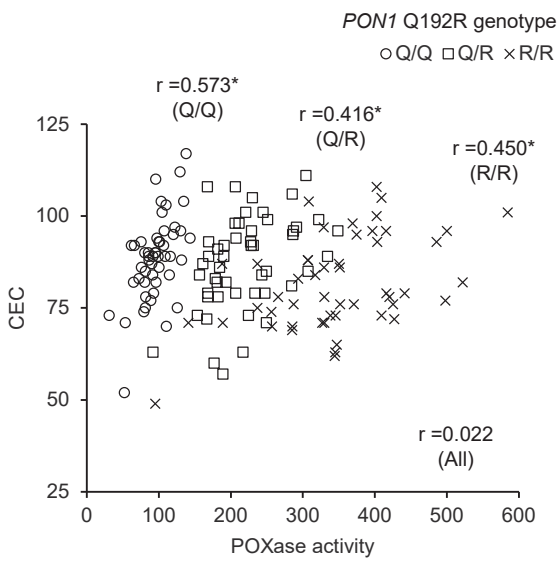

Fig. 1. The correlation between CEC and the POXase activity

CEC, cholesterol efflux capacity; POXase, paraoxonase (activity); *PON1*, paraoxonase 1 (gene); r, correlation coefficient. *$P<0.05$.

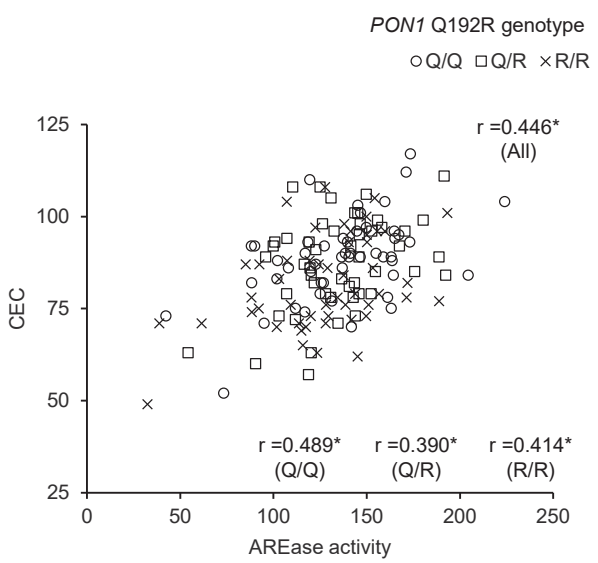

Fig. 2. The correlation between CEC and the AREase activity

CEC, cholesterol efflux capacity; AREase, arylesterase; *PON1*, paraoxonase 1 (gene); r, correlation coefficient. *$P<0.05$.

Table 2. The association of CEC with HDL-C, POXase and AREase activities stratified by *PON1* Q192R genotype using a simple or multiple regression analysis

	Simple regression analysis				Multiple regression analysis			
	β	B	SE	*P*	β	B	SE	*P*
All subjects ($n=150$)								
HDL-C	0.525	0.407	0.054	<0.001	0.427	0.331	0.054	<0.001
POXase activity	0.055	0.006	0.009	0.506	-	-	-	-
AREase activity	0.444	0.187	0.031	<0.001	0.308	0.130	0.029	<0.001
Q/Q genotype carriers ($n=50$)								
HDL-C	0.516	0.311	0.075	<0.001	0.297	0.180	0.080	0.030
POXase activity	0.573	0.279	0.058	<0.001	0.418	0.203	0.065	0.003
AREase activity	0.489	0.172	0.044	<0.001	-	-	-	-
Q/R genotype carriers ($n=50$)								
HDL-C	0.434	0.412	0.123	0.002	0.339	0.322	0.123	0.012
POXase activity	0.416	0.098	0.031	0.003	0.313	0.074	0.031	0.020
AREase activity	0.390	0.181	0.061	0.005	-	-	-	-
R/R genotype carriers ($n=50$)								
HDL-C	0.633	0.466	0.082	<0.001	0.540	0.398	0.086	<0.001
POXase activity	0.450	0.060	0.017	0.001	0.246	0.033	0.016	0.040
AREase activity	0.414	0.160	0.051	0.003	-	-	-	-

CEC, cholesterol efflux capacity; POXase, paraoxonase (activity); AREase, arylesterase; *PON1*, paraoxonase 1 (gene); HDL-C, high-density lipoprotein cholesterol; β, standardized partial regression coefficient; B, unstandardized partial regression coefficient; SE, standard error.

activity ratio. **(Supplemental Fig. 3)**. Although there was no correlation between CEC and the POXase/AREase activity ratio, the range of the ratio was completely divided among the *PON1* Q192R genotypes, with the Q/Q genotype showing ≤ 1.15, the Q/R genotype showing 1.26-2.05, and the R/R genotype showing ≥ 2.21 **(Supplemental Fig. 3)**.

