APC1 T45S polymorphism is associated with reduced obesity indices and lower plasma concentrations of leptin and apolipoprotein C-I in aboriginal Canadians

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Abstract  Apolipoprotein (apo) C-I is a constituent of chylomicrons, very low density lipoprotein, and high density lipoprotein. The role of apo C-I in human metabolism is incompletely defined. We took advantage of a naturally occurring amino acid polymorphism that is present in aboriginal North Americans, namely apo C-I T45S. We assessed the hypothesis that metabolic traits, including obesity-related and lipoprotein-related traits, would differ between carriers and noncarriers of apo C-I T45S. A genotyping assay was developed for APC1 T45S and genotypes were determined in a sample of 410 Canadian Oji-Cree subjects. The allele frequency of the apo C-I S45 allele was ~8% in this sample. We observed the apo C-I S45 allele was significantly associated with) lower percent body fat (P < 0.05), 2) lower waist circumference (P = 0.058), 3) lower serum leptin levels (P < 0.05), and 4) lower plasma apo C-I levels (P < 0.0001), using a newly developed ELISA-based method. Taken together, these results suggest that at the whole human phenotype level, apo C-I is associated with the complex metabolic trait of obesity as well as with serum leptin levels. —Lahiry, P., H. Cao, M. R. Ban, R. L. Pollex, M. Mamakeesick, B. Zinman, S. B. Harris, A. J. G. Hanley, M. W. Huff, P. W. Connelly, and R. A. Hegele. APC1 T45S polymorphism is associated with reduced obesity indices and lower plasma concentrations of leptin and apolipoprotein C-I in aboriginal Canadians. J. Lipid Res. 2010, 51: 843–848.

Supplementary key words nonsynonymous variant • leptin • Canadian First Nation population • genetics • serum levels • hypertriglyceridemic waist

Complex quantitative traits such as obesity are influenced by both genetic and environmental factors. Moreover, evaluation of the genetic contribution identifies gene products and their interactions in biological pathways, aiding in overall understanding of these complex traits. We have previously identified significant associations between genomic variants and complex traits in the Oji-Cree, an isolated Canadian First Nations population (1, 2). The Oji-Cree are an ideal population to study association of genetic factors with complex traits because their background genetic and environmental variation is relatively low.

Apolipoprotein (apo) C-I is a protein constituent of chylomicrons, VLDL, and HDL (3). Apo C-I is a member of the human apo C family, which also includes apo C-II and apo C-III. In contrast to other extensively investigated apolipoproteins such as apo E, B, and A1, and even apo C-II and C-III, the physiological role of apo C-I is less well established. In vitro, apo C-I has been suggested to be positively involved in HDL metabolism through activation of LCAT (4), inhibition of HL (5, 6), and inhibition of cholesteryl ester (CE) transfer protein (CETP) activity (7). Using in vivo models of apo C-I-deficient and apo C-I-over-expressing mice, apo C-I has also been suggested to have a positive role in cholesterol metabolism (8).

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Abbreviations: apo, apolipoprotein; BMI, body-mass index; CE, cholesteryl ester; CETP, CE transfer protein; NCEP ATP, National Cholesterol Education Program Adult Treatment Panel; HTGW, hypertriglyceridemic waist; MetS, metabolic syndrome; TC, total cholesterol; TG, triglyceride.

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relationship with LDL. Apo C-I has been observed to affect metabolism of apo B-containing lipoproteins by attenuating VLDL clearance by inhibiting LPL, directly (8) or indirectly (9), and by inhibiting liver-specific LDL receptor (LDLR) (10) and LDLR-related protein (LRP) (11), as well as the peripheral tissue-specific VLDL receptor (VLDLR) (12). Overall, apo C-I has been shown to increase the production of VLDL (13), triglyceride (TG) and cholesterol in mice (3, 8).

