ORIGINAL RESEARCH

COMMON VARIANTS *APOC3, APOA5, APOE* AND *PON1* ARE ASSOCIATED WITH VARIATION IN PLASMA LIPOPROTEIN TRAITS IN GREENLANDERS

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ABSTRACT

Objectives. We undertook studies of the association between common genomic variations in *APOC3, APOA5, APOE* and *PON1* genes and variation in biochemical phenotypes in a sample of Greenlanders.

Study design. Genetic association study of quantitative lipoprotein traits.

Methods. In a sample of 1,310 adult Greenlanders, fasting plasma lipid, lipoprotein and apolipoprotein (apo) concentrations were assessed for association with known functional genomic variants of *APOC3*, *APOA5, APOE* and *PON1*. For significantly associated polymorphisms, between-genotype differences were examined in closer detail.

Results. We found that (1) the *APOE* restriction isotype was associated with variation in plasma total and LDL cholesterol and apo B (all p<.0001); (2) the *APOC3* promoter genotype was associated with variation in plasma triglycerides, HDL cholesterol and apo A-I (all p<.002); (3) the *APOA5* codon 19 genotype was associated with variation in plasma triglycerides (p=.027); and (4) the *PON1* codon 192 genotype was associated with variation in total and LDL cholesterol and apo B (all p<.05).

Conclusions. Taken together, our results suggest that common genetic variations in *APOC3*, *APOA5*, *APOE* and *PON1* are associated with significant variation in intermediate traits in plasma lipoprotein metabolism in Greenlanders; the associations are similar to those observed for these variants in other populations. *(Int J Circumpolar Health 2007; 66(5): 390-400)*

Keywords: apolipoproteins, genetics, single nucleotide polymorphisms, Greenland, complex disease, atherosclerosis

INTRODUCTION

Efforts to understand the genetic contribution to complex diseases will likely lead to improved diagnosis and prevention (1). One strategy to identify genetic determinants has been to study the association of single nucleotide polymorphisms (SNPs) of candidate genes involved in intermediate biochemical phenotypes, such as plasma lipoprotein concentration, that are risk factors for the development of complex diseases like atherosclerosis (2). While numerous candidate gene association studies of plasma lipoproteins have been reported in various populations, these studies have not been widely performed or reported among circumpolar people. There is a strong likelihood that genetic factors in these populations may provide stronger association signals because of relatively low variability in background genetic and environmental factors.

Numerous genetic polymorphisms (2–21) have been tested for their associations with plasma lipids and lipoproteins. Only a few have shown fairly consistent replicable results across many populations. Among the more replicable genetic associations with plasma lipoproteins are those reported for *APOC3*, *APOA5, APOE* and *PON1* genes encoding apolipoprotein (apo) E, apo C-III, apo A-V and serum paraoxonase-1, respectively. The role of these gene products in lipoprotein metabolism is shown schematically in Figure 1. *APOE* encodes apo E, a polymorphic protein that is a component of chylomicrons, very low density lipoproteins (VLDL) and high density lipoproteins (HDL) and also mediates their cellular uptake (3,4). The genetic polymorphisms of apo E result in common protein isoforms called E4, E3 and E2. In several populations, the E4 allele of *APOE* has been associated with higher plasma concentrations of total cholesterol (TC) (3,4), low-density lipoprotein (LDL) cholesterol and apo B (4,5), while correlating with lower concentrations of HDL cholesterol (4,6). *APOC3* encodes apo C-III, a protein that inhibits intravascular lipoprotein lipase (LPL) activity (7). The promoter polymorphism -455C>T within *APOC3* has been associated with increased concentration of plasma triglyceride (TG) (8) and decreased concentration of HDL cholesterol (9). *APOA5* encodes apo A-V, a component of chylomicrons, VLDL and HDL. The *APOA5* p.S19W polymorphism has been associated with elevated TG and lowered HDL cholesterol concentrations (10,11). Finally, serum paraoxonase-1, the product of *PON1*, is a component of a subfraction of HDL particles. The *PON1* p.Q192R polymorphism *PON1* is associated with variation in HDL and LDL cholesterol, TG and apo B (12,13).

In the present study, we evaluated whether common genomic variation in *APOC3*, *APOA5*, *APOE* and *PON1* would be associated with a variation in concentrations of plasma lipids (total cholesterol and TG), lipoproteins (LDL and HDL) and apolipoproteins (apos) in a sample of Greenlanders. Moreover, SNPs were selected based on (1) published association with plasma lipoproteins in other populations, and (2) evidence for function or biochemical impact of the polymorphism.