The Association of CEC with HDL-C, POXase and AREase Activities using either a Simple or Multiple Regression Analysis

Since the stratified analysis according to the *PON1* Q192R genotype showed that CEC was correlated with the POXase activity in each individual genotype **(Fig. 1)**, we examined the associations of CEC with POXase and AREase activities and HDL-C stratified by *PON1* Q192R genotype using either a simple or multiple regression analysis **(Table 2)**. In all *PON1* Q192R genotypes, CEC was associated with the POXase and AREase activities and the HDL-C level according to a simple regression analysis **(Table 2)**. Moreover, in all individual *PON1* Q192R genotypes, CEC was associated with the POXase activity and HDL-C in all multiple regression models **(Table 2)**. CEC was associated with the POXase activity even after adjusting for HDL-C **(Table 2)**.

The Association of CEC with the *PON1* Q192R Genotype using a Multiple Regression Analysis

As shown in **Table 1**, we found that CEC was lower in the *PON1* Q192R R/R genotype than in the Q/Q and Q/R genotypes **(Table 1)**. Therefore, we examined the multivariable association of CEC with the *PON1* Q192R genotype using a multiple regression analysis **(Table 3)** and found an association between the *PON1* Q192R genotype and CEC (Q/Q > Q/R > R/R) independent of the HDL-C and POXase activities **(Table 3)**.

The Associations of CEC, HDL-C, POXase, and AREase Activities with the Laboratory Values, Comorbidities, and Habits

To explore the factors involved in the POXase and AREase activities, we analyzed the associations of CEC, HDL-C, POXase, and AREase activities with the laboratory values, comorbidities, and habits **(Supplemental Tables 1, 2, 3, 4)**. Since the POXase activity varies widely among the *PON1* Q192R genotypes, the associations of the POXase activity with the laboratory values, complications, and habits were analyzed and stratified by genotype **(Supplemental Tables 3 and 4)**. The AREase activity was associated with BMI, sex, and eGFR <60 ml/min/1.73 m2 **(Supplemental Tables 1 and 2)**. The POXase activity was associated with age, BMI, and sex

Table 3. The association of CEC with HDL-C, POXase activity and *PON1* Q192R genotype using multiple regression analysis

		Multiple regression analysis			
		β	B	SE	*P*
PON1	Q/Q genotype	0			
	Q/R genotype	-0.238	-6.31	2.68	0.020
	R/R genotype	-0.685	-18.16	4.06	<0.001

Adjusted by HDL-C and POXase activity.
CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol; POXase, paraoxonase (activity); *PON1*, paraoxonase 1 (gene); β, standardized partial regression coefficient; B, unstandardized partial regression coefficient; SE, standard error.

in the *PON1* Q/Q genotype and with uric acid and sex in the Q/R genotype **(Supplemental Tables 3 and 4)**.

Discussion

The present study, conducted in Japanese subjects without ASCVD, showed the POXase activity to be positively correlated with CEC, independent of HDL-C, in a stratified analysis by the *PON1* Q192R genotype **(Fig. 1 and Table 2)**. *PON1* Q192R R/R genotype carriers had a lower CEC than Q/Q or Q/R genotype carriers, independent of the POXase activity **(Table 3)**. The AREase activity was positively correlated with CEC regardless of the *PON1* Q192R genotype **(Fig. 2 and Table 2)**.

It has been reported that HDL particles bound to PON1 prevent the oxidation of LDL and inhibit the generation of monocyte chemotactic protein 1, which is implicated in the prevention of atherosclerosis[25]. PON1 has also been shown to protect against lipid peroxidation and increase CEC in HDL, but not in lipoprotein-deficient serum[26]. Additionally, PON1 interacts with ATP-binding cassette protein A1 (ABCA1)[27] and scavenger receptor class B type I (SR-BI)[28, 29]. In the present study, since J774.1 cells without any upregulation of ABCA1 by cAMP were used in the CEC measurement, we speculate that PON1 is involved in cholesterol efflux mediated by SR-BI and/or passive diffusion. Indeed, it has been reported that SR-BI is a key mediator of the ability of HDL to acquire PON1 [30] and that the anti-inflammatory activity of PON1 is mediated via the interaction with SR-BI[31]. Nevertheless, to elucidate this mechanism in detail, it is necessary to examine the relationship between PON1 and SR-BI-specific CEC using Fu5H cells[32]. Furthermore, the relationship between PON1 and ABCA1-specific CEC should be examined.

