

Association between *PON1* L/M55 Polymorphism and Plasma Lipoproteins in Two Canadian Aboriginal Populations

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Serum paraoxonase circulates on a subfraction of high density lipoproteins and appears to use phospholipids on both low and high density lipoprotein particles as a physiological substrate. This functional relationship could explain the reported associations between common variation in the *PON1* gene – at codons 55 and 192 – and phenotypes related to atherosclerosis and lipoprotein metabolism. We evaluated associations between plasma lipoproteins and *PON1* L/M55, *PON1* Q/R192 and *PON2* A/G148 polymorphisms in samples from two Canadian aboriginal populations, namely the Oji-Cree and the Inuit. In diabetic Oji-Cree, we found that carriers of *PON1* M55 had a higher mean plasma triglyceride concentration than non-carriers. In non-diabetic Oji-Cree, we found that carriers of *PON1* M55 had higher mean plasma concentrations of total and low density lipoprotein cholesterol and apo B than non-carriers. In Inuit, we found that carriers of *PON1* M55 had higher mean plasma concentrations of total and low density lipoprotein cholesterol than non-carriers. The other polymorphic markers were not associated with variation in any plasma lipoprotein trait. Thus, the *PON1* M55 allele appeared to be associated with deleterious changes in the plasma lipoprotein profile from two independent Canadian aboriginal samples. These results suggest that common variation in *PON1* codon 55 is associated with variation of intermediate traits in plasma lipoprotein metabolism in aboriginal Canadians.

Key words: Metabolism; Atherosclerosis; Oxidation; Polygenic disease.

Abbreviations: BMI, body mass index; CHD, coronary heart disease; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride.

Introduction

The paraoxonase gene family on chromosome 7q21.3–22.1 contains three genes, namely *PON1*, *PON2*, and *PON3* (1, 2). The *PON1* gene product is serum paraoxonase. Serum paraoxonase is associated with high density lipoprotein (HDL) particles, probably via interaction with apo AI (3–6). Serum paraoxonase functions as an esterase towards organophosphate insecticides (5). *In vitro*, paraoxonase can destroy the pro-inflammatory modified lipids on low density lipoproteins (LDL) that can participate in the development of atherosclerosis (5–9). In particular, substrates for paraoxonase include an oxidized arachidonic acid derivative at the sn-2 position of LDL phospholipids (10), which can initiate the cellular interactions that are characteristic of the early stages of atherosclerosis (3, 11). This latter activity of serum paraoxonase suggests a role in atherogenesis. In contrast, the physiological roles of the *PON2* and *PON3* gene products are unknown (5).

The activity and expression of the *PON1* gene product appears to be modulated by two polymorphisms within the *PON1* gene (3, 5, 7, 12). The first polymorphism is at codon 54 or 55, depending upon the nucleotide taken to be the transcription start site (3), where a T→A nucleotide base change gives rise to a L→M amino acid change. The second polymorphism is at codon 191 or 192, depending upon the nucleotide taken to be the transcription start site (3), where an A→G nucleotide base change gives rise to a Q→R amino acid change. The *PON1* Q/R192 polymorphism has been associated with a wide range of inter-individual differences in activity toward paraoxon (5, 8, 13). Some studies have shown an association between the *PON1* Q/R192 polymorphism and coronary heart disease (CHD) (14, 15), while others have not (16, 17). The *PON1* L/M55 polymorphism has been associated with variation in serum concentrations of paraoxonase, with carriers of the L55 allele showing higher concentrations than carriers of the M55 allele (18–20). As with the *PON1* Q/R192 polymorphism, there are inconsistent reports of associations between the *PON1* L/M55 polymorphism and CHD (21–23).

We have previously reported an association between *PON1* Q/R192 and variation in plasma total and LDL cholesterol and apo B in Canadian Oji-Cree children (24). We have also previously reported the absence of an association between *PON1* Q/R192 and plasma lipoproteins in Canadian Inuit (25). In the present study, we evaluated the association between variation in levels of fasting plasma lipoprotein traits and the *PON1* codon 55 genotype in Oji-Cree with and without type 2 diabetes, and in Inuit.

Subjects and Methods

Study subjects

The Oji-Cree sample was comprised of residents of the Sandy Lake reserve in northwestern Ontario. Sandy Lake is an isolated community approximately 2000 km northwest of Toronto, in the subarctic boreal forest of central Canada.