Human genetic studies have had limited success in better elucidating the physiological role of apo C-I. This is due in part to the paucity of naturally-occurring human variants in apo C-I compared with numerous common and rare variants affecting the protein sequences of apo E, B, A-I, A-II, A-IV, A-V, C-II, and C-III (14). The human variants of these other apolipoproteins have often served to identify and specify key pathways and mechanisms for more intensive study (15). Common nonsynonymous DNA variants of APOC1 located in the promoter region were among the first to have been reported for genes affecting lipoprotein metabolism (16, 17) with somewhat variable associations with plasma lipoproteins (18–20) and neurological phenotypes (21–24). One naturally occurring structural variant of apo C-I, namely T45S, was correlated with the clinical trait of increased body mass index (BMI) in Native Americans and Mexican descendants (25). Thus far, this variant has not been associated with biochemical traits nor has this association been replicated in other ethnic populations.

In the process of systematically screening candidate genes in lipoprotein metabolism for new DNA sequence variants in Canadian subpopulations, we identified the nonsynonymous variant designated APOC1 T45S in the Oji-Cree. We took advantage of this naturally occurring polymorphism to genotype Oji-Cree who were nondiabetic in order to detect associations with clinical and biochemical traits. We found that individuals with the APOC1 T45S variant, when compared with other nondiabetic subjects, had lower indices for waist circumference, hypertriglyceridemic waist (HTGW) prevalence, percent body fat, as well as lower serum concentrations of leptin and apo C-I, the latter of which was measured using a newly developed quantitative assay.

**METHODS**

**Study subjects**

All subjects in the current study had formerly been participants in the original 1993–1995 Sandy Lake Health and Diabetes Study (26). The Oji-Cree community of Sandy Lake, Ontario, is located ~2000 km northwest of Toronto, in the subarctic boreal forest of central Canada. Seven hundred twenty-eight members of this community (72% of the total population) ≥10 years of age participated in the original survey. Detailed information on demographics, dietary habits, and physical fitness of the study participants has been previously reported (27–29). The studies were approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee and signed informed consent was obtained from all participants.

Clinical characteristics and biochemical analysis

Body weight, height, waist circumference, and blood pressure were measured by standardized procedures (26). For waist circumference measurement, the natural waist was considered as the minimal circumference between the umbilicus and the xiphoid process. Hypertensive individuals were defined as those subjects having either blood pressure exceeding 130 mmHg systolic and/or 80 mmHg (diastolic), or those taking antihypertensive medications. Measurements of fasting blood analyses, including glucose, cholesterol, TGs, and leptin were performed as described elsewhere (26).

Genetic analysis of APOC1 T45S

Leukocyte DNA was prepared as described elsewhere (30, 31) and was used for genotype analysis. Genotypes for APOC1 codon 45 (T45S, exon 3) were determined using PCR. Exon 3 was amplified following established procedures (31) using primers 5′-GGG AGG TAG CTG CAC ACA GT and 3′-GTT GTG GGA AAT TTC AGA GG, followed by amplicon digestion using endonuclease BsmAI (New England Biolabs Inc., Ipswich, MA), as per manufacturer’s recommendations. The digested fragments were electrophoresed in a 2% agarose gel. Using this restriction isotyping, the smaller (65 bp and 89 bp) and larger (154 bp) fragments represented the T45 and S45 variants, respectively.

HTGW case definition

According to Lemieux et al. (32), subjects have a HTGW if they present with both of the following: 1) abdominal obesity, defined by waist circumference ≥ 90 cm, and 2) hypertriglyceridemia, defined by plasma TG concentration ≥ 2 mmol/L. On the other hand, National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III guidelines for HTGW is gender-specific such that the waist circumference cut-off for males is >102 cm and >88 cm for females (33).

MetS case definition

According to the NCEP ATP III criteria (33), metabolic syndrome (MetS) was identified if a subject had ≥3 of: 1) increased waist circumference [>102 cm (≥40 inches) for men, >88 cm (≥35 inches) for women]; 2) elevated plasma triglycerides [≥1.69 mmol/L (≥150 mg/dl)]; 3) low plasma HDL cholesterol [<0.40 mmol/L (<40 mg/dl) for men, <1.29 mmol/L (<50 mg/dl) for women]; 4) increased blood pressure (≥130 mmHg systolic and/or ≥85 mmHg diastolic) or on antihypertensive treatment; and 5) impaired fasting glucose [≥6.1 mmol/L (≥110 mg/dl)].