Interestingly, CEC was lower in carriers of the R/R genotype, a group with a high POXase activity, than that in carriers of the Q/Q genotype, a group with low POXase activity **(Tables 1 and 3)**. However, a correlation between the POXase activity and CEC was only observed when stratified according to the *PON1* Q192R genotype **(Fig. 1 and Table 2)**. These phenomena suggest that differences in the POXase activity based on the *PON1* Q192R genotype do not affect CEC.

In the structure of the PON1 protein, the amino acid at position 192 is at the edge of the active site of the POXase activity and it is also close to the presumed binding site of PON1 and HDL[33]. A previous study of recombinant PON1 proteins (rePON1) showed a lower binding affinity of rePON1-192Q with HDL than rePON1-192R[34]. Therefore, it is speculated that the *PON1* Q192R polymorphism affects not only the POXase activity but also the binding of PON1 to HDL, and this difference in binding properties may partly affect CEC. However, since other factors may be directly or indirectly involved, further studies are needed to elucidate the detailed mechanisms of the association between the *PON1* genotype and CEC.

Previous reports have shown that the PON1 protein in carriers of the *PON1* Q192R R allele has a weaker antioxidant capacity than that in carriers of the Q allele and that the antioxidant capacity is associated with CEC[35-38]. A study conducted in healthy human volunteers reported that HDL derived from the *PON1* Q192R R/R genotype carriers was less protective against LDL oxidation than that derived from Q/Q genotype carriers[35]. Furthermore, the addition of PON1 protein from the serum of healthy volunteers to homogenates of coronary and carotid lesions from patients undergoing coronary artery bypass surgery reduced the lipid peroxide levels in the lesions, with serum from volunteers with the *PON1* Q192R Q/Q genotype being more effective than that from volunteers with the R/R genotype[36]. In contrast, an increase in oxidized LDL has been reported to cause a

decrease in the HDL function[37, 38]. Abdominal macrophages of mice loaded with oxidized LDL showed impaired HDL-mediated cholesterol efflux[37], while another report showed that increased levels of oxidized LDL are involved in the generation of dysfunctional HDL due to oxidative modification[38]. Based on the above information, we speculate that the decreased protective effect of the *PON1* Q192R R allele on LDL oxidation and lipid peroxides may be associated with the decreased CEC of HDL.

The AREase activity was positively correlated with CEC regardless of the *PON1* Q192R genotype **(Fig. 2 and Table 2)**. Since the AREase activity was positively correlated with the serum PON1 protein concentration[39], the AREase activity may reflect PON1 protein-induced CEC enhancement. Meanwhile, since the active site of the AREase activity is involved in the hydrolysis of cholesterol- and choline-based compounds[11], the AREase activity is not merely an indicator of PON1 protein concentration, but it may also be indirectly related to CEC[40]. Further studies are required to elucidate the mechanistic details of the association between the AREase activity and CEC.

The correlation coefficient between the HDL-C level and the AREase activity was lower than that between CEC and the AREase activity **(Fig. 2 and Supplemental Fig. 1)**, and in analyses stratified by the *PON1* Q192R genotype, the correlation coefficient between the HDL-C level and the POXase activity was also lower than that between CEC and POXase in each genotype **(Fig. 1 and Supplemental Fig. 1)**. Furthermore, the AREase activity was not necessarily higher in participants with higher HDL-C levels, and the same phenomenon was observed for POXase activity when stratified by *PON1* Q192R genotype **(Supplemental Fig. 1)**. This suggests that CEC is more closely associated with the POXase and AREase activities than with the HDL-C levels.

To explore the factors involved in the POXase and AREase activities, we analyzed the associations of their activities with laboratory values, comorbidities, and habits **(Supplemental Tables 1-4)**. The AREase activity was associated with BMI, sex, and eGFR <60 ml/min/1.73 m^2 **(Supplemental Tables 1 and 2)**. The POXase activity was associated with age, BMI, and sex in the *PON1* Q/Q genotype and with uric acid and sex in the Q/R genotype **(Supplemental Tables 3 and 4)**. These factors may be associated with CEC via changes in the AREase and POXase activities, although further investigation to identify any causal relationships is needed.