Seven hundred and twenty eight community members, or 72% of eligible subjects, ranging in age from 10 to 79 years, participated in the Sandy Lake Health and Diabetes Project, which was a survey of diabetes and cardiovascular risk factors (26). The survey included an interviewer-administered questionnaire and a clinical examination. Fasting blood samples were obtained for measurement of biochemical traits and determination of genotypes as previously described (26). Diabetes was diagnosed according to pre-1997 criteria (26). Subjects diagnosed with impaired glucose tolerance were excluded from the present study. The project was approved by the University of Toronto Ethics Review Committee.

The Inuit study sample was comprised of residents of eight communities from the Keewatin region of the Northwest Territories, primarily from the eastern areas adjacent to Hudson Bay. A total of 516 unrelated individuals ranging in age from 18 to 80 years, participated in this study. From these, 281 reported themselves as Inuit, 92 of European (white) origin, 112 of mixed Inuit/European background, and the remainder were of another ethnic background. The proportions were representative of the ethnic architecture of the Northwest Territories determined from a 1986 population census (25). An interviewer-administered questionnaire and a clinical examination of all individuals were included in the study. Examinations included the determination of body mass index (BMI), defined as weight/height² (kg/m²). Fasting plasma samples were obtained with informed consent. Only non-diabetic subjects with at least two grandparents of Inuit origin were included in the present analysis. The project had ethical approval from the Universities of Manitoba and Toronto.

Biochemical and genetic analyses

For the Oji-Cree sample, sufficient DNA and phenotypic information were obtained from 115 diabetic subjects (of whom 45 were newly diagnosed) and from 478 non-diabetic subjects. Fasting (12 hours) plasma concentrations of total, HDL and LDL cholesterol, triglyceride (TG), apo B, and apo AI were determined as previously described (27, 28). For the Inuit sample, sufficient DNA and phenotypic information were obtained from 243 subjects with at least two Inuit grandparents. Fasting plasma concentrations of total, HDL and LDL cholesterol and TG in the Inuit subjects were determined as previously described (25).

Published primer sequences and PCR protocols (13) were used to determine *PON1* codon 55 genotypes, with the only modification being a decrease in the annealing temperature from 61°C to 55°C. Restriction analysis was performed on all PCR products using *NotI* digestion on a 10% polyacrylamide gel. A fragment derived from the L55 allele yielded an uncut, 170 bp band. A fragment derived from the *PON1* M55 allele yielded two fragments of 134 and 36 bp. *PON1* codon 192 and *PON2* codon 148 genotypes were determined as described (29).

Statistical analyses

Statistical Analysis System software SAS (version 6.12, SAS Institute, Cary, North Carolina, USA) was used for all statistical comparisons. Significant deviations of observed genotype

frequencies from those predicted by the Hardy-Weinberg equation were evaluated with χ^2 analysis. In addition, χ^2 analysis was used to determine potential differences in proportions of genotypes between subjects with and without type 2 diabetes from the Sandy Lake sample, and between subjects of Inuit and non-Inuit (white) origin from the Inuit sample.

Each lipoprotein variable had a distribution that was significantly non-normal. Consequently, each variable was logarithmically transformed and subjected to analysis of normality. As a result, total, LDL, and HDL cholesterol, TG, apo AI, and apo B had distributions that were not significantly different from normal.

Stepwise multivariate regression analysis in SAS was used to determine sources of variation for the log transformed lipoprotein traits in Oji-Cree subjects with and without type 2 diabetes and in Inuit subjects. In the analysis of Oji-Cree subjects, the independent variables were sex, diabetes status, BMI, age, *PON1* codon 55, *PON1* codon 192, and *PON2* codon 148 genotypes, and family of origin, which was included to correct for the influence of relatedness. In the analysis of Inuit subjects, the independent variables were sex, BMI, age, and the *PON1* codon 55, *PON1* codon 192, *PON2* codon 148 genotypes. For the Inuit sample, *PON1* codon 55 genotype was tested assuming a dominant model of inheritance, by setting the genotype variable to 0 for L55/L55 subjects and 1 for both L55/M55 and M55/M55 subjects. The other genetic variables were treated as co-dominant determinants of plasma lipoprotein traits. In *post hoc* analyses, other models of inheritance were also tested. ANOVA, with age, sex and BMI as co-variables, was used to compare mean values of quantitative traits according to genotypes.

