OBJECTIVE 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.
Genetic determinants of lipoproteins in Greenlanders

**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–

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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'.
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 A1 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 (0.010) (24) but less frequent when compared with Caucasians (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/m²                 | 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 Inuita | Caucasianb |
|---------------|--------|-----------|-----------------|------------|
| APOE<sup>1</sup> | E4     | 0.220     | 0.230           | 0.138      |
| (n=1301)      | E3     | 0.751     | 0.760           | 0.780      |
|               | E2     | 0.029     | 0.010           | 0.082      |
| APOC3<sup>2</sup> | -455T  | 0.593     | 0.478           | 0.650      |
| (n=1308)      | -455C  | 0.407     | 0.522           | 0.350      |
| APOA5<sup>3</sup> | S19    | 0.947     | 0.968           | 0.941      |
| (n=1293)      | W19    | 0.053     | 0.032           | 0.059      |
| PON1<sup>4</sup> | Q192   | 0.492     | 0.223           | 0.710      |
| (n=1305)      | R192   | 0.508     | 0.777           | 0.290      |

<sup>1</sup> APOE, apo E gene exon 4 restriction isotype.
<sup>2</sup> APOC3, apo C-III gene -455 nt genotype; -445T,T at nt -455; -455C, C at nt -455.
<sup>3</sup> APOA5, apo A-V gene codon 19 genotype; S19, serine at codon 19; W19, tryptophan at codon 19.
<sup>4</sup> PON1, serum paroxonase-1 gene codon 192 genotype; Q192, glutamine at codon 192; R192, arginine at codon 192.

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).

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 homozgyotes (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. \( APOC3 -455C \) homogyotes (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.

| Source of Variation | df | Total cholesterol | Triglycerides | LDL cholesterol | HDL cholesterol |
|---------------------|----|-------------------|---------------|-----------------|-----------------|
|                     F | P<.05 | F | P<.05 | F | P<.05 |
| 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)         |

**P<.05 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<sup>1</sup> |
|          |      | E3/2 | 44 | 5.65±0.40<sup>1</sup> |
|          |      | E3/3 | 742| 5.82±0.36  |
|          |      | E4/2 | 18 | 5.25±0.44<sup>1</sup> |
|          |      | E4/3 | 426| 5.99±0.36<sup>2</sup> |
|          | *PON1*, codon 192 | Q/Q | 332| 5.55±0.38  |
|          |      | Q/R | 620| 5.50±0.37  |
|          |      | R/R | 353| 5.74±0.38<sup>3</sup> |
| **TG, mmol/L** | *APOC3*, -455nt | C/C | 226| 1.29±0.21<sup>4</sup> |
|          |      | T/C | 614| 1.18±0.21  |
|          |      | T/T | 468| 1.06±0.21  |
| **LDL-C, mmol/L** | *APOE* restriction isotype | E2/2 | 7  | 2.60±0.50<sup>1</sup> |
|          |      | E3/2 | 44 | 3.67±0.37<sup>1</sup> |
|          |      | E3/3 | 742| 3.90±0.33  |
|          |      | E4/2 | 18 | 3.33±0.41<sup>1</sup> |
|          |      | E4/3 | 426| 4.09±0.34<sup>2</sup> |
|          |      | E4/4 | 64 | 4.39±0.36<sup>2</sup> |
|          | *PON1*, codon 192 | Q/Q | 332| 3.62±0.35  |
|          |      | Q/R | 620| 3.56±0.34  |
|          |      | R/R | 353| 3.82±0.35<sup>3</sup> |
| **HDL-C, mmol/L** | *APOC3*, -455nt | C/C | 226| 1.34±0.14<sup>4</sup> |
|          |      | 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<sup>1</sup> |
|          |      | E3/2 | 44 | 0.82±0.27<sup>1</sup> |
|          |      | E3/3 | 742| 0.91±0.23  |
|          |      | E4/2 | 18 | 0.77±0.14<sup>1</sup> |
|          |      | E4/3 | 426| 0.95±0.23<sup>2</sup> |
|          |      | E4/4 | 64 | 1.06±0.29<sup>2</sup> |
|          | *PON1*, codon 192 | Q/Q | 332| 0.92±0.25  |
|          |      | Q/R | 620| 0.91±0.21  |
|          |      | R/R | 353| 0.95±0.25<sup>3</sup> |
| **Apo A-I, g/L** | *APOC3*, -455nt | C/C | 226| 1.69±0.29<sup>4</sup> |
|          |      | 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.

<sup>1</sup>*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.

<sup>2</sup>*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.

<sup>3</sup>*PON1* R/R genotype had significantly higher (p<0.05) mean TC and LDL-C than the Q/Q genotype.

<sup>4</sup>*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.

<sup>5</sup>*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
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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.
REFERENCES

1. Schork NJ. Genetically complex cardiovascular traits. Origins, problems, and potential solutions. Hypertension 1997;29:145–149.