The results of the analysis of the POXase/AREase activity ratio showed that the range of the ratio was completely divided by *PON1* Q192R genotype **(Supplemental Fig. 3)**, and the genotype may be determined based on the ratio without genomic DNA. In addition, measuring the POXase and AREase activities may allow us to estimate the effect of the *PON1* Q192R genotype on CEC.

The present study is associated with some limitations. First, it included a relatively small number of participants. Second, this study only examined relatively healthy subjects, which limited our ability to assess the relationship between *PON1* Q192R polymorphism and ASCVD. The plasma levels of myeloperoxidase (MPO), which causes oxidative modification of the lipid and protein components of HDL, are known to be low in healthy individuals and elevated in ASCVD patients[41]. Oxidative modification of ApoAI by MPO reduces its properties, thus affecting its CEC and anti-inflammatory function and promoting the development of atherosclerosis[42, 43]. Furthermore, MPO, PON1, and HDL form ternary complexes that inactivates PON1 [44]. Therefore, the relationship between CEC, the *PON1* Q192R genotype, and the ternary complex of MPO, PON1, and HDL should be further investigated in patients with ASCVD. Third, it is uncertain whether the results of this study can be generalized to other racial groups. Previous epidemiological evidence has shown that the genetic impact of the *PON1* Q192R polymorphism on ASCVD risk varies by race[18-21]. A previous study showed that the effect of the POXase activity on CHD risk varied according to race[15]. The frequency of the *PON1* Q192R genetic polymorphism also varies by race; for example, the R allele frequency in East Asians is approximately 65%, while that in Europeans is approximately 31%[45]. Therefore, the association between the *PON1* Q192R genotype and CEC may vary by race and may also be influenced by racial differences in the association between POXase activity and CEC and the frequency of the *PON1* Q192R R allele. It is noteworthy that this study was conducted in Japanese subjects with a high *PON1* Q192R R allele frequency[46]. It is also noteworthy that the subjects were matched not only by age and sex, but also by alcohol intake and smoking status, both of which are known to affect CEC[16, 24]. Larger prospective studies involving diverse populations are necessary to confirm our findings.

Conclusions

The present study showed that the *PON1* Q192R R allele is associated with a decreased CEC independent of the POXase activity. We speculate that the decrease in CEC with the *PON1* Q192R R allele

may be due to changes in the stability of the PON1 protein in binding to HDL rather than a genotype-derived difference in the POXase activity. However, it is unclear whether the *PON1* Q192R genotype is directly associated with CEC, and the detailed mechanism underlying the association between the *PON1* Q192R genotype and CEC needs to be further clarified. Furthermore, it is necessary to elucidate whether the association between the *PON1* Q192R genotype and CEC affects the severity of atherosclerosis and the development of ASCVD.

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Conflicts of Interest

Mariko Harada-Shiba received stock holdings or options from Liid Pharma Inc. and honoraria from Amgen, MEDPACE, and Novartis. Masatsune Ogura received honouraria from Amgen and Kowa.