Quantitation of serum apo C-I concentration

Apo C-I levels were quantified from frozen Oji-Cree serum samples by sandwich ELISA as described elsewhere (34). Briefly, polyclonal rabbit anti-human apo C-I antibody (Academy Bio-medical Co., Houston, TX) was coated overnight at 7°C onto 96-well polystyrene plates (Nunc-Immuno MaxiSorp, NUNC, Rochester, NY) at a dilution of 1:200, followed by one wash with 300 µL PBS per well. Each well was then blocked for 1 h at 26°C with 300 µL PBS containing 0.05% Tween 20 (PBST) and 0.5% BSA (Sigma, St. Louis, MO) and followed by one wash with 300 µL PBS. Subsequently, serum samples (dilution, 1:8000) and apo C-I standard (dilution, 1-60 ng/ml; Biomedical Co., Houston, TX) diluted with PBST and 0.1% BSA and, respectively, were added to each well and incubated for 1 h at 450 rpm at 26°C. Wells were then washed three times with 300 µL PBST and 0.5% BSA, followed by the addition of HPR-conjugated polyclonal goat anti-human apo C-I antibody (dilution, 1:8000; Academy Bio-medical) diluted in PBST and 0.1% BSA. After two washes in PBST and one wash in PBS, color was developed by addition of
RESULTS

The baseline clinical and biochemical attributes of the 410 unrelated nondiabetic Oji-Cree subjects are shown in Table 1. This subset of the Oji-Cree had nearly even distribution of males and females. In addition to the traits indicated, their MetS status and their HTGW values were also recorded in Table 1.

In this sample of Oji-Cree, 348 had the APOC1 T45/T45 genotype, 62 had the T45/S45 genotype, and none had the S45/S45 genotype (Table 2). Because there were no S45 homozygotes, T45 was treated as a dominant allele for the purpose of subsequent analyses. Overall, the APOC1 T45S genotype frequencies in the Oji-Cree do not deviate significantly from predictions of the Hardy-Weinberg equation. In addition, the allele frequencies for T45 and S45 carriers are indicated in Table 2.

The clinical and biochemical attributes and significance of the nondiabetic Oji-Cree according to their APOCI genotype are shown in Table 3. Significance for each biochemical or clinical trait was derived after adjustment for age and sex. There were no significant differences (P > 0.05) in any of the plasma lipid concentrations. In addition, there were no significant between-genotype differences in the prevalence of MetS or in either mean (BMI) or waist-to-hip ratio. The lower values in waist circumference and NCEP-defined HTGW prevalence in S45 carriers, compared with T45 homozygotes, was close to being statistically significant with \( P = 0.070 \) (P = 0.066, using logarithmic adjustment) and \( P = 0.077 \), respectively. There were significant differences \( (P < 0.05) \) in percent body fat, serum leptin levels, and HTGW prevalence, with lower levels of these traits in the APOCI S45 carriers. In addition, we found that the APOCI S45 allele was associated with a significantly lower serum apo C-I concentration \( (P < 0.0001) \).

DIscussion

The principal findings of this study in nondiabetic Oji-Cree subjects were significant associations between the APOCI T45S polymorphism and variations in obesity indices, serum adipose-secreted hormone levels, and apo C-I levels. Specifically, in subjects carrying the APOCI S45 allele we found: 1) lower waist circumference, including lower frequency of HTGW, 2) lower percent body fat, 3) lower serum leptin concentrations, and 4) lower serum apo C-I concentrations, using an ELISA-based quantitative method. Thus, the nonsynonymous T45S variant in APOC1 among the Canadian First Nations is associated with variations in adiposity, obesity, and lower serum apo C-I levels.