2. Borjigt AP, Connelly PW, Brunt JH, Scherer SW, Tsui LC, Hegele RA. Genetic variation in paraoxonase-1 and paraoxonase-2 is associated with variation in plasma lipoproteins in Alberta Hutterites. Atherosclerosis 1998;139:131–136.

3. Kamboh MI, Aston CE, Ferrell RE, Hamman RF. Impact of apolipoprotein E polymorphism in determining interindividual variation in total cholesterol and low density lipoprotein cholesterol in Hispanics and non-Hispanic whites. Atherosclerosis 1993;98:201–211.

4. Larson IA, Ordovas JM, DeLuca C, Barnard JR, Feussner G, Schaefer EJ. Association of apolipoprotein (Apo)E genotype with plasma apo E levels. Atherosclerosis 2000;148:327–335.

5. Gerdes LU, Klausen IC, Sihm I, Faergeman O. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genet Epidemiol 1992;9:55–167.

6. Hallman DM, Boerwinkle E, Saha N et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 1991;49:338–349.

7. Wang CS, McConathy WJ, Kloer HU, Alauopovic P. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. J Clin Invest 1985;75:384–390.

8. Ito Y, Azrolan N, O’Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990;249:790–793.

9. Fiegenbaum M, de Andrade FM, Hutz MH. Association between plasma lipid parameters and APOC3 genotypes in Brazilian subjects: effect of gender, smoking and APOE genotypes. Clin Chim Acta 2002;380:175–181.

10. Talmud PJ, Hawe E, Martin S et al. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. Hum Mol Genet 2002;11:3039–3046.

11. van der Veuten GM, Isaacs A, Zeng VW et al. Haplotype analyses of the APOA5 gene in patients with familial combined hyperlipidemia. Biochim Biophys Acta 2007;1772:81–88.

12. Hegele RA, Brunt JH, Connelly PW. Multiple genetic determinants of variation in plasma lipoproteins in Alberta Hutterites. Arterioscler Thromb Vasc Biol 1995;15:861–871.

13. Ruiz J, Blanche H, James RW et al. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet 1995;346:869–872.

14. Jorgensen ME, Moustgaard H, Bjerringaard P, Bjor D-Christensen K. Gender differences in the association between westernization and metabolic risk among Greenland Inuit. Eur J Epidemiol 2006;21:741–748.

15. Bjerringaard P, Curtis T, Bor D-Christensen K et al. Inuit health in Greenland: a population survey of lifestyle and disease in Greenland and among Inuit living in Denmark. Int J Circumpolar Health 2003;62(Suppl 1):3–79.

16. Hegele RA, Evans AJ, Tu L, Ip G, Brunt JH, Connelly PW. A gene–gender interaction affecting plasma lipoproteins in a genetic isolate. Arterioscler Thromb 1994;14:671–678.

17. Hegele RA, Brunt JH, Connelly PW. A polymorphism of the paraoxonase gene associated with variation in plasma lipoproteins in a genetic isolate. Arterioscler Thromb Vasc Biol 1995;15:89–95.

18. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hpal I. J Lipid Res 1990;31:545–548.

19. Hegele RA, Connelly PW, Hanley AJ, Sun F, Harris SB, Zinnman B. Common genomic variation in the APOC3 promoter associated with variation in plasma lipoproteins. Arterioscler Thromb Vasc Biol 1997;17:2753–2758.

20. Hubacek JA, Skodova Z, Adamkova V, Lanska V, Poledne R. The influence of APOAV polymorphisms (T-1131>C and S19>W) on plasma triglyceride levels and risk of myocardial infarction. Clin Genet 2004;65:126–130.

21. Humbert R, Adler DA, Distech CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993;3:73–76.

22. Hill WG, Robertson A. Linkage disequilibrium in finite populations. Theor Appl Genet 1968;38:226–231.

23. SAS Institute Inc. SAS/STAT User’s Guide, Version 9.0: SI Inc.; 2007.

24. Hegele RA, Young TK, Connelly PW. Are Canadian Inuit at increased genetic risk for coronary heart disease? J Mol Med 1997;75:364–370.

25. Anuurad E, Lu G, Rubin J, Pearson TA, Berglund L. ApoE genotype affects allele-specific apo[a] levels for large apo[a] sizes in African Americans: the Harlem-Basset Study. J Lipid Res 2007;48:693–698.

26. Miller M, Rhyne J, Khatma M, Parekh H, Zeller K. Prevalence of the APOC3 promoter polymorphisms T-455C and C-482T in Asian-Indians. Am J Cardiol 2001;87:220–221, A228.

27. Chandak GR, Ward KJ, Yajnik CS et al. Triglyceride associated polymorphisms of the APOA5 gene have very different allele frequencies in Pune, India compared to Europeans. BMC Med Genet 2006;7:76.

28. Hegele RA, Connelly PW, Hanley AJ, Sun F, Harris SB, Zinnman B. Common genomic variants associated with variation in plasma lipoproteins in young aboriginal Canadians. Arterioscler Thromb Vasc Biol 1997;17:1060–1066.

29. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. Hum Mol Genet 2002;11:3031–3038.

30. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining plasma lipoproteins. Arterioscler Thromb Vasc Biol 1995;15:89–95.

31. de Knijff P, Johansen LG, Rosseneu M, Frants RR, Jepsen J, Havekes LM. Lipoprotein profile of a Greenland Inuit population. Influence of anthropometric variables, Apo E and A4 polymorphism, and lifestyle. Arterioscler Thromb 1992;12:1371–1379.
Genetic determinants of lipoproteins in Greenlanders

32. Ou T, Yamakawa-Kobayashi K, Arinami T et al. Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. Atherosclerosis 1998;137:23–28.

33. Maeda N, Li H, Lee D, Oliver P, Quarfordth SD, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. J Biol Chem 1994;269:23610–23616.

34. van Dijk KW, Rensen PC, Voshol PJ, Havekes LM. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? Curr Opin Lipidol 2004;15:239–246.

35. Qi L, Liu S, Rifai N, Hunter D, Hu FB. Associations of the apolipoprotein A1/C3/A4/A5 gene cluster with tri- glyceride and HDL cholesterol levels in women with type 2 diabetes. Atherosclerosis 2007;192:204–210.

36. Ruiz-Narvaez EA, Yang Y, Nakanishi Y, Kirchdorfer J, Campos H. APOC3/A5 haplotypes, lipid levels, and risk of myocardial infarction in the Central Valley of Costa Rica. J Lipid Res 2005;46:2605–2615.

37. Li WW, Dammerman MM, Smith JD, Metzger S, Breslow JL, Leff T. Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. J Clin Invest 1995;96:2601–2605.

38. Olivieri O, Martinelli N, Sandri M et al. Apolipoprotein C-III, n-3 polyunsaturated fatty acids, and “insulin-resistant” T-455C APOC3 gene polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of type 2 diabetes. Atherosclerosis 1998;137:2363–2367.

39. Fruchart-Najib J, Bauge E, Niculescu LS et al. Mechanism of triglyceride lowering in mice expressing human apolipoprotein A5. Biochem Biophys Res Commun 2004;319:360–367.

40. Pennacchio LA, Olivier M, Hubacek JA et al. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 2001;294:169–173.

41. Talmud PJ, Palmen J, Putt W, Lins L, Humphries SE. Determination of the functionality of common APOA5 polymorphisms. J Biol Chem 2005;280:28215–28220.

42. Hubacek JA. Apolipoprotein A5 and triglyceridemia. Focus on the effects of the common variants. Clin Chem Lab Med 2005;43:897–902.

43. Klos KL, Hamson S, Clark AG, Boerwinkle E, Liu K, Sing CF. APOA5 polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study. J Lipid Res 2005;46:564–571.

44. Lai CQ, Demissie S, Cupples LA et al. Influence of the APOA5 locus on plasma triglyceride, lipoprotein sub-classes, and CVD risk in the Framingham Heart Study. J Lipid Res 2004;45:2096–2105.

45. Waterworth DM, Talmud PJ, Bujac SR, Fisher RM, Miller GJ, Humphries SE. Contribution of apolipo- protein C-III gene variants to determination of triglyceride levels and interaction with smoking in mid- aged men. Arterioscler Thromb Vasc Biol 2000;20:2663–2669.

46. Lai CQ, Parnell LD, Ordovas JM. The APOA1/C3/ A4/A5 gene cluster, lipid metabolism and cardiovascular disease risk. Curr Opin Lipidol 2005;16:153–166.

47. Blatter MC, James RW, Messmer S, Barja P, Pometta D. Identification of a distinct human high-density li- poprotein sub-species defined by a lipoprotein-associ- ated protein, K-45. Identity of K-45 with paraoxo- nase. Eur J Biochem 1993;211:871–879.

48. Carlson CS, Heagerty PJ, Hatsukami TS et al. TagSNP analyses of the PON gene cluster: effects on PON1 activity, LDL oxidative susceptibility, and vascular disease. J Lipid Res 2006;47:1014–1024.

49. Watson AD, Berliner JA, Hama SY et al. Protective effect of high density lipoprotein associated paraox -onase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest 1995;96:2882–2891.

50. Pfohl M, Koch M, Enderle MD et al. Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery dis- ease, and myocardial infarction in type 2 diabetes. Di- abetes 1999;48:623–627.

51. Zama T, Murata M, Matsubara Y et al. A 192Arg vari- ant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. Arterioscler Thromb Vasc Biol 1997;17:3565–3569.

52. Fanella S, Harris SB, Young TK et al. Association be- tween PON1 L/M55 polymorphism and plasma lipo- proteins in two Canadian aboriginal populations. Clin Chem Lab Med 2000;38:413–420.

53. Rice GI, Ossei-Gerning N, Stickland MH, Grant PJ. The paraoxonase Gln-Arg polymorphism in sub- jects with ischaemic heart disease. Corron Artery Dis 1997;8:677–682.

54. Young TK, Moffatt ME, O’Neil JD. Cardiovascular diseases in a Canadian Arctic population. Am J Public Health 1993;83:881–887.