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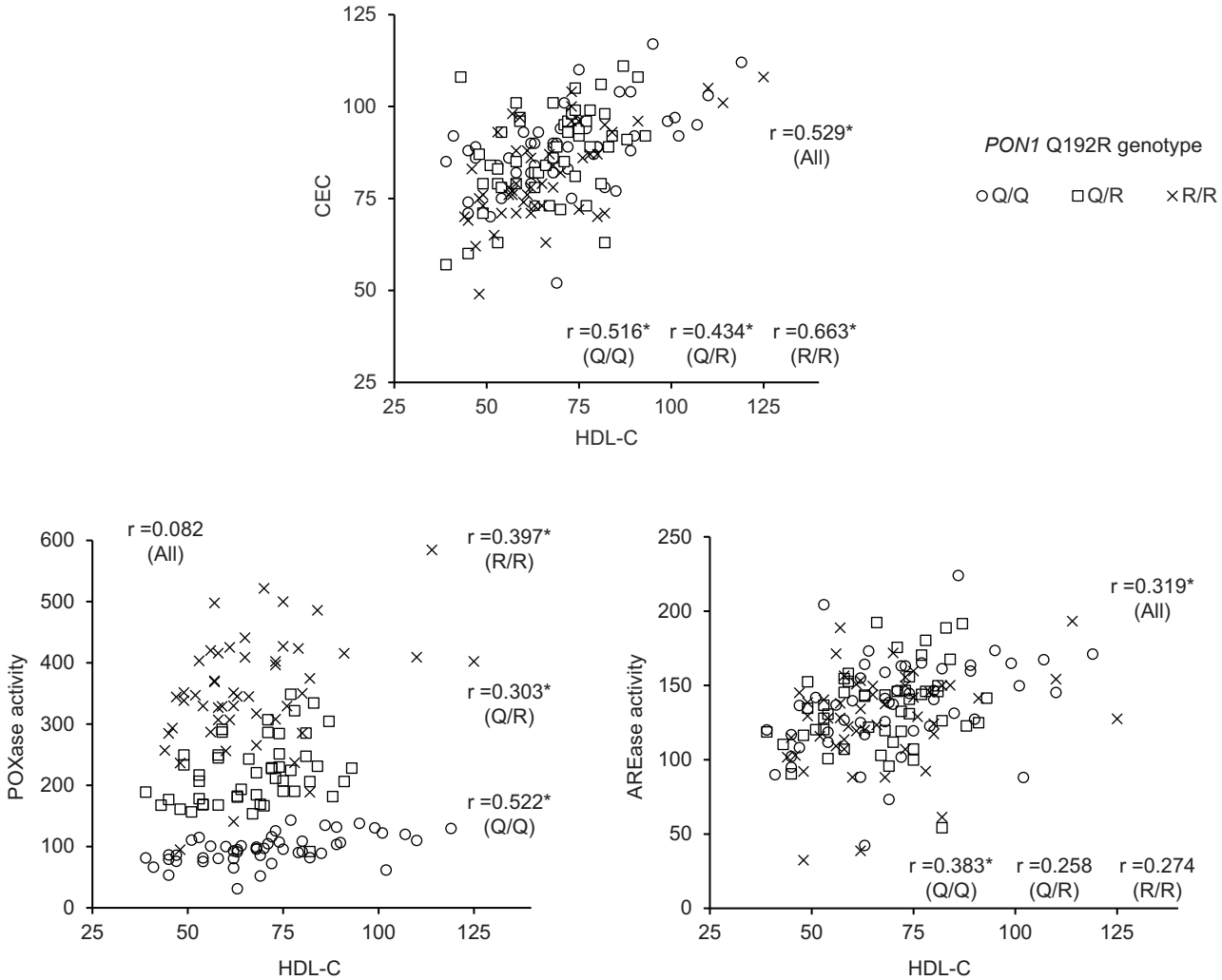
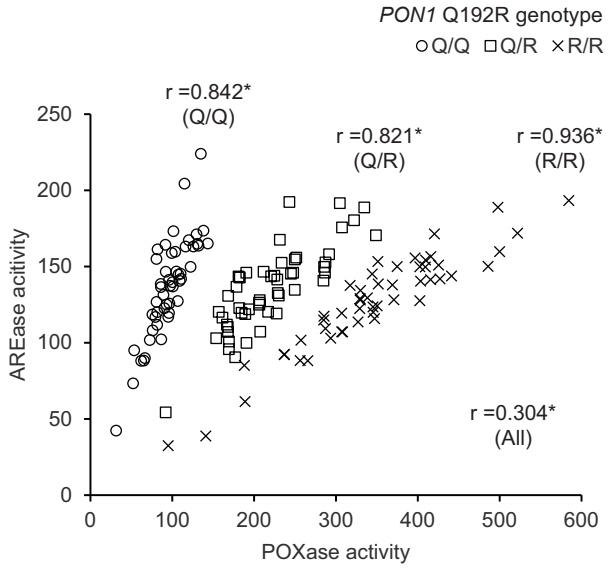