Human apo C-I, C-II, and C-III, are protein constituents of chylomicrons, VLDL, and HDL. Although much is known about the roles of the apo C-II and C-III in lipoprotein metabolism, less is known about the biological function(s) of apo C-I. The gene coding for human apo C-I, APOCI, is part of a 48-kb gene cluster on chromosome 19. The gene cluster also includes APOC2, APOE, and the pseudo-APOCI gene (3, 35). The 4.7 kb APOCI gene is

### Table 1. Baseline clinical and biochemical traits of male and female nondiabetic Oji-Cree

| Measurement                        | Males          | Females         |
|------------------------------------|----------------|-----------------|
| n                                  | 192            | 217             |
| Age, y                             | 26.9 ± 13.2\(^a\) | 23.7 ± 11.5     |
| Triglycerides, mmol/L              | 1.32 ± 0.73    | 1.17 ± 0.46     |
| Total cholesterol, mmol/L          | 4.43 ± 1.01    | 4.45 ± 0.71     |
| LDL cholesterol, mmol/L            | 2.58 ± 0.87    | 2.34 ± 0.60     |
| HDL cholesterol, mmol/L            | 1.25 ± 0.29    | 1.28 ± 0.26     |
| apo A1, g/L                        | 1.46 ± 0.22    | 1.48 ± 0.21     |
| apo B, g/L                         | 1.05 ± 0.32    | 0.94 ± 0.21     |
| BMI, kg/m\(^2\)                    | 24.7 ± 4.95\(^b\) | 25.8 ± 5.80     |
| Percent body fat, %                | 24.4 ± 9.00\(^c\) | 39.3 ± 11.8     |
| WHR—iliac crest                    | 0.95 ± 0.07\(^d\) | 0.94 ± 0.05     |
| WHR—natural waist                  | 0.91 ± 0.07\(^d\) | 0.86 ± 0.05     |
| Waist circumference, cm            | 89.9 ± 14.0\(^e\) | 86.5 ± 13.2     |
| Leptin, ng/ml                      | 6.70 ± 6.77\(^c\) | 18.8 ± 11.6     |
| Apo C-I, µg/ml                     | 220.7 ± 62.8\(^c\) | 203.9 ± 38.8\(^e\) |
| MetS, %                            | 1.86 ± 0.35    | 1.87 ± 0.34     |
| HTGW, %                            | 1.84 ± 0.56    | 1.96 ± 0.19     |
| HTGW—NCEP waist, %                 | 1.91 ± 0.28    | 1.96 ± 0.19     |

\(^a\) n = 193.
\(^b\) n = 191.
\(^c\) n = 77.
\(^d\) n = 102.

**BMI**, body mass index; **WHR**, waist to hip ratio; **MetS**, diagnosis of metabolic syndrome using the National Cholesterol Education Program Adult Treatment Panel III criteria (34); **HTGW**—diagnosis of ‘hypertriglyceridemic waist’; **HTGW—NCEP**—diagnosis of hypertriglyceridemic waist using cut values from the National Cholesterol Education Program Adult Treatment Panel III criteria (34).

### Table 2. Genotype and allele frequencies in Oji-Cree

| Genotype Frequencies\(^a\) | Allele Frequencies |
|----------------------------|-------------------|
| T45/T45   | T45/S45 | S45/S45 |
| Male       | 166 (0.86) | 27 (0.14) | 0 (0)       |
| Female     | 182 (0.84) | 35 (0.16) | 0 (0)       |
| Total      | 348 (0.85) | 62 (0.15) | 0 (0)       |

\(^a\) percent in their respective populations given in parentheses.
TABLE 3. Clinical and biochemical traits of nondiabetic Oji-Cree according to APOCI T45S genotypes