Estimates of pairwise linkage disequilibrium between the markers used in this study were done using the Hill and Robertson's coefficient of linkage disequilibrium (30). The nominal level of significance for all *a priori* analyses was $p < 0.05$.

Results

Baseline phenotypic characteristics

The baseline clinical and biochemical characteristics of the 115 Oji-Cree subjects with type 2 diabetes, the 478 Oji-Cree subjects without type 2 diabetes and the 243 Inuit subjects are shown in Table 1.

Allele and genotype frequencies

The *PON1* codon 55 allele and genotype frequencies for Oji-Cree and Inuit samples are shown in Table 2. The *PON1* codon 55 genotype frequencies in all subgroups of both aboriginal samples did not deviate significantly from those predicted by the Hardy-Weinberg equation (Table 2). The *PON1* codon 55 genotype frequencies did not differ between Oji-Cree with and without diabetes ($\chi^2 = 0.043$ $p = NS$).

Among Inuit subjects, nine had the *PON1* M55/M55 genotype, 36 had the L55/M55 genotype, and 253 had the L55/L55 genotype. Due to the relatively small number of M55/M55 homozygotes, these subjects were analysed together with subjects with the L55/M55 genotype. This created a dominant model of inheritance for the *PON1* M55 allele for subsequent hypothesis testing. In one *post hoc* analysis, all three genotypes

Tab. 1 Baseline clinical and biochemical characteristics of the Oji-Cree and Inuit (means \pm SD).

	Oji-Cree		Inuit
	With diabetes	Without diabetes	
Number	115	478	243
Age (years)	44.5 \pm 15.4	25.4 \pm 12.6	37.1 \pm 15.4
BMI (kg/m ²)	30.2 \pm 4.8	25.5 \pm 5.6	26.5 \pm 4.5
Cholesterol (mmol/l)			
– Total	5.08 \pm 0.87	4.29 \pm 0.91	4.98 \pm 1.03
– LDL	2.95 \pm 0.71	2.46 \pm 0.74	3.05 \pm 0.89
– HDL	1.19 \pm 0.32	1.27 \pm 0.56	1.44 \pm 0.43
Triglycerides (mmol/l)	2.04 \pm 0.90	1.26 \pm 0.61	1.07 \pm 0.45
Apolipoproteins (g/l)			
– A1	1.51 \pm 0.31	1.48 \pm 0.24	ND
– B	1.31 \pm 0.32	0.99 \pm 0.30	ND

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; ND, not determined; SD, standard deviations; BMI, body mass index

Tab. 2 *PON1* and *PON2* allele and genotype frequencies of the Oji-Cree and Inuit.

	Oji-Cree		Inuit
	With diabetes	Without diabetes	
<i>PON1</i> codon 55			
L55 allele	0.9913	0.9927	0.963
M55 allele	0.0087	0.0073	0.037
L55/L55	0.9826	0.9854	0.934
L55/M55	0.0174	0.0146	0.066
<i>PON1</i> codon 192			
Q192 allele	0.80	0.76	0.70
R192 allele	0.20	0.24	0.30
Q192/Q192	0.64	0.578	0.49
Q192/R192	0.32	0.365	0.42
R192/R192	0.04	0.057	0.09
<i>PON2</i> codon 148			
A148 allele	0.72	0.72	0.73
G148 allele	0.28	0.28	0.27
A148/A148	0.518	0.52	0.533
A148/G148	0.403	0.40	0.394
G148/G148	0.078	0.08	0.073

were analyzed separately under a codominant model of expression. In a second *post hoc* analysis, the L55/M55 heterozygotes were grouped together with the L55/L55 homozygotes, creating a recessive model of expression. In contrast, in the Oji-Cree sample, no M55/M55 homozygotes were found (Table 2), so that only a dominant model was evaluated.

Linkage disequilibrium

For the Oji-Cree sample, Hill and Robertson's pairwise coefficients of linkage disequilibrium (D) were as follows: *PON1* codon 55 vs. *PON1* codon 192, D=0.004, r=0.09 (p=6 x 10⁻⁶); *PON1* codon 55 vs. *PON2* codon 148, D=-0.001, r=-0.024 (p=0.52); for *PON1* codon 192 vs.