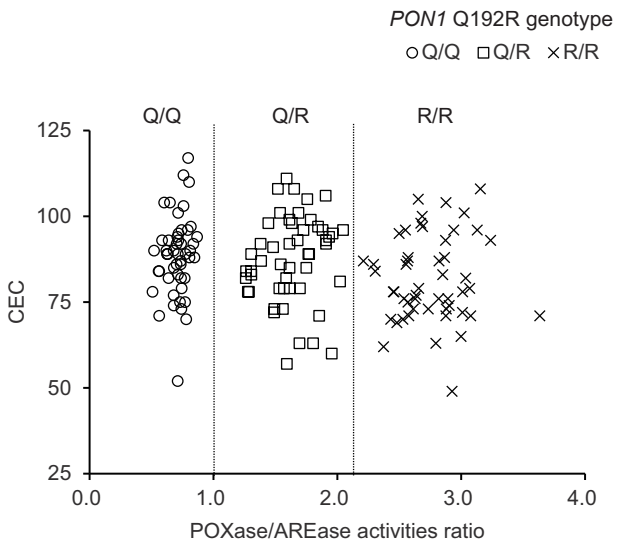
Supplemental Fig. 1. The correlations of HDL-C with CEC, POXase and AREase activities

CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol; *PON1*, paraoxonase 1 (gene); POXase, pataoxonase (activity); AREase, arylesterase; r, correlation coefficient. **P*<0.05.



Supplemental Fig. 2. The correlation between POXase and AREase activities

POXase, paraoxonase (activity); AREase, arylesterase; *PON1*, paraoxonase 1 (gene); r, correlation coefficient. *$P<0.05$.



Supplemental Fig. 3. The correlation of CEC with POXase/AREase activities ratio

The ranges of POXase/AREase activities ratios for the PON1 Q192R Q/Q, Q/R and R/R genotypes are 0.51-0.87, 1.26-2.05 and 2.21-3.63, respectively.
CEC, cholesterol efflux capacity; POXase, paraoxonase (activity); AREase, arylesterase; *PON1*, paraoxonase 1 (gene).

Supplemental Table 1. Correlations of HDL-C, CEC, and AREase activities with variables

	HDL-C (mg/dL)	CEC	AREase activity (nmol/mL/min)
Age (years)	0.166*	0.084	0.074
BMI (kg/m^2)	-0.402*	-0.134	-0.201*
LDL-C (mg/dL)	-0.046	-0.059	0.118
TGs (mg/dL)	-0.410*	0.031	0.010
FBG (mg/dL)	-0.132	-0.064	-0.138
AST (U/L)	-0.102	0.015	-0.016
ALT (U/L)	-0.301	-0.071	-0.078
GGT (U/L)	-0.143	0.122	0.038
Albumin (g/dL)	-0.001	0.102	0.104
hs-CRP (mg/L)	-0.229*	-0.168*	-0.015
UA (mg/dL)	-0.334*	-0.198*	-0.112
eGFR (ml/min/1.73m^2)	0.061	0.048	-0.007
Alcohol intake (g/day)	0.011	0.075	0.023

Values are Pearson's correlation coefficients. *$P<0.05$
HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; AREase, arylesterase; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol: TGs, triglycerides; FBG, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; hs-CRP, high-sensitive C-reactive protein; UA, uric acid; eGFR, estimated glomerular filtration rate.

Supplemental Table 2. Associations of HDL-C, CEC, and AREase activity with categorical variables

		HDL-C (mg/dL)	*P*	CEC	*P*	AREase activity (nmol/mL/min)	*P*
Sex	Male	61.3 ± 14.6	< 0.001	84 (49-125)	0.020 [b]	129.3 (32.5-224.0)	0.017 [b]
	Female	75.0 ± 15.9		90 (63-117)		141.3 (38.8-193.3)	
Smoking status	Never	70.9 ± 16.6	0.003 [a]	87.5 (60-125)	0.701 [c]	137.1 (38.8-204.4)	0.784 [c]
	Ex	67.7 ± 16.8		86 (49-108)		134.3 (32.5-224.0)	
	Current	56.7 ± 11.7		84 (62-108)		142.8 (90.0-188.9)	
eGFR	≥ 60 ml/min/1.73m^2	69.5 ± 16.3	0.033	87 (52-117)	0.289 [b]	137.6 (38.8-224.0)	0.020 [b]
	< 60 ml/min/1.73m^2	60.8 ± 17.9		83 (49-125)		118.8 (32.5-193.3)	
Type 2 diabetes	Absent	69.0 ± 16.7	0.129	87 (49-125)	0.229 [b]	136.9 (32.5-204.4)	0.944 [b]
	Present	62.0 ± 15.3		84 (62-117)		134.7 (42.4-224.0)	
Dyslipidemia	Absent	68.7 ± 15.4	0.846	87 (52-110)	0.879 [b]	131.0 (38.8-193.3)	0.142 [b]
	Present	68.2 ± 17.8		86 (49-125)		138.6 (32.5-224.0)	
Hypertension	Absent	69.9 ± 16.8	0.126	88 (62-117)	0.019 [b]	138.6 (38.8-224.0)	0.137 [b]
	Present	65.4 ± 16.3		83 (49-125)		130.8 (32.5-188.8)	

Values are means ± standard deviation. [a]One-way ANOVA. [b]Mann-Whitney *U*-test. [c]Kruskal-Wallis test (otherwise, Student's *t*-test was used).
HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; AREase, arylesterase; eGFR, estimated glomerular filtration rate.

