

## Variable association between genetic variation in the *CYP7* gene promoter and plasma lipoproteins in three Canadian populations

Robert A. Hegele<sup>a,\*</sup>, Jian Wang<sup>a</sup>, Stewart B. Harris<sup>b</sup>, J. Howard Brunt<sup>c</sup>,  
T. Kue Young<sup>d</sup>, Anthony J.G. Hanley<sup>e</sup>, Bernard Zinman<sup>e,g</sup>, Philip W. Connelly<sup>f,g,h</sup>,  
Carol M. Anderson<sup>a</sup>

<sup>a</sup> Department of Medicine, Blackburn Cardiovascular Genetics Laboratory, John P. Robarts Research Institute, University of Western Ontario, 406-100 Perth Drive, London, Ont., Canada N6A 5K8

<sup>b</sup> Thames Valley Family Practice Research Unit, University of Western Ontario, London, Ont., Canada  
<sup>c</sup> School of Nursing, University of Victoria, Victoria, BC, Canada

<sup>d</sup> Northern Health Research Unit, Department of Community Health Sciences, University of Manitoba, Winnipeg, Man., Canada

<sup>e</sup> Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ont., Canada

<sup>f</sup> St. Michael's Hospital, University of Toronto, Toronto, Ont., Canada

<sup>g</sup> Department of Medicine, University of Toronto, Toronto, Ont., Canada

<sup>h</sup> Department of Biochemistry, University of Toronto, Toronto, Ont., Canada

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### Abstract

The promoter sequence variant -278A in the *CYP7* gene, which encodes cholesterol 7- $\alpha$  hydroxylase, was previously reported to be associated with reduced plasma low density lipoprotein (LDL) cholesterol concentration. We tested for association of *CYP7*-278A with plasma lipoprotein traits in samples taken from three distinct Canadian populations: 594 Alberta Hutterites, 325 Ontario Oji-Cree and 190 Keewatin Inuit. The *CYP7*-278A allele frequencies in these three groups were 0.708, 0.466 and 0.490, respectively. The frequencies of *CYP7*-278A/A homozygotes were 0.481, 0.215 and 0.247, respectively. In the Hutterites, *CYP7*-278A was associated with reduced plasma HDL-cholesterol and apolipoprotein AI concentration. In the Oji-Cree, *CYP7*-278A was not significantly associated with any plasma lipoprotein trait. In the Inuit *CYP7*-278A was associated with elevated plasma total and LDL-cholesterol. There was no consistent relationship between the population mean plasma LDL-cholesterol concentration and the population *CYP7*-278A frequency. Our findings suggest that the common -278A promoter variant of *CYP7* was inconsistently associated with variation in plasma LDL- and HDL-cholesterol in samples from three independent populations. The inconsistencies could be due to differences in genetic background or to unspecified environmental or genetic factors. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Despite many published association studies, there has been a notable lack of consensus about the role of common genomic variation in the susceptibility to atherosclerosis and its related intermediate phenotypes [1]. Few alleles of any gene studied to date have been consistently associated with variation in intermediate phenotypes of atherosclerosis, such as plasma lipoproteins, across diverse populations [2,3]. Part of the

inconsistency may be due to the fact that many of the DNA markers studied do not have a functional impact on the structure or expression of the gene product. Thus, most positive associations have been attributed to linkage disequilibrium with putative functional changes elsewhere at the genetic locus. Since linkage disequilibrium may vary between populations, factors such as admixture can produce falsely positive genetic associations [3,4]. One way to reduce such confounding would include selecting DNA markers that directly mark a functional change in the gene of interest. Also, consistent results from complementary experimental ev-

\* Corresponding author. Tel.: +1-519-6633461; fax: +1-519-6633789.

E-mail address: robert.hegele@trri.on.ca (R.A. Hegele).

idence, such as using independent statistical approaches, independent study samples, *in vitro* studies and/or animal studies may increase confidence in the biological validity of genetic associations.

