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patient-oriented and epidemiological research

Association of the novel cardiovascular risk factors paraoxonase 1 and cystatin C in type 2 diabetes[S]

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Abstract Paraoxonase 1 (PON1) has been reported to be associated with proteinuria in subjects with type 2 diabetes mellitus (T2DM). Plasma cystatin C is more accurate than creatinine for identifying stage 3 kidney disease in T2DM.[jlr] We tested the hypothesis that PON1 and cystatin C would be associated in T2DM subjects from an Aboriginal Canadian community, who are at high risk for the development of nephropathy. *PON1* A(-162)G and *PON2* Ala148Gly genotypes, cystatin C, HbA1c, high density lipoprotein cholesterol (HDLC), waist circumference (waist), and duration of diabetes were included in the regression analysis with $\log_e$ (ln) of PON1 mass as the dependent variable. A regression model including *PON2* Ala148Gly genotype, HDLC, and ln cystatin C explained 25.8% of the variance in PON1 mass. Conversely, waist, age, ln HbA1c, ln duration of diabetes, and ln PON1 mass, but not *PON2* genotype, explained 38% of the variance in cystatin C. Subjects with cystatin C estimated glomerular filtration rate (eGFR) <60 ml/min per 1.73 m^2 (stage 3 kidney disease) had significantly lower PON1 mass compared with subjects with cystatin C-eGFR >60 ml/min per 1.73 m^2. The lower mass of PON1, an anti-inflammatory HDL-associated enzyme, in T2DM with cystatin C-eGFR <60 ml/min per 1.73 m^2 may contribute to their increased risk for cardiovascular disease.—Connelly, P. W., B. Zinman, G. F. Maguire, M. Mamakeesick, S. B. Harris, R. A. Hegele, R. Retnakaran, and A. J. G. Hanley. **Association of the novel cardiovascular risk factors paraoxonase 1 and cystatin C in type 2 diabetes.** ***J. Lipid Res.*** **2009.** 50: **1216–1222.**

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Paraoxonase 1 (PON1) (arylesterase; EC 3.1.1.2) is an HDL-associated enzyme that hydrolyzes organophosphate compounds and fatty acid lactones (1–4). PON1 activity has been inversely associated with cardiovascular disease (5), and PON1-deficient mice are more prone to develop atherosclerosis (6). In human studies, PON1 activity is significantly lower in subjects with the metabolic syndrome (7). PON1 activity was recently shown to be reduced in subjects with type 1 and type 2 diabetes mellitus (T1DM and T2DM) (8) and was inversely associated with glucose concentrations in subjects with type 1 diabetes (9). Subjects with the Arg192 allele of the *PON1* gene had an odds ratio of 3.21 for the presence of retinopathy and/or nephropathy compared with subjects who were homozygous for the Gln192 allele in a Japanese cohort. The Japanese are known to have a higher frequency of the minor Arg192 allele compared with Caucasians (10). Hofer et al. (9) reported that homozygosity for the minor A allele of the *PON1* A(-162)G promoter polymorphism was associated with microalbuminuria in an adolescent Australian cohort of subjects with T1DM. Furthermore, several studies have reported the association of *PON1* gene variants with increased diabetic complications in T1DM (11) and in T2DM (10, 12).

We have reported that the *PON2* polymorphism Ala148Gly is associated with diabetes in a cohort of Aboriginal Canadians (13, 14). In a prospective study of 3,374 predominantly Caucasian subjects (the United Kingdom Prospective

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✤ ***Author's Choice****— Final version full access.*

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Abbreviations: apoAI, apolipoprotein AI; cysC, cystatin C; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; ln, $\log_e$; PON1, paraoxonase 1; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of two figures.

Diabetes Study), with a median duration of diabetes of 14 years, it was found that heterozygotes for *PON2* Ala148Gly polymorphism had a 1.25 relative risk for the development of microalbuminuria (15). However, there are no previous studies of *PON2* polymorphisms and PON1 mass.