Supplemental Table 3. Correlations of POXase activity with variables stratified by *PON1* Q192R genotype

	POXase activity (nmol/mL/min)		
	Q/Q genotype	Q/R genotype	Q/R genotyp
Age (years)	0.372*	0.177	0.194
BMI (kg/m^2)	-0.425*	-0.081	-0.154
LDL-C (mg/dL)	-0.125	0.261	0.256
TGs (mg/dL)	-0.207	-0.066	-0.160
FBG (mg/dL)	-0.249	-0.040	-0.012
AST (U/L)	-0.125	-0.232	0.218
ALT (U/L)	-0.264	-0.221	0.174
GGT (U/L)	-0.123	-0.135	0.179
Albumin (g/dL)	-0.153	0.100	0.176
hs-CRP (mg/L)	-0.205	-0.263	0.154
UA (mg/dL)	-0.274	-0.339*	0.008
eGFR (ml/min/1.73m^2)	-0.077	0.197	-0.195
Alcohol intake (g/day)	-0.268	0.022	0.089

Values are Pearson's correlation coefficients. *$P < 0.05$
POXase, paraoxonase (activity); *PON1*, paraoxonase 1 (gene); BMI, body mass index; LDL-C, low-density lipoprotein cholesterol: TGs, triglycerides; FBG, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; hs-CRP, high-sensitive C-reactive protein; UA, uric acid; eGFR, estimated glomerular filtration rate.

Supplemental Table 4. Associations of POXase activity with categorical variables stratified by *PON1* Q192R genotype

		POXase activity (nmol/mL/min)					
		Q/Q genotype	*P*	Q/R genotype	*P*	R/R genotype	*P*
Sex	Male	83.9 (31.4-134.9)	0.003	190.6 (92.0-284.7)	0.014	346.3 (95.1-521.9)	0.985
	Female	102.5 (62.0-143.4)		229.6 (153.6-348.9)		347.8 (141.0-584.5)	
Smoking status	Never	97.4 (31.4-143.3)	0.256 [a]	217.1 (92.0-348.9)	0.882 [a]	350.8 (141.0-584.5)	0.736 [a]
	Ex	96.5 (52.2-134.9)		211.6 (161.3-286.6)		330.0 (95.1-521.9)	
	Current	81.6 (53.4-116.2)		208.1 (156.8-322.1)		345.5 (329.8-497.9)	
eGFR	≥ 60 ml/min/1.73m^2	96.5 (31.4-143.4)	0.202	220.9 (92.0-348.9)	0.126	346.3 (141.0-521.9)	0.896
	<60 ml/min/1.73m^2	86.5 (53.4-93.0)		179.2 (153.6-284.7)		349.2 (95.1-584.5)	
Type 2 diabetes	Absent	96.5 (52.2-143.4)	0.804	214.3 (92.0-348.9)	0.653	347.9 (95.1-584.5)	0.940
	Present	91.6 (31.4-138.1)		203.2 (156.8-249.7)		345.8 (344.3-347.2)	
Dyslipidemia	Absent	93.5 (52.2-130.7)	0.379	199.9 (92.0-322.1)	0.060	329.0 (141.0-584.5)	0.454
	Present	97.9 (31.4-143.4)		231.0 (156.8-348.9)		350.3 (95.1-521.9)	
Hypertension	Absent	100.2 (31.4-143.4)	0.250	216.1 (92.0-348.9)	0.931	350.8 (141.0-584.5)	0.236
	Present	89.2 (52.2-132.0)		214.3 (156.8-334.6)		337.5 (95.1-500.0)	

Values are means ± standard deviation. [a] Kruskal-Wallis test (otherwise, Mann-Whitney *U*-test was used).
POXase, paraoxonase (activity); *PON1*, paraoxonase 1 (gene); eGFR, estimated glomerular filtration rate.