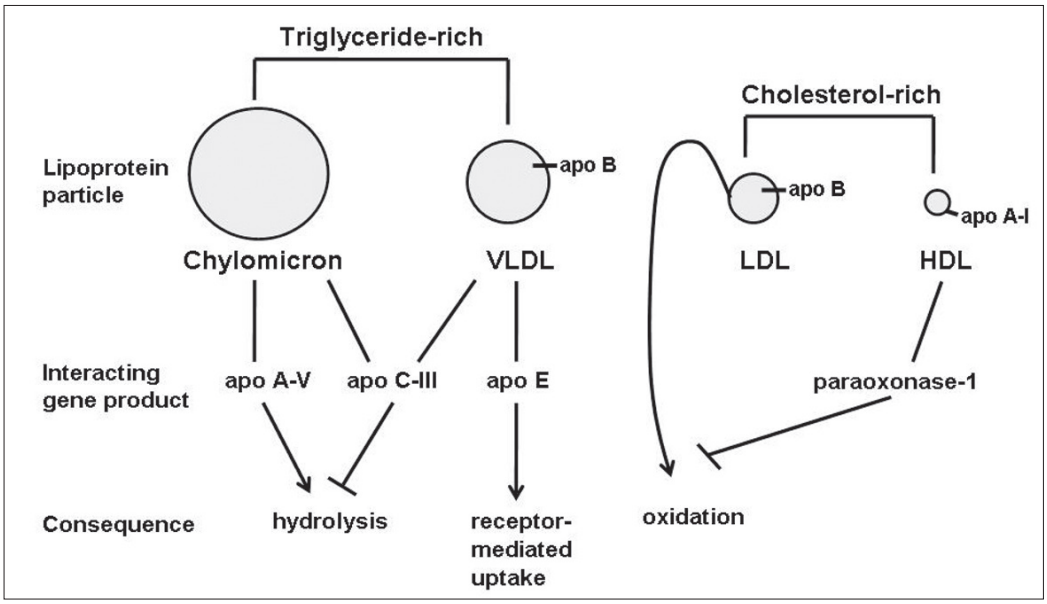

Figure 1. Overview of lipoprotein metabolism and evaluated gene products. The 2 main circulating lipids, namely triglyceride (TG) and cholesterol, are solubilised in plasma by being packaged in lipoprotein particles. The main TG-rich lipoproteins are chylomicrons – which carry intestine-derived TG, and very-low density lipoprotein (VLDL) – which carries liver-derived TG. The main cholesterol-rich lipoproteins are low-density lipoprotein (LDL) – which transports cholesterol from the liver to peripheral cells, and high-density lipoprotein (HDL) – which transports cholesterol from the periphery back to the liver. LDL can become oxidized and then can be deposited in arterial walls, leading to atherosclerosis, while HDL can remove cholesterol from arterial plaques. The family of proteins that are associated with lipoproteins are called apolipoproteins (apos). Apo B and A-I are the main proteins found on VLDL/LDL and HDL, respectively. Apos have many functions, including solubilisation of core lipids, activation of enzymes and acting as ligands for receptors. Apo B and A-I are measured with increasing frequency in clinical labs as an adjunct to LDL and HDL cholesterol. Apo A-V, encoded by the APOA5 gene, is a component of TG-rich lipoproteins that mediates hydrolysis by the enzyme lipoprotein lipase (LPL), as indicated by the arrow. On the other hand, apo C-III, encoded by the APOC3 gene, is a component of TG-rich lipoproteins that inhibits hydrolysis, as indicated by the blunt-ended line. Apo E, encoded by the APOE gene, is a structural component of VLDL that directs uptake of lipoproteins through cell surface receptors, as indicated by the arrow. Some HDL particles carry paraoxonase-1 encoded by the PON1 gene, an enzyme that slows LDL oxidation, as indicated by the blunt-ended line, and explains why HDL is considered 'good cholesterol'.