*CYP7* encodes cholesterol 7 $\alpha$ -hydroxylase (EC 1.14.13.17). Recently *CYP7* was linked to variation in plasma concentrations of low-density lipoprotein (LDL) cholesterol [5]. The *CYP7* gene product is the rate-limiting enzyme in the biosynthesis of bile acids, and pharmacologic manipulation of cholesterol 7 $\alpha$ -hydroxylase activity by bile acid binding resins is associated with lowered plasma LDL-cholesterol [6]. A common polymorphism in the promoter region of *CYP7*, namely -278C/A, was found to be associated with variation in plasma LDL-cholesterol concentration in independent samples [5]. In particular, the *CYP7*-278A allele was found to be associated with lower plasma total and LDL-cholesterol, with -278A/A homozygotes having lower levels of these traits, by ~10%, than -278C/C homozygotes [5]. We wanted to determine the generalizability of this association, and thus performed an association analysis between the *CYP7* promoter variant and plasma lipoproteins in samples taken from three distinct and independent Canadian populations: Alberta Hutterites, Sandy Lake Oji-Cree and Keewatin Inuit.

## 1. Methods

### 1.1. Subjects

#### 1.1.1. Hutterites

Eight hundred and forty six individuals from 21 colonies of Alberta Hutterites aged 18–80 years participated in the Canadian Heart Health Survey for CHD risk assessment [7–13]. These individuals came from two sects, called Lehrerleut and Dariusleut [7–13]. Almost every one of the study subjects could be placed into a defined sibship representing one of 187 nuclear families. The average inbreeding coefficient for members of the study sample was 0.05 [7–13]. The survey included an interviewer-administered questionnaire and a clinical examination. Medical histories and physical examinations were performed on all individuals. Fasting blood samples were obtained for measurement of biochemical traits and determination of genotypes [7–13]. The clinical, biochemical and genetic attributes of these subjects are more fully described elsewhere [7–13]. The study had ethical approval from the Universities of Alberta and Toronto.

#### 1.1.2. Sandy Lake Oji-Cree

Seven hundred and twenty eight members (or 72% of eligible subjects) from the isolated native community of Sandy Lake, Ontario were enrolled in a survey of

diabetes and cardiovascular risk factors. The survey included an interviewer-administered questionnaire and a clinical examination. Medical histories and physical examinations were performed on all individuals. Fasting blood samples were obtained for measurement of biochemical traits and determination of genotypes [14–20]. The clinical, biochemical and genetic attributes of these subjects are described elsewhere [14–20]. The study had ethical approval from the University of Toronto.

#### 1.1.3. Keewatin Inuit

Five hundred and sixteen randomly selected individuals, not known to be related, from the Keewatin region aged 18–80 years participated in a comprehensive health interview and examination survey. Of these, 92 reported themselves as being of European background (white) and 31 reported themselves as being of an ethnic background other than Inuit or white. Only subjects with >50% self-reported Inuit background were included in the analysis. The survey included an interviewer-administered questionnaire and a clinical examination. Medical histories and physical examinations were performed on all individuals. Fasting blood samples were obtained for measurement of biochemical traits and determination of genotypes [21,22]. The clinical, biochemical and genetic attributes of these subjects are described elsewhere [21,22]. The project had ethical approval from the Universities of Manitoba and Toronto.

### 1.2. Biochemical analyses

Blood for lipoprotein analyses from all studies was centrifuged at 2000 rpm for 30 min. The plasma was either shipped in ice packs and analysed for lipids and lipoproteins, (in the case of the Hutterites) or separated into aliquots, which were frozen on site and shipped on dry ice (in the case of the Oji-Cree and Inuit). Plasma apo AI and B were measured on aliquots that had been stored at –70°C. Plasma concentrations of lipids and lipoproteins in all three study samples, and of apolipoproteins in the Hutterites and Oji-Cree only, were determined in the J. Alick Little Lipid Research Laboratory at St. Michael's Hospital, as previously described [7–21]. LDL-cholesterol concentrations were calculated using the Friedewald equation and apo B and AI determinations were performed using nephelometry [7–13]. Exclusion criteria for all studies included an insufficient sample for all determinations, age below 18 years and a diagnosis of either diabetes or impaired glucose tolerance in the Oji-Cree, according to World Health Organization and National Diabetes Data Group criteria, respectively [14]. In all samples, subjects taking lipid-lowering medications were excluded. Most subjects treated for hypertension in each sample were taking angiotensin-converting enzyme inhibitors.

### 1.3. Genetic analyses

Sufficient DNA and phenotypic information were obtained from 594 Hutterites, 325 non-diabetic Oji-Cree and 190 Inuit. An established procedure, utilizing published primer sequences, PCR conditions and digestion with *BsaI*, was used to genotype the *CYP7* promoter [5] (hereafter designated as the -278C/A polymorphism for consistency with previous reports [5], although the position of the polymorphism differs from this designation by ~10 bp). Known genotypic controls were run with each reaction.