Relatively little information is available regarding circulating PON1 mass in subjects with and without diabetic complications (16, 17). This issue is of interest due to the hypothesized role of oxidative stress in the development of the complications of diabetes (18). Furthermore, we are unaware of any studies of PON1 mass and diabetic complications among Aboriginal populations, who are at high risk for diabetes and associated sequelae (19, 20). In particular, it is well documented that Aboriginal Canadian subjects with diabetes have high rates of microalbuminuria and end stage renal disease (19–21), suggesting that kidney dysfunction may be the predominant microvascular complication of diabetes in this population. Our objective, therefore, was to determine the association of PON1 mass with cystatin C, a marker of renal function, in a sample of Aboriginal Canadians with T2DM.

RESEARCH DESIGN AND METHODS

The methodology of the Sandy Lake Diabetes Complications Study (2001–2002) has been presented in detail previously (21). Briefly, the research project was conducted in partnership with a Canadian Aboriginal community currently experiencing high rates of T2DM, with an early age of disease onset and high rates of complications (21, 22). All community members known to have T2DM were invited to participate; 189 of 250 (76%) eligible subjects were enrolled. Serum samples were available for the measurement of PON1 mass for 106 of these subjects (44 males/62 females). Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and the Mount Sinai Hospital Ethics Review Committee.

Validated measures were used to assess nephropathy as described previously (21). Diabetic nephropathy was determined by measuring the albumin:creatinine ratio in a single random daytime urine sample (23) using the Bayer DCA 2000 Point-of-Care Analyzer, which had been validated ($r = 0.95$) against laboratory techniques (24).

HbA1c was determined using the DCA 2000 analyzer ($r = 0.90$–0.98 vs. laboratory measures) (25). Lipid, lipoprotein, and creatinine concentrations were determined using standard laboratory methods. The Cockcroft-Gault equation was used for estimated glomerular filtration rate (eGFR) (26). Height, weight, and waist circumference, systolic and diastolic blood pressure, duration of diabetes, and method of diabetes and hypertension treatment were determined as previously described (21). Cystatin C, a novel surrogate measure of GFR (27), was determined using the Behring N latex Cystatin C reagent and the Behring BN100 instrument according to manufacturer's protocols. The cystatin C-estimated GFR was calculated using the Arnal equation as follows: Cys-eGFR = $74.835/(\text{CysC}^{1.333})$ (28).

PON1 mass was determined using SDS-electrophoresis and Western blot with anti-human PON1 monoclonal antibody 4C10 as the primary antibody and Alexa Fluor 680 anti-mouse IgG (catalog number A21065; Alexa) as the second antibody (29) (see supplementary Fig. I). Detection and quantitation was done with the Licor Odyssey (Omaha, NE). Between-day coefficient of variation was 7.8%. A reference serum was calibrated using recombinant human paraoxonase-1 (Cedarlane Laboratories, Ontario, Canada).

Genotypes of *PON1* Gln192 Arg, Met55Leu, and A(-162)G polymorphisms and *PON2* Ala148Gly polymorphism were determined using established methods (9, 30, 31).

Statistical methods

Analyses were conducted using PON1 mass as well as the ratios of PON1/HDLC and PON1/apo AI, which were calculated to account for the demonstrated stabilizing effect of HDL and apolipoprotein AI (apoAI) on PON1. Genotypes of *PON1* A(-162)G and *PON2* Ala148Gly were entered into models as discrete independent variables. Differences between means were evaluated using *t*-tests, and univariate associations between continuous variables were assessed using Spearman correlation analysis. Independent associations of PON1 with the continuous outcomes cystatin C and eGFR were determined using multiple linear regression, with covariates included based on previous analysis of diabetic kidney complications in this population (21). All analyses were conducted using SAS version 9.1 (SAS Institute, Carey, NC).

RESULTS

Although study participants were relatively young (mean age of 46 years), the average duration of diabetes was 8.4 years and metabolic control was suboptimal (**Table 1**). In univariate correlation analysis, PON1/HDLC and PON1/apoAI were significantly positively correlated with eGFR-CG ($r = 0.37$ and $r = 0.28$, respectively, both $P < 0.001$), while PON1, PON1/HDLC, and PON1/apoAI showed significant inverse correlations with cystatin C ($r = -0.22$, $P < 0.05$; $r = -0.30$ and $r = -0.29$, both $P < 0.01$, respectively) (**Table 2**). PON1 showed no correlation with urinary albumin/creatinine ratio.