MATERIALS AND METHODS

Study subjects

Data were collected from March 1999 to September 2002, in random samples of adult Inuit from Denmark and 3 selected areas in Greenland (14). The total population of Greenland is 56,000 of whom 90% are ethnic Greenland Inuit (Greenlanders) (14). Greenlanders have Inuit (Eskimo) genetic background with a substantial admixture of European-Danish genes. Moreover, they are closely related to the Inuit and Yupik in Canada, Alaska and Siberia. The study sample comprised Greenlanders aged >35 years living in 3 areas of West Greenland: Nuuk (population 14,000), Qasigiannguit (population 1,400) and 4 villages in the district of Uummannaq (population 240–

275 each). In Nuuk a random sample of the population was invited to participate, while in Qasigiannguit and Uummannaq everyone was invited. The details of this study have been described elsewhere (15). Body mass index (BMI) was defined as weight/height^2 (kg/m^2). All participants provided informed consent in writing and orally. The relevant ethical review committees approved the study.

Biochemical and genetic analyses

Blood for lipoprotein analyses was centrifuged at 2,000 rpm for 30 minutes and the plasma was stored at -70°C. Plasma concentrations of lipids and lipoproteins were determined as described elsewhere (12,16,17). Apolipoproteins AI and B were measured using turbidimetric kits using a Cobas Mira S autoanalyzer from Roche Diagnostics (Laval, Quebec, Canada). Procedures, previously established, were used to extract leukocyte DNA and to determine genotypes of *APOE* exon 4 (E4, E3 and E2) (18); *APOC3* position -455nt (-455C>T) (19); *APOA5* codon 19 (p.S19W) (20); and *PON1* codon 192 (p.R192Q) (21).

Statistical analyses

Significance of the deviation of observed genotype frequencies from Hardy-Weinberg equilibrium were assessed using chi-square tests. Linkage disequilibrium between *APOC3* and *APOA5* alleles was determined using correlation coefficients of gene frequencies as described elsewhere (22). ANOVA was performed using the general linear models method to determine the sources of variation for plasma LDL cholesterol, HDL cholesterol, TC and TG, with F tests computed from the type III sums of squares (23). Independent covariates for each ANOVA were sex, age and BMI. In addition, independent class variables for ANOVA were 4 genotypes: *APOC3* position -455nt, *APOA5* codon 19, *APOE* restriction isotype and *PON1* codon 192. All statistical analyses were performed using SAS version 9.0 (Cary, NC), with a nominal level of significance of $p<0.05$.

RESULTS

Baseline demographic features

Baseline clinical and biochemical attributes of the study sample stratified by sex are shown in Table I. It is notable that mean HDL cholesterol and apo A-I concentrations in men were relatively higher than in other populations.

Baseline genetic attributes

All observed genotype frequencies did not deviate significantly from predictions of the Hardy-Weinberg equation. Therefore, only allele frequencies are shown in Table II.

Some allele frequencies were markedly different from those reported in other populations. The *APOE* E4 allele frequency for the Greenlanders was similar to Canadian Inuit (0.230) (24) but higher than in Caucasians (0.138) (25). The *APOE* E2 allele in Greenlanders was somewhat more frequent compared with Canadian Inuit (0.010) (24) but less frequent when compared with Caucasians (0.082) (25). The *APOC3* -455C allele was less frequent in Greenlanders compared with Canadian Inuit (0.478) (Pollex and Hegele, unpublished data) but more frequent when compared with Caucasians (0.35) (26). The *APOA5* p.W19 allele frequency in Greenlanders is higher than in Canadian Inuit (0.032) (Pollex and Hegele, unpublished data) but similar to Caucasians (0.059) (27). The *PON1* p.R192 allele frequency

in Greenlanders was considerably lower compared with Canadian Inuit (0.777) (28) but higher when compared with American-Caucasians (0.270) (17).

Determinants of plasma lipoproteins

A summary of the ANOVA is shown in Table III. Age and BMI were significant determinants of all plasma lipids, lipoproteins and apolipoproteins, while sex was significantly associated only with triglycerides and HDL cholesterol. The *APOE* restriction isotype was significantly associated with plasma total and LDL cholesterol and apo B concentrations. The *APOC3* genotype was significantly associated with plasma TG, HDL cholesterol and apo A-I concentrations. The *APOA5* p.S19W genotype was significantly associated with plasma TG concentration. Lastly, the *PON1* p.Q192R genotype was significantly associated with plasma total and LDL cholesterol and apo B concentrations.

Table I. Baseline characteristics (mean±SD) of male and female Greenlanders.

Measurement	Males	Females
n	576	734
Age, yrs	43.6±14.2	43.4±14.1
BMI, kg/m^2	26.0±4.5	26.4±5.3
Total cholesterol, mmol/L	5.89±1.17	5.91±1.15
Triglycerides, mmol/L	1.19±0.68	1.13±0.64
LDL cholesterol, mmol/L	3.82±1.07	3.80±1.07
HDL cholesterol, mmol/L	1.53±0.47	1.60±0.42
Apo B, g/L	0.89±0.08	0.89±0.08
Apo A-I, g/L	1.56±0.10	1.68±0.10

Table II. Allele frequencies in Greenland, Canadian Inuit and Caucasian populations.