PON2 codon 148, D=0.058, r=0.31 (p=2 x 10⁻²⁵). For the Inuit sample, the pairwise coefficients of linkage disequilibrium (D) were as follows: *PON1* codon 55 vs. *PON1* codon 192, D=0.051, r=0.07 (p=5 x 10⁻¹⁵); *PON1* codon 55 vs. *PON2* codon 148, D=-0.015, r=-0.015 (p=0.54); for *PON1* codon 192 vs. *PON2* codon 148, D=0.011, r=0.049 (p=0.052). Therefore, in both samples, there was linkage disequilibrium between the two markers in *PON1* and also between the *PON1* codon 192 and *PON2* codon 148 polymorphisms, but no significant linkage disequilibrium between the *PON1* codon 55 and *PON2* codon 148 polymorphisms. Rather than being an accurate reflection of relationships between alleles, these latter results might have reflected the small numbers of subjects with the *PON2* M55 allele.

Regression analysis for sources of variation in plasma lipoproteins

The results from the stepwise multivariate regression analysis from the Oji-Cree are shown in Table 3. For the Oji-Cree with diabetes, each plasma trait, except total cholesterol, had at least one significantly associated independent variable. The *PON1* codon 55 genotype was

a significant source of variation only for total TG ($p=0.018$). The proportion of TG variation that was due to the *PON1* codon 55 genotype was ~27% of the attributable variation, or ~4.5% of the total variation (Table 3a).

For Oji-Cree without diabetes, each plasma trait had at least three significantly associated independent variables (Table 3b). Age, sex and BMI were significant de-

Tab. 3a Stepwise regression analysis for determinants of variation in plasma lipoproteins in Oji-Cree with diabetes.

	Determinant	Partial r^2	F-value	Probability>F
log total cholesterol	*			
log LDL cholesterol	Sex	0.0394	4.38	0.039
log HDL cholesterol	Sex	0.0949	12.6	0.0006
	Age	0.0948	11.4	0.001
	Family ID	0.0225	3.05	NS(0.08)
log triglycerides	Age	0.1045	12.7	0.0005
	Family ID	0.0168	2.16	NS(0.14)
	<i>PON1</i> codon 55	0.0455	5.78	0.0179
log apo B	Sex	0.0322	3.62	NS(0.06)
log apo AI	Sex	0.0847	10.1	0.002
	Age	0.0488	6.08	0.015
	Family ID	0.0215	2.72	NS(0.100)

*All p-values were NS ($p>0.15$); abbreviations as Table 1; NS, not significant

Tab. 3b Stepwise regression analysis for determinants of variation in plasma lipoproteins in Oji-Cree without diabetes.

	Determinant	Partial r^2	F-value	Probability>F
log total cholesterol	Age	0.2676	173.6	0.0001
	BMI	0.0182	12.1	0.0006
	Sex	0.0096	6.44	0.011
	<i>PON1</i> codon 55	0.0054	3.66	NS(0.056)
	Family ID	0.0047	3.16	NS(0.076)
log LDL cholesterol	Age	0.2442	153.1	0.0001
	BMI	0.0363	23.9	0.0001
	Sex	0.0167	11.2	0.0009
	<i>PON1</i> codon 55	0.0085	5.76	0.017
	Family ID	0.0051	3.47	NS(0.063)
log HDL cholesterol	BMI	0.0985	51.9	0.0001
	Age	0.0198	10.6	0.001
	Sex	0.0153	8.34	0.004
log triglycerides	BMI	0.2139	129.2	0.0001
	Age	0.0247	15.4	0.0001
	Sex	0.0055	3.47	NS(0.063)
log apo B	Age	0.2474	156.2	0.0001
	BMI	0.0737	51.5	0.0001
	Sex	0.0260	18.9	0.0001
	<i>PON1</i> codon 55	0.01	7.13	0.008
	Family ID	0.0065	4.82	0.029
log apo AI	Age	0.0370	18.2	0.0001
	BMI	0.0243	12.2	0.0005
	Sex	0.0114	5.80	0.016

Abbreviations as Table 1; NS, not significant

Tab. 4 Stepwise regression analysis for determinants of variation in plasma lipoproteins in Inuit.