TABLE 1. Characteristics of participants: Sandy Lake Complications Prevalence and Risk Factor Study, 2001–2002

Variable	Mean	(SD)	Median
Age (years)	46.0	(11.5)	46.0
Duration of diabetes (years)	8.4	(6.5)	9.0
HbA1c (%)	8.4	(2.3)	8.1
Albumin:creatinine ratio (mg/mmol)	11.2	(17.6)	3.6
BMI (kg/m^2)	30.4	(5.8)	29.6
Waist (cm)	103.4	(11.6)	102.5
Cholesterol (mmol/L)	4.74	(0.82)	4.77
LDL-cholesterol (mmol/L)	2.72	(0.7)	2.68
HDL-cholesterol (mmol/L)	1.19	(0.27)	1.17
Triglyceride (mmol/L)	1.82	(0.82)	1.7
apoAI (g/L)	1.55	(0.3)	1.53
apoB (g/L)	1.00	(0.22)	0.98
Systolic BP (mmHg)	125.7	(15.9)	125
Diastolic BP (mmHg)	75.2	(9.1)	75
Cystatin C (mg/L)	0.87	(0.21)	0.84
Creatinine (μmol/L)	64.0	(15.4)	61
eGFR (ml/min)[a]	140.5	(43.8)	136.0
eGFR-MDRD[b]	110.2	(25.4)	106.7
PON1 (mg/L)	176.9	(35.2)	175.1
PON/HDL	154.8	(42.7)	151.5
PON/apoAI	117.6	(30.0)	117.0
	n		
Gender (male/female)	44/62		

BMI, body mass index; BP, blood pressure.
[a] Cockcroft-Gault equation.
[b] Modification of diet in renal disease equation.

TABLE 2. Univariate associations of PON1, PON1/HDL, PON1/ApoA1, and other demographic and metabolic risk factors with urine albumin/creatinine ratio, estimated glomerular filtration rate, and cystatin C

	Albumin-Creatinine Ratio	Estimated Glomerular Filtration Rate		Cystatin C
		CG[a]	MDRD[b]	
Risk Factor	*r*	*r*		*r*
Age	0.06	−0.61¶	−0.36‡	0.43¶
Diabetes duration	0.33‡	0.34‡	−0.09	0.17
HbA1c	0.30†	0.12	0.25*	−0.27†
Systolic BP	0.24*	−0.02	−0.03	0.10
HDL	−0.10	−0.31†	−0.10	0.10
apoAI	−0.10	−0.27†	−0.14	0.15
PON1	−0.03	0.12	0.10	−0.22*
PON1/HDL	0.10	0.37¶	0.17	−0.30†
PON1/apoAI	0.08	0.28†	0.16	−0.29†

Univariate Spearman correlation analysis.
*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; ¶$P < 0.0001$. BP, blood pressure.
[a] Cockcroft-Gault equation.
[b] Modification of diet in renal disease equation.

TABLE 3. Univariate analysis of PON1 mass by PON2 Ala148Gly and PON1 A(-162)G genotypes

		PON1 mg/L		
Genotype	n	Mean	(SD)	Median
PON2 Ala148Gly				
Ala/Ala*	58	165	(33.7)	165
Ala/Gly	27	192	(27.4)	196
Gly/Gly	18	192	(36.7)	208
PON1 A(-162)G				
G/G†	83	171	(34.4)	171
G/A	18	199	(31.4)	209
A/A	3	197	(20.5)	207

*Wilcoxon nonparametric test $P = 0.0002$. †Wilcoxon nonparametric test $P = 0.004$.

The minor allele frequencies of *PON1* amino acid polymorphisms Gln192Arg and Leu55Met and promoter polymorphism A(-162)G were 0.22(Arg192), 0.019 (Met55), and 0.11 (A(-162)), respectively. The minor allele frequency of the *PON2* amino acid polymorphism Ala148Gly was 0.31 (Ala148). The *PON1* A(-162)G and *PON2* Ala148Gly showed significant but modest allelic association (linkage disequilibrium constant D = 0.06, $P < 0.01$), so these markers was considered to be independent for further analyses. *PON1* Gln192Arg and Leu55Met genotypes were not significantly associated with PON1 mass (data not shown). Because of the very low frequencies (and low absolute counts) of the minor allele for *PON1* Arg192 and *PON1* Leu55 polymorphisms in this sample of Aboriginal Canadians, these polymorphisms were not evaluated further. However, genotypes of *PON1* A(-162)G and *PON2* Ala148Gly polymorphisms were each significantly associated with PON1 mass (**Table 3**). The *PON2* polymorphism had been previously shown to be associated with diabetes in this cohort (13, 14), and among the genetic markers evaluated, it had the strongest statistical relationship with PON1 mass.