| Trait                          | TT          | TS          | P          |
|-------------------------------|-------------|-------------|------------|
|                              | n = 547     | n = 62      |            |
| Triglycerides, mmol/L         | 1.25 ± 0.61 | 1.15 ± 0.57 | NS (0.15)  |
| log triglycerides             |             |             |            |
| Total cholesterol, mmol/L     | 4.28 ± 0.88 | 4.27 ± 0.84 | NS (0.62)  |
| log total cholesterol         |             |             |            |
| LDL cholesterol, mmol/L       | 2.45 ± 0.75 | 2.46 ± 0.72 | NS (0.82)  |
| log LDL                        |             |             |            |
| HDL cholesterol, mmol/L       | 1.26 ± 0.27 | 1.29 ± 0.29 | NS (0.56)  |
| log HDL                       |             |             |            |
| BMI, kg/m²                    | 25.4 ± 5.5  | 24.7 ± 5.1  | NS (0.15)  |
| log BMI                       |             |             |            |
| Percent body fat, %           | 32.7 ± 12.9 | 30.0 ± 13.0 | 0.0081     |
| log percent body fat          |             |             | 0.0131     |
| WHR – iliac crest             | 0.94 ± 0.06  | 0.95 ± 0.06  | NS (0.89)  |
| WHR – normal waist            | 0.88 ± 0.07  | 0.89 ± 0.07  | NS (0.68)  |
| log WHR (natural)             |             |             |            |
| Waist circumference, cm       | 88.5 ± 13.6  | 86.2 ± 13.8  | NS (0.070) |
| log waist                     |             |             | NS (0.066) |
| Leptin, ng/ml                 | 13.6 ± 11.9  | 10.7 ± 7.8   | 0.0065     |
| log leptin                    |             |             | 0.029      |
| Apo C-I, µg/ml                | 221.7 ± 49.6 | 190.2 ± 47.7 | <0.0001    |
| MetS, %                       | 14.1        | 9.09        | NS (0.24)  |
| HTGW, %                       | 10.4        | 3.23        | 0.047      |
| HTGW – NCEP waist, %          | 6.92        | 1.61        | NS (0.077) |

BMI, body mass index; WHR, waist to hip ratio; HTGW, diagnosis of hypertriglyceridemic waist; HTGW-NCEP waist, diagnosis of hypertriglyceridemic waist using cut values from the National Cholesterol Education Program Adult Treatment Panel III criteria (33); NS, not significant with nominal P > 0.05. Data are means ± SD. *P-values are adjusted for age and sex. The P-values in bold indicates the difference observed for the trait is significant or close to significant.

primarily expressed in the liver, with lower amounts expressed in the lung, skin, testes, and spleen (35). The pseudo-APOC1' gene 7.5 kb downstream from APOC1 has no detectable mRNA products in any tissue (35). APOC1 gene expression is regulated by an array of elements found throughout the whole APOE/C1/C2 gene cluster, such as the hepatic control region (36). The 6.6 kDa apo C-I protein is a polypeptide of 57 amino acid residues with residues 7 to 24 and 35 to 53 of apo C-I having importance in binding lipoproteins (37, 38).

Little is known about naturally occurring mutations in the APOC1 gene contributing to lipid-related abnormalities in humans. Dumon et al. (39) reported a patient with chylomicronemia who had a naturally occurring mutation in APOC1 resulting in a deficiency of apo C-I. However, this patient concurrently suffered from apo C-II deficiency due to an APOC2 defect, which was more likely to have been the cause of the chylomicronemia (39). Mass spectrometry studies elucidated the first structural non-synonymous variant of apo C-I T45S that was observed to have increased N-terminal truncation and increased in vitro distribution to the VLDL fraction in Native Americans and Mexicans (25). From mass spectrometry identification of the apo C-I T45S polymorphism in 228 Native Americans and in five sibling pairs of Mexican ancestry, S45 was associated with higher BMI (25). The authors commented that more comprehensive studies of the APOC1 T45S polymorphism in independent samples were necessary (25).

Our results in a Canadian First Nation population indicate that the apo C-I T45S variant has the opposite effect, so that S45 carriers had lower percent body fat and BMI. This ‘flip-flop’ association has been previously reported across different ethnic groups because differences in the genetic background and/or environment cause heterogeneous effects due to the same variant (40). Impact of multiple loci may also affect association, causing the flip-flop phenomenon (40). In addition, our study was carried out in 424 individuals, which was a larger study than that reported in the initial study of Native American subjects.