	Determinant	Partial r ²	F-value	Probability>F
log total cholesterol	Age	0.2109	63.1	0.0001
	<i>PON1</i> codon 55	0.0193	5.89	0.016
	BMI	0.0135	4.18	0.042
	Sex	0.0072	2.23	NS(0.136)
log LDL cholesterol	Age	0.1423	38.5	0.0001
	BMI	0.0237	6.55	0.011
	<i>PON1</i> codon 55	0.0224	6.35	0.012
log HDL cholesterol	Age	0.1012	26.1	0.0001
	BMI	0.0938	26.9	0.0001
	Sex	0.0231	6.78	0.010
log triglycerides	BMI	0.1178	31.0	0.0001
	Sex	0.0192	5.13	0.024

Abbreviations as in Table 1; NS, not significant at p<0.05.

terminants for each plasma trait Oji-Cree without diabetes. The *PON1* codon 55 genotype was a source of significant variation for LDL cholesterol and apo B (p=0.02 and 0.008, respectively). *PON1* codon 55 also tended to be associated with variation in plasma total cholesterol (p=0.056). The proportion of plasma LDL cholesterol and apo B variation that was due to the *PON1* codon 55 genotype was ~2.7% and ~2.8% of attributable variation, or ~0.9% and ~1.0% of total variation, respectively.

Results from the stepwise multivariate regression analysis from the Inuit sample, under a dominant model of inheritance for *PON1* M55 are shown in Table 4. BMI was a significant source of variation for each dependent variable. The *PON1* codon 55 genotype was found to be a significant source of variation for both total and LDL cholesterol. In separate analysis, *PON1* codon 55 was also found to be a significant source of variation in these traits under a co-dominant model of inheritance but not a recessive model. In both dominant and co-dominant models, the proportion of the attributable variation that was due to *PON1* codon 55 genotype was ~8% for total and ~12% for LDL cholesterol, or ~1.5% and ~2% of the total variation, respectively.

Clinical and biochemical variables according to PON1 codon 55 genotype in Oji-Cree

The clinical and biochemical variables for Oji-Cree subjects with and without type 2 diabetes are shown in Table 5. For Oji-Cree with diabetes, there were no significant differences between *PON1* codon 55 genotypes for either age or BMI (Table 5a). Plasma total, LDL, and HDL cholesterol, apo B and apo AI were not significantly different between genotypes. However, *PON1* L55/M55 heterozygotes had significantly higher mean plasma TG, by ~50%, compared to L55/L55 homozygotes (p=0.023).

For Oji-Cree without type 2 diabetes, the mean age and BMI were not significantly different between genotypes (Table 5b). Plasma concentrations of total and HDL cholesterol, and apo AI were also not significantly

Tab. 5a Clinical features and plasma lipoproteins in Oji-Cree with diabetes grouped according to *PON1* codon 55 genotype (mean±SD).

	L55/M55	L55/L55
Number/females	2/1	113/69
Age (years)	42.8±0.97	44.5±15.5
BMI (kg/m ²)	29.8±0.74	30.2±4.81
Cholesterol (mmol/l)		
– Total	5.80±0.89	5.06±0.92
– LDL	2.91±0.89	2.94±0.72
– HDL	0.99±0.11	1.19±0.30
Triglycerides (mmol/l)	3.87±1.27	2.00±0.91**
Apolipoproteins (g/l)		
– AI	1.49±0.41	1.51±0.25
– B	1.48±0.11	1.30±0.31

Abbreviations as in Table 1; **p<0.05

Tab. 5b Clinical features and plasma lipoproteins in Oji-Cree without diabetes grouped according to *PON1* codon 55 genotype (mean±SD).

	L55/M55	L55/L55
Number/females	7/4	471/260
Age (years)	25.9±13.4	25.4±12.6
BMI (kg/m ²)	24.5±6.8	25.5±5.6
Cholesterol (mmol/l)		
– Total	4.79±0.92	4.27±0.87**
– LDL	3.00±0.83	2.44±0.75**
– HDL	1.19±0.36	1.27±0.27
Triglycerides (mmol/l)	1.30±0.55	1.25±0.61
Apolipoproteins (g/l)		
– AI	1.43±0.31	1.48±0.21
– B	1.20±0.26	0.99±0.27**

Abbreviations as in Table 1; **p<0.05

Tab. 6 Plasma lipoprotein traits in Inuit grouped according to *PON1* genotype.