To analyze the determinants of PON1 mass, regression analysis was performed with genotype variables coded as 0 or 1 (e.g., presence or absence of genotypes 148Ala/Ala, 148Ala/Gly, and 148Gly/Gly). Analysis was performed with age, gender, $\log_e$ (ln) duration of diabetes, ln HbA1c, waist circumference, high density lipoprotein cholesterol (HDLC), and ln cystatin C. It was noted that the distribution of duration of diabetes was skewed (see supplementary Fig. II), with most subjects having had diabetes of <15 years' duration. Thus, analysis is also reported with restriction to subjects with duration of diabetes <15 years. The strongest determinant of PON1 mass was the presence of the *PON2* 148Ala/Ala genotype, which accounted for 12.6% of the variation in PON1 mass, followed by HDLC, ln cystatin C, and ln duration of diabetes (**Table 4**). The remaining variables did not reach significance at the $P < 0.05$ level. The order of the variables was the same when the analysis was carried out for those subjects with duration of diabetes <15 years; however, duration of diabetes was no longer significant. Similarly, when the analysis was done with *PON1* A(-162)G, the presence of the *PON1* -162G/G genotype was the strongest determinant of mass, accounting for 8.9% of the variation in PON1 mass, followed by HDLC, ln cystatin C, and ln duration of diabetes (**Table 5**). When both genotypes were included in the analysis, the *PON2* 148Ala/Ala genotype remained the strongest determinant of PON1 mass, whereas the *PON1* -162G/G genotype had a $P = 0.06$.

The cross-sectional design of this study makes it difficult to assign a cause–effect relationship between variables. Thus, the complementary question was also addressed; that is, what are the determinants of cystatin C? The determinants of cystatin C were also analyzed by regression analysis with forward selection for the full cohort and with restriction to those subjects with duration of diabetes <15 years (**Table 6**). Both analyses ranked age, ln HbA1c, and waist circumference as the most influential variables, accounting for 15.6, 11.2, and 5.3%, respectively, of the variation in cystatin C for the full cohort. The impact of the subjects with duration of diabetes >15 years is seen, in that, when these subjects are included in the analysis, duration enters the regression model at the fourth step, whereas when they are excluded, it is not significant. PON1 mass accounts for a similar partial r^2 in both analyses (2.3 and 3.5% for the full cohort and subjects with duration <15 years, respectively) but has a *P* value < 0.05 only for the analysis restricted to subjects with duration of diabetes of <15 years. Notably, there was no relationship of *PON2* genotype with cystatin C.

Beauvieux et al. (28) reported that the Arnal-Dade formula for cystatin C-estimated GFR (cysC-eGFR) resulted in the correct estimation of GFR in both the high (GFR 67–164 ml/min per 1.73 m^2) and medium (GFR 34–67 ml/min per 1.73 m^2) range. Thus, the data were analyzed with subjects classified as cysC-eGFR < 60 or > 60 ml/min per 1.73 m^2, which identifies stage 3 kidney disease (32) (**Table 7**). Classification of subjects by cysC-eGFR was of

TABLE 4. Regression analysis of PON1 mass including PON2 genotype

Step	Variable	Partial r^2	Model r^2	β	SEM	t	*P*
(n = 102)	Intercept			5.0	0.084	59.04	<0.0001
1	PON2 "AA"	0.147	0.147	−0.149	0.035	−4.26	<0.0001
2	HDLC	0.0647	0.2116	0.239	0.064	3.72	0.0003
3	ln cystatin C	0.0605	0.2720	−0.2	0.0765	−2.62	0.0103
4	ln duration	0.036	0.308	−0.044	0.02	−2.23	0.0278
Duration <15 years							
(n = 92)	Intercept			4.95	0.088	56.43	<0.0001
1	PON2 "AA"	0.126	0.126	−0.138	0.037	−3.75	0.0003
2	HDLC	0.0756	0.2016	0.214	0.068	3.14	0.0023
3	ln cystatin C	0.0562	0.2578	−0.217	0.084	−2.58	0.0115

comparable significance to HDLC concentrations in explaining variance in plasma PON1 concentrations for both the total cohort and for those subjects with a duration of diabetes <15 years.