In vitro studies using, for instance, ligand blotting assays, artificial bilayer vesicles, and cultured fibroblasts have shown that apo C-I has an inhibitory and/or stimulatory effect on many receptors and enzymes involved in lipoprotein metabolism, suggesting a complex role for apo C-I in human disease (3, 10, 11, 41). One such example is the potential HDL-raising effect that apo C-I has by regulating the activity of HL, CETP, as well as LCAT, a protein known to esterify cholesterol in HDL such that it increases HDL levels and particle size (4).

New insights into the metabolic properties of apo Cs have been provided by the technologies of gene targeting through human transgenic mice and knockout mouse models. Human APOC1–transgenic animals (on a wild-type or APOE-deficient background) have been used to elucidate several roles in lipoprotein metabolism, such as inhibition of LPL (8, 13), inhibition of apo E-mediated lipid clearance via LRP (12), and blocking the binding of VLDL to the VLDLR (12). These roles have been associated with hyperlipidemia and increased atherosclerotic lesion development (9). In addition to hyperlipidemia, APOC1–transgenic animals exhibit several abnormalities, consisting of elevated plasma free fatty acid levels, inflammatory skin disorders, atrophic sebaceous glands, and subcutaneous adipose tissue (42), which suggests an additional role for apo C-I in epidermal lipid synthesis as well as adipose tissue formation. Because transgenic mice over-expressing ApoC1 develop hyperlipidemia (43), a hypolipidemic phenotype was expected in ApoC1 null mice. However, ApoC1 null mice on a high-fat and high-cholesterol diet developed hypercholesterolemia due to impaired in vivo hepatic uptake of VLDL (44). In addition, a recent study demonstrated that ApoC1 null mice had decreased serum HDL concentration (43). In contrast, human APOC1 transgenic mice have increased HDL concentrations due to inhibition of scavenger receptor class B type 1, a protein receptor that facilitates the flux of HDL through the plasma membrane (45).

Overall, in vitro, in vivo, and clinical evidence demonstrates that apo C-I inhibits the uptake of TG-rich lipoproteins via hepatic receptors and as a consequence, the presence of apo C-I on the lipoprotein particle may prolong their residence time in the circulation and subsequently facilitate their conversion to LDL (3). Apo C-I also functions to activate LCAT, which exhibits two activities in normal plasma: α-LCAT activity leads to mature-HDL production whereas β-LCAT activity has a positive effect on
LDL concentrations (46). In addition, apo C-I has a role in adipose biology as observed in human transgenic mice (42).

Therefore, our study suggests that the S45 variant in the Canadian First Nations results in lower concentrations of apo C-I and leptin, which may lead to lower percent body fat and waist circumference. However, a previous study has suggested the S45 variant to have greater N-terminal truncation and preferential distribution to the VLDL fraction compared with the APOCI T45 variant (47); increased concentration of apo C-I was recently observed to be associated with decreased visceral fat in men with MetS (48).

Based on the studies thus far, apo C-I seems to promote HDL as well as LDL production due to its effects on CETP and on remnant lipoprotein clearance and LCAT activity. A prospective study of a genotype-determined concentration will be required to prove this, as circulating levels will be affected by increasing or decreasing lipoprotein concentrations. Further biochemical studies are required to uncover the effects of the T45S polymorphism on the structure and distribution of apo C-I in Canadian First Nations and other ethnicities using mass spectrometry and plasma distribution of apolipoproteins in order to understand the clinical consequences of this variant. In addition, long-term prospective studies might help to clarify the long-term effect of this structural variant on the propensity for obesity and diabetes. Overall, such studies using geographically isolated populations help further elucidate the physiological role of this relatively poorly defined protein in complex metabolic disorders.

In summary, although the precise function of apo C-I is unknown, the human genetic data presented herein suggest a possible role for the APOCI S45 variant in obesity and adipocyte regulation as demonstrated by the decrease in body fat, waist circumference, decreased prevalence of HTGW, as well as a decreased serum leptin levels. Moreover, this is the first demonstration of a direct relationship of APOCI genetic variation with the serum concentration of apo C-I.

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