Gene	Allele	Greenland	Canadian Inuit[a]	Caucasian[b]
APOE[1]	E4	0.220	0.230	0.138
(n=1301)	E3	0.751	0.760	0.780
	E2	0.029	0.010	0.082
APOC3[2]	-455T	0.593	0.478	0.650
(n=1308)	-455C	0.407	0.522	0.350
APOA5[3]	S19	0.947	0.968	0.941
(n=1293)	W19	0.053	0.032	0.059
PON1[4]	Q192	0.492	0.223	0.710
(n=1305)	R192	0.508	0.777	0.290

[1]*APOE*, apo E gene exon 4 restriction isotype.
[2]*APOC3*, apo C-III gene -455 nt genotype; -445T, T at nt -455; -455C, C at nt -455.
[3]*APOA5*, apo A-V gene codon 19 genotype; S19, serine at codon 19; W19, tryptophan at codon 19.
[4]*PON1*, serum paroxonase-1 gene codon 192 genotype; Q192, glutamine at codon 192; R192, arginine at codon 192.
[a]For *APOE*, n=175 (24); for *APOC3*, n=139 (Pollex and Hegele, unpublished data); for *APOA5*, n=140 (Pollex and Hegele, unpublished data); for *PON1*, n=509 (28).
[b]For *APOE*, n=336 (25); for *APOC3*, n=200 (26); for *APOA5*, n=237 (27); for *PON1*, n=793 (17).

The nature of the significant associations was further explored with comparisons of genotypic means, shown in Table IV.

Least-squares (adjusted for age, sex and BMI) are shown in Table IV, so these differ slightly from mean values shown in Table I. *APOE4* genotype showed the well-established gradient of concentrations for total and LDL cholesterol and apo B, specifically E4/E4 > E4/E3 > E3/E3 > E3/E2 > E2/E2, with E4/E2 subjects showing relatively low mean levels of these biochemical traits. In addition, *PON1* genotypes showed an association with total and LDL cholesterol and apo B, with p.R192 homozygotes (R/R) showing the highest level of these traits; the absolute levels were higher than those previously observed in Canadian Inuit (28) but similar to those reported in Hutterite-Caucasians (17). *APOC3* and *APOA5* genotypes both showed associations with plasma TG. *APOC3* -455C homozygotes (C/C) had the highest plasma TG and lowest plasma HDL cholesterol and apo A-I concentrations, as previously observed (19). In addition, the *APOA5* p.W19 carriers had the highest plasma TG, as has been repeatedly reported in other populations (10,29).

Table III. Determinants of plasma lipoproteins in Greenlanders according to the analysis of variance.

Dependent Variable: Source of Variation	*df*	Total cholesterol		Triglycerides	
		F	P≥F	F	P≥F
Sex	1	0.30	ns(.58)	5.15	0.024
Age	1	109.0	<.0001	30.5	<.0001
BMI	1	42.8	<.0001	225.8	<.0001
APOE restriction isotype	5	7.18	<.0001	2.20	ns(.052)
APOC3 genotype	2	0.90	ns(.41)	11.2	<.0001
APOA5 genotype	2	0.76	ns(.47)	3.62	.027
PON1 genotype	2	5.69	.0035	0.09	ns(.91)
		LDL cholesterol		HDL cholesterol	
Sex	1	0.030	ns(.86)	11.0	.0009
Age	1	70.9	<.0001	109.5	<.0001
BMI	1	69.1	<.0001	160.0	<.0001
APOE restriction isotype	5	8.90	<.0001	1.42	ns(.21)
APOC3 genotype	2	1.15	ns(.32)	9.07	.0001
APOA5 genotype	2	0.86	ns(.42)	1.33	ns(.27)
PON1 genotype	2	7.54	.0006	0.63	ns(.53)
		Apo B		Apo A-I	
Sex	1	0.030	ns(.85)	53.8	<.0001
Age	1	5.21	.023	41.0	<.0001
BMI	1	83.3	<.0001	81.3	<.0001
APOE restriction isotype	5	12.2	<.0001	0.82	ns(.54)
APOC3 genotype	2	2.55	ns(.078)	6.28	.0019
APOA5 genotype	2	0.09	ns(.91)	1.45	ns(.24)
PON1 genotype	2	3.10	.045	1.31	ns(.27)

P≥F indicates probability of a greater between-group F value using ANOVA; ns, not significant with nominal p<.05; *APOE*, apo E gene exon 4 restriction isotype; *APOC3* apo C-III gene nt -455 genotype; *APOA5*, apo A-V gene codon 19 genotype; and *PON1*, serum paraoxonase-1 gene codon 192 genotype.