DISCUSSION

Although several previous reports have documented associations of *PON1* gene polymorphisms with diabetic complications (9–12), less information is available from studies measuring circulating concentrations of PON1, and no previous study of subjects with diabetes has looked specifically at the relationship of PON1 mass and cystatin C. In the present analysis, we demonstrated a significant, independent inverse association of PON1 mass with cystatin C, a novel marker of kidney function, in Aboriginal subjects with type 2 diabetes. It was found that this was explained by lower PON1 mass for subjects with a cysC-eGFR <60 ml/min per 1.73 m^2 compared with subjects with cysC-eGFR >60 ml/min per 1.73 m^2.

Previous research on PON1 and diabetic kidney disease has focused on microalbuminuria (10, 11). We did not find an association between PON1 mass and urinary albumin: creatinine ratio in our study. The most likely explanation for an absence of association of PON1 mass with microalbuminuria is the relatively short duration of diabetes in this cohort, with a median time of 8 years, compared with 14 years for other studies.

Serum cystatin C concentrations are an indicator of glomerular filtration rate and strongly correlate with iothalamate clearance (28, 33–35). There are several possible explanations for the association of PON1 mass and of the ratio of PON1/apoAI with GFR as reflected by plasma cystatin C concentrations. PON1 expression has been reported to be reduced by interleukin 1-β and tumor necrosis factor-α (TNF-α) (36). Keller et al. (37) reported a significant correlation between cystatin C and TNF-α in elderly individuals. Thus, an inflammatory cytokine, such as TNF-α, could link reduced renal function and reduced PON1 concentrations.

Serum PON1 is correlated with HDL in normal subjects and is found in both apoAI- and apoAII-containing lipoproteins (38). Association of PON1 with apoAI has been shown to be important for the stability of the enzyme (39), and in vitro studies have suggested that this stability is greater for PON1 associated with LpAI:AII compared with LpAI HDL particles (38). Subjects with diabetes have been reported to have lower LpAI:AII concentrations (40), whereas LpAI has been reported to be reduced in subjects with chronic renal failure with or without diabetes (41). We have recently shown that the PON1 knockout mouse has an inflammatory vascular phenotype on chow diet (42). Clearly there is potential for an interaction between the inflammatory and metabolic changes of diabetes to result in lower serum PON1. The extent to which the lower PON1 and the subsequent reduction in anti-inflammatory function of HDL contribute to vascular and renal changes remains to be determined.

The genetic polymorphisms of the paraoxonase gene family locus are complex (43, 44). Linkage disequilibrium exists not only within the *PON1* gene, but between *PON1* and the neighboring *PON2* gene, although these relationships vary widely across different geographical ancestries.

TABLE 5. Regression analysis of PON1 mass including PON1 A(-162)G genotype

Step	Variable	Partial r^2	Model r^2	β	SEM	t	*P*
(n = 103)	Intercept			5.03	0.09	56.9	<0.0001
1	PON1 "GG"	0.089	0.089	−0.156	0.044	−3.52	0.0007
2	HDLC	0.091	0.17	0.257	0.065	3.94	0.0002
3	ln cystatin C	0.062	0.23	−0.19	0.074	−2.52	0.013
4	ln duration	0.043	0.29	−0.048	0.02	−2.42	0.0173
Duration <15 years							
(n = 93)	Intercept			5.0	0.094	53.12	<0.0001
1	HDLC	0.087	0.087	0.25	0.07	3.59	0.0005
2	PON1 "GG"	0.083	0.17	−0.134	0.047	-2.84	0.0057
3	ln cystatin C	0.051	0.221	−0.19	0.0984	−2.42	0.0176