	M55/M55 & L55/M55	L55/L55
Number/females	17/11	226/121
Age (years)	34.2±12.9	37.3±15.5
BMI (kg/m ²)	26.3±4.18	26.6±4.48
Cholesterol (mmol/l)		
– Total	5.34±0.92	4.95±1.05**
– LDL	3.42±0.83	3.02±0.92**
– HDL	1.39±0.48	1.44±0.40
Triglycerides (mmol/l)	1.12±0.54	1.06±0.55

Abbreviations as in Table 1; **p<0.05.

different. However, significant differences between genotypes were seen for the non-diabetic subjects for LDL cholesterol (p=0.045) and apo B (p=0.032). Plasma LDL cholesterol was ~20% higher in L55/M55 heterozygotes than in the L55/L55 homozygotes. As well, for the non-diabetic subjects, plasma apo B levels were significantly higher in the heterozygotes than in the homozygotes by ~16%. In contrast to the diabetic subjects, plasma TG did not vary significantly between genotypes.

Clinical and biochemical variables according to PON1 codon 55 genotype in Inuit

For the Inuit, the mean biochemical and clinical traits according to *PON1* codon 55 genotype are shown in Table 6. There were no significant differences between genotypes for either age or BMI. There were no significant differences between genotypes in HDL cholesterol. Significant differences were seen between L55/L55 homozygotes and the L55/M55 and M55/M55 subjects for total and LDL cholesterol (p=0.027 and 0.020, respectively). Plasma total and LDL cholesterol were ~7% and ~12% higher in M55 carriers than in L55/L55 homozygotes.

Discussion

The principal finding in this analysis of Oji-Cree and Inuit was the significant association between variation in *PON1* at codon 55 and variation in fasting plasma concentrations of certain lipoproteins. In particular, a consistent finding in the non-diabetic Oji-Cree and Inuit was higher total and LDL cholesterol (and apo B in Oji-Cree) among carriers of the *PON1* M55 allele. In Oji-Cree subjects with diabetes, carriers of the *PON1* M55 allele had higher mean plasma TG than subjects with the other genotypes. The *PON1* codon 55 genotype contributed up to 25% of the attributable variation of these lipoprotein variables. In these study samples, there was no significant association with *PON1* codon 192 and *PON2* codon 148 genotypes.

There are at least two possible interpretations of the significant associations between variation in *PON1*

codon 55 and variation in plasma lipoproteins. First, this polymorphism might have a functional impact *in vivo* that could affect plasma lipoprotein metabolism. For example, *PON1* codon 55 genotype has been associated with variation in serum concentration of paraoxonase, which appears to be a consequence of production of different amounts of mRNA from the two alleles (20). This difference in serum concentration might affect oxidation of, lipid transfer between, or uptake of lipoprotein particles. The second interpretation of these associations is that in both Oji-Cree and Inuit samples, the *PON1* codon 55 alleles might have been in linkage disequilibrium with other functional mutations, either within the *PON* gene family, or with another gene locus elsewhere on chromosome 7. However, despite the evidence for strong linkage disequilibrium with *PON1* codon 192 genotype in both samples, the regression model indicated that the association was significant for *PON1* codon 55 genotype only. The linkage disequilibrium with *PON2* codon 148 was even less consistent in these samples. Therefore, if there was another functional allele at this locus that accounted for the associations with plasma lipoproteins, it was not likely to have been either *PON1* codon 192 or *PON2* codon 148.

While plasma LDL cholesterol and apo B were higher in Oji-Cree without diabetes who carried *PON1* M55, plasma TG were higher in Oji-Cree with diabetes who carried *PON1* M55. The difference in the association could be related to the different metabolic background in the diabetic subjects. It is possible that the metabolic perturbations that are characteristic of diabetes, such as increased flux of free fatty acids to the liver, increased hepatic lipogenesis, decreased LPL activity, and even altered serum paraoxonase activity (31), could have affected the genetic associations with the biochemical end point. It is of interest that the *PON1* M55 allele was associated with the more deleterious biochemical phenotype in Oji-Cree both with and without diabetes. It is plausible that in diabetic Oji-Cree the perturbed metabolic background influenced the association of lipoprotein phenotypes with variation in *PON1*-codon 55 genotype.