Table IV. Biochemical variables with significant associations with genotype in Greenlanders.

Variable	Gene	Genotype	n	Mean±SD
TC, mmol/L	*APOE* restriction isotype	E2/2	7	4.55±0.54[1]
		E3/2	44	5.65±0.40[1]
		E3/3	742	5.82±0.36
		E4/2	18	5.25±0.44[1]
		E4/3	426	5.99±0.36[2]
		E4/4	64	6.32±0.39[2]
	PON1, codon 192	Q/Q	332	5.55±0.38
		Q/R	620	5.50±0.37
		R/R	353	5.74±0.38[3]
TG, mmol/L	*APOC3*, -455nt	C/C	226	1.29±0.21[4]
		T/C	614	1.18±0.21
		T/T	468	1.06±0.21
	APOA5, codon 19	S/S	1157	1.18±0.05
		S/W	135	1.33±0.73[5]
		W/W	1	1.03±0.61
LDL-C, mmol/L	*APOE* restriction isotype	E2/2	7	2.60±0.50[1]
		E3/2	44	3.67±0.37[1]
		E3/3	742	3.90±0.33
		E4/2	18	3.33±0.41[1]
		E4/3	426	4.09±0.34[2]
		E4/4	64	4.39±0.36[2]
	PON1, codon 192	Q/Q	332	3.62±0.35
		Q/R	620	3.56±0.34
		R/R	353	3.82±0.35[3]
HDL-C, mmol/L	*APOC3*, -455nt	C/C	226	1.34±0.14[4]
		T/C	614	1.40±0.14
		T/T	468	1.48±0.14
Apo B, g/L	*APOE* restriction isotype	E2/2	7	0.65±0.16[1]
		E3/2	44	0.82±0.27[1]
		E3/3	742	0.91±0.23
		E4/2	18	0.77±0.14[1]
		E4/3	426	0.95±0.23[2]
		E4/4	64	1.06±0.29[2]
	PON1, codon 192	Q/Q	332	0.92±0.25
		Q/R	620	0.91±0.21
		R/R	353	0.95±0.25[3]
Apo A-I, g/L	APOC3, -455nt	C/C	226	1.69±0.29[4]
		T/C	614	1.74±0.30
		T/T	468	1.78±0.31

Abbreviations are as defined in Table II. LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.
[1]*APOE* E2/E2, E3/E2 and E4/E2 restriction isotypes had significantly lower ($p<0.05$) TC, LDL-C and Apo B than the E3/E3 genotype.
[2]*APOE* E4/E3 and E4/E4 restriction isotypes had significantly higher ($p<0.05$) TC, LDL-C and Apo B than the E3/E3 genotype.
[3]*PON1* R/R genotype had significantly higher ($p<0.05$) mean TC and LDL-C than the Q/Q genotype.
[4]*APOC3* C/C genotype had significantly higher ($p<0.05$) mean TG but lower HDL-C and Apo A-I than the T/T genotype.
[5]*APOA5* S/W genotype had significantly higher ($p<0.05$) mean TG than the S/S genotype.

DISCUSSION

The principal finding in this study of the Greenlanders was the identification of several significant associations between candidate genomic variants and fasting plasma lipoprotein concentrations, specifically: (1) the *APOE* restriction isotype was associated with variation in total and LDL cholesterol and apo B; (2) the *APOC3* -455nt genotype was associated with variation in HDL cholesterol and apo A-I; (3) the *APOA5* codon 19 genotype was associated with variation in TG; and (4) the *PON1* codon 192 genotype was associated with variation in total and LDL cholesterol and apo B. The nature and direction of all associations was similar to previous reports in other populations.

We found that *APOE* genetic variation was associated with variation in plasma concentrations of total and LDL cholesterol and apo B in a manner consistent with many other reports from diverse populations (5,30). Specifically, we found the E4 allele was associated with higher total and LDL cholesterol whereas the E2 allele was associated with lower total and LDL cholesterol (5,6). Overall, our results contrast with results from a previous study with 133 Greenlanders, in which the *APOE* restriction isotype did not associate with plasma lipoproteins (31). However, our study included a much larger number of subjects. Overall, the E4 allele of *APOE* has been established to be a consistent determinant of plasma apo B-containing lipoproteins and cardiovascular disease risk (32).