TABLE 6. Regression analysis with ln cystatin C as the dependent variable

Step	Variable	Partial r^2	Model r^2	β	SEM	t	*P*
(n = 106)	Intercept			0.44	0.53	0.83	NS
1	Age	0.1564	0.1564	0.0079	0.0017	4.58	<0.0001
2	ln HbA1c	0.112	0.268	−0.328	0.075	−4.37	<0.0001
3	Waist	0.0532	0.3215	0.00531	0.00169	3.14	0.0022
4	ln duration	0.0361	0.3576	0.0486	0.024	2.02	0.0456
5	ln PON1	0.0227	0.3803	−0.1776	0.0928	−1.91	0.058
Duration <15 years							
(n = 95)	Intercept			0.68	0.58	1.18	NS
1	ln HbA1c	0.1139	0.1139	−0.295	0.08	−3.68	0.0004
2	Age	0.1173	0.2312	0.007	0.0018	4.23	<0.0001
3	Waist	0.0631	0.2943	0.005	0.0018	2.91	0.0046
4	ln PON1	0.0383	0.3326	−0.23	0.101	−2.27	0.026

NS, not significant.

The *PON1* promoter polymorphism at position −162 nucleotides has been suggested to affect expression of *PON1* mRNA (44). The linkage disequilibrium of this polymorphism with the *PON2* Ala148Gly polymorphism prevents us from ascribing the genetic effects on *PON1* concentration to a single polymorphism. However, the lack of an association between the *PON1* and *PON2* polymorphisms and cystatin C concentration suggests that genetic determinants of *PON1* concentration are not affecting renal function, within the limitations of the relatively small sample size studied here. The observed relative risk of 1.25 for the development of microalbuminuria for heterozygotes for the *PON2* Ala148Gly polymorphism in the United Kingdom Prospective Diabetes Study (15) is consistent with variation at the *PON1/PON2* locus contributing to susceptibility for diabetic nephropathy. However, our data would suggest that the effect of renal function on PON1 concentration is of a significantly greater magnitude than that of the effect of *PON1/PON2* genetic variation on renal function.

It has been demonstrated that PON1 activity is lower in subjects on dialysis compared with controls (45). In contrast, PON1 activity was not different from controls in kidney transplant patients (45). This is consistent with impaired renal function resulting in reduced PON1 and suggests that PON1 is a biomarker of renal impairment. The extent of reduction in PON1 is likely to be important, as it has been reported that lower PON1 mass predicted higher mortality in maintenance hemodialysis patients (46) and lower PON1 activity predicted occurrence of combined cardiovascular end points (5). The mean PON1 mass, adjusted for age, sex, duration of diabetes, and HDLC, was 143 mg/L for the subjects with the PON2 148Ala/Ala genotype and cysC-eGFR <60 ml/min per 1.73 m^2 (n = 5) compared with 186 mg/L for the subjects with the PON2 GG148 genotype and cysC-eGFR >60 ml/min per 1.73 m^2 (n = 15). This reduction in PON1 mass is comparable to that observed in patients on dialysis (dialysis 129.5 ± 43.3 vs control 189.5 ± 44.1, mg/L PON1) (J. Kanampuza et al., unpublished observations). Thus, in patients with diabetic nephropathy, but without renal failure, the presence of the PON2 AA148 genotype is associated with a metabolic profile that is indistinguishable from renal failure requiring dialysis.

In conclusion, we have found a significant, independent inverse association of PON1 with cystatin C, a novel marker of GFR. Future studies of subjects at risk for vascular disease will be required to determine whether the association of PON1 with inflammatory markers is part of a complex including decreased renal function.

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TABLE 7. Regression analysis with ln PON1 as the dependent variable and cystatin C eGFR as less than or greater than 60 ml/min/1.73m^2

Step	Variable	Partial r^2	Model r^2	β	SEM	t	*P*
(n = 106)	Intercept			4.91	0.099	49.45	<0.0001
1	PON2 "AA"	0.1469	0.1469	−0.15	0.035	−4.29	<0.0001
2	CysC-eGFR <60	0.0652	0.2121	0.161	0.061	2.62	0.01
3	HDLC	0.0627	0.2747	0.215	0.064	3.35	0.0011
4	ln duration	0.0330	0.3078	−0.042	0.02	−2.15	0.034
Duration <15 years							
(n = 95)	Intercept			4.83	0.107	45.35	<0.0001
1	PON2 "AA"	0.126	0.126	−0.14	0.037	−3.82	0.0003
2	HDLC	0.0756	0.2016	0.193	0.068	2.84	0.0057
3	CysC-eGFR <60	0.0586	0.2602	0.196	0.074	2.64	0.0098

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