There have been some reported associations between *PON1* codon 55 variation and the risk of CHD. Interestingly, different alleles were associated with CHD in different studies. For example, two studies have shown that the *PON1* L55 allele was associated with increased risk of CHD in subjects with diabetes (18) and carotid atherosclerosis (22). This was consistent with *in vitro* observations, which found that HDL from L55/L55 homozygotes were the least effective at preventing LDL oxidation *in vitro* (7). However, a third study reported that the *PON1* M55 allele was associated with increased risk of CHD (23). Differences between these reported associations may have resulted from fundamental, underlying differences in the populations from which samples were collected. For example, the previously mentioned studies used either very few study subjects (7) or used subjects from heterogeneous populations (7, 18, 22, 23). In addition to possible differences in linkage disequilibrium with functional alleles,

the Oji-Cree and Inuit may have other unique attributes, such as distinctive environments. Our ability to detect relatively modest associations between variation in *PON1* codon 55 and plasma lipoproteins might have been related to smaller differences in background genetic and environmental variation than would be observed in these other heterogeneous populations.

The *PON1* L55 allele frequencies exceeding 0.95 in both of these aboriginal populations were similar to the reported frequency in Chinese subjects (21), but were different from the reported frequencies in Caucasians, which ranged from 0.57 to 0.74 (7, 13, 18, 22, 23). The low frequency of *PON1* M55 might be consistent with the historically low prevalence of CHD in Canadian aboriginal people. Also, the distinctive *PON1* allele frequencies in both Sandy Lake and Inuit might reflect the frequencies of the ancestral founders of these study samples. Ancestors of the current Oji-Cree population settled in the Sandy Lake region about 6000 years ago (32). Lifestyle was hunter-gatherer based until about 70 years ago. The current inhabitants were largely descended from one clan, whose allele frequencies would be reflected in contemporary allele frequencies. For the Inuit, this explanation has been proposed for the unique distribution of alleles of serological markers (33). The Inuit arrived in the central arctic about 5000 years ago. Ultimately, all North American Inuit are descended from migrants who crossed the land bridge from Asia, at various times during the Pleistocene Age (34). This could explain why the aboriginal subjects had comparable allele frequencies to present day Pacific Rim, but not Caucasian samples.

Another explanation for the distinctive *PON1* allele frequencies in both populations could be related to the impact of selection pressures in the local environments. It is possible that the *PON1* L55 allele might have imparted a survival benefit to carriers in the past. It is possible that higher serum paraoxonase concentrations in *PON1* L55 allele carriers in the past, might have imparted protection vs. an environmental toxin with a chemical structure related to OP compounds. It is also possible that infectious diseases have influenced the present distributions of *PON1* codon 55 alleles in both aboriginal populations. Infectious diseases, such as tuberculosis, are significant contributors to the mortality rate in Canadian aboriginal communities (35). If any "disease-resistance" genes were in linkage disequilibrium with the *PON1* or *PON2* markers, mortality due to susceptibility to infection could have produced past changes in allele frequencies. As well, persistent infections have been suggested to permanently alter lipoprotein profiles (36). Taken together, this may represent a potentially important gene-environment interaction where study subjects with particular chromosomal haplotypes who survived infection have changes in both their serum paraoxonase and lipoprotein phenotypes.

In summary, we report associations between *PON1* codon 55 genotype and variation in plasma lipoprotein concentrations in two independent Canadian aboriginal populations. While the number of subjects with the

PON1 M55 allele was small, the results indicated a deleterious influence of the *PON1* M55 allele upon plasma lipoproteins, and would be consistent with those previously reported studies that suggest a deleterious impact of the *PON1* M55 allele on vascular disease and related phenotypes. These results emphasize the importance of understanding even modest contributions of genetic variation to complex traits such as atherosclerosis. Over time, even these modest contributions have the potential to be associated with a significantly abnormal or deleterious phenotype, appearing later on in life as a result of the additional influence of secondary genetic or environmental factors. This is of particular importance for aboriginal populations, in which a continuing westernization of lifestyle may lead to an increased prevalence of metabolic diseases (35), and for which elucidating genotype-phenotype associations might aid in designing disease prevention protocols.

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