Although the precise function of apo C-III in lipid metabolism is not fully understood, apo C-III positively correlates with plasma TG concentrations (7). It has been further observed that overexpression of human apo C-III in mice results in hypertriglyceridemia (8), whereas the absence of *APOC3* in knockout mice leads to reduced TG concentrations (33). In addition, population studies have elucidated genetic association of *APOC3* with HDL cholesterol levels (9). The human *APOC3* gene has been mapped on chromosome 11 and a number of SNPs have been described within and around the gene as possible genetic markers of hypertriglyceridemia (9). One such common SNP has been described within the insulin response element of the promoter region -455T>C (34). The elevated TG and depressed HDL cholesterol and apo A-I associated with the -455C allele of the *APOC3* gene are consistent with associations seen in other populations (19,28). Association studies have repeatedly shown that the -455T allele is associated with lower plasma TG (35,36). In vitro studies have shown a 40-50% insulin-mediated decrease in *APOC3* gene expression in -455T-containing promoter constructs, which is lost in -455C-containing promoter constructs (37). Moreover, metabolic syndrome patients carrying the -455C allele are at an increased risk of cardiovascular disease (38). These observations suggest that the functional *APOC3* SNP at the insulin-response element mediates the widely replicated associations of hypertriglyceridemia and lower HDL cholesterol.

Apo A-V is predominantly located on TG-rich particles, such as chylomicrons and VLDL. The importance of apo A-V in hyperlipoproteinemia has been suggested by mice overexpressing human *APOA5* that exhibit a 35% decrease in cholesterol (39) and a 33% reduction in TG levels (40). Although many SNPs have been reported in the coding region of the *APOA5* gene, the p.S19W polymorphism is unique in that it is common, alters the amino acid sequence and is dysfunctional (41). Since this SNP is localized close to the N-terminal signal sequence, it

potentially affects the *APOA5* export rate from the liver (42). We found that the *APOA5* S19/W19 genotype was significantly associated with elevated plasma TG concentrations compared with the S19/S19 genotype. The significantly higher TG levels in S19/W19 heterozygotes is comparable to findings in Caucasian and African Americans (20,43,44).

Overall, major insight into the functions of both apolipoproteins (apo), C-III and A-V, were elucidated in transgenic and knockout mouse models (34). Consistent with our results, multiple studies have shown that SNPs of *APOC3* and *APOA5* are consistently associated with variations in plasma triglyceride (TG) levels (29,40,45,46). However, pair-wise linkage disequilibrium comparison performed in this study between *APOC3* and *APOA5* demonstrated that they are not linked (r=0.002, NS), suggesting independent mechanisms for the associations with plasma lipoproteins and related traits.

Finally, the *PON1* p.Q192R SNP is known to modulate the activity and expression of serum paraoxonase-1 (2), which is itself associated with variation in plasma lipid and lipoprotein concentrations (17,47). Moreover, serum paraoxonase-1 inhibits LDL oxidation through metabolism of bioactive lipid hydroperoxidases (48,49), thus potentially attenuating the initiation of atherosclerosis (19). While most studies have shown a positive association between the *PON1* R192 allele and coronary heart disease (50,51), a few have not (52,53). We found in Greenlanders that the high activity variant of serum paraoxonase-1, encoded by *PON1* R192, was associated with higher plasma concentrations total and LDL cholesterol and apo B, as observed previously in populations.

In summary, we have observed that common variants of *APOC3, APOA5*, *APOE* and *PON1* genes are associated with variation in the intermediate phenotypes in plasma lipoprotein metabolism among Greenlanders. Variations of these genes may further be associated with the development of atherosclerosis, when secondary genetic or environmental factors are present, although our sample was essentially healthy; furthermore, we did not have variables related to atherosclerosis end points for this analysis. Understanding the impact of environmental factors on a background of genetic predisposition is even more important in native populations, which may develop an increased prevalence of metabolic diseases as their lifestyles modernize (54). Furthermore, evaluating the genotype-phenotype associations may prove to be helpful in understanding potential cardiovascular risk and designing prevention strategies for this population (54).

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Conflict of interest disclosures

The authors declare no conflict(s) of interest.

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