### 1.4. Statistical analysis

The significance of deviations of observed genotype frequencies from those predicted by the Hardy–Weinberg equation were evaluated with chi-square tests. SAS (Version 6.12) was used for all statistical comparisons involving biochemical variables [23]. Quantitative variables were transformed and subjected to analysis of normality as described [10,19,22]. All quantitative traits from all study samples had significantly non-normal distributions. For each sample, taking the natural logarithm (log) of each variable resulted in a distribution that was not significantly different from normal in the case of all variables but triglycerides (TG), in which case no transformation gave a distribution that was more normal than the log (data not shown).

For each sample, multivariate regression analysis was performed to determine the sources of variation for fasting plasma total, LDL- and high density lipoprotein (HDL-) cholesterol, and TG [23]. For the Hutterite and Oji-Cree sample, additional regression analyses were performed to determine the sources of variation for apo B and apo AI. Covariates were age, body mass index (BMI) and the use of antihypertensive medications. We also included covariates that would correct, at least in part, for the influence of relatedness within each study sample. Thus, in the Hutterites and Oji-Cree, respectively, we included the colony of origin and the family of origin as covariates. We have previously used these variables in order to control for shared background genetic and environment effects. Also included as an independent variable for each ANOVA was the *CYP7* gene promoter genotype. Three models of inheritance were tested for each sample: (1) for a dominant model, the genotype variable was set at one for subjects with -278A/A and -278A/C genotypes and zero for subjects with the -278C/C genotype; (2) for a co-dominant model, the genotype variable was set at one for subjects with the -278A/A genotype, at 0.5 for subjects with the -278A/C genotype and at zero for subjects with the -278C/C genotype; and (3) for a recessive model, the genotype

variable was set at one for subjects with -278A/A genotype and zero for subjects with the -278A/C and -278C/C genotypes. The proportion of variation in a plasma lipoprotein due to a covariate was estimated from partial regression coefficients. Because of the gender difference in plasma lipoproteins, men and women were analysed separately for pairwise comparisons.

We also wished to determine whether there was sufficient power within our samples to declare a true absence of an association. We took into account the genotype frequencies, the means and the standard deviations for each trait within each sample in order to determine a priori statistical power to detect a between-genotype difference of 10%, or 0.3 mmol/l for LDL-cholesterol and 0.09 g/l for apo B. Thus, in the Hutterites, we had >85% power to detect a 10% difference in mean LDL-cholesterol between *CYP7*-278A/A homozygotes and -278A/C heterozygotes ( $\alpha = 0.05$ , two-tailed). Furthermore, in the Hutterites, we had >90% power to detect a 10% difference in mean apo B between *CYP7*-278A/A homozygotes and -278A/C heterozygotes ( $\alpha = 0.05$ , two-tailed). Similarly, in the Oji-Cree, we had >60% power to detect a 10% difference in LDL-cholesterol between *CYP7*-278A/A homozygotes and -278A/C heterozygotes ( $\alpha = 0.05$ , two-tailed), but >90% power to detect a 10% difference in LDL-cholesterol and a 10% difference in apo B between *CYP7*-278A/A homozygotes and -278C/C homozygotes ( $\alpha = 0.05$ , two-tailed). Finally, in the Inuit, we had >40% power to detect a 10% difference in LDL-cholesterol between *CYP7*-278A/A homozygotes and -278A/C heterozygotes ( $\alpha = 0.05$ , two-tailed), but >80% power to detect a 10% difference in LDL-cholesterol and a 10% difference in apo B between *CYP7*-278A/A homozygotes and -278C/C homozygotes ( $\alpha = 0.05$ , two-tailed). We concluded that our samples had adequate statistical power to detect differences in LDL-cholesterol and apo B that were on the order of magnitude of those reported by Wang et al. [5].

## 2. Results

### 2.1. Baseline clinical and biochemical characteristics

A summary of the mean  $\pm$  standard deviation (SD) of the clinical and biochemical characteristics of the three study samples is shown in Table 1. While the mean ages of male and female Hutterites and Inuit were not different, both were higher than those in male and female Oji-Cree ( $P < 0.01$ ). While the BMI in male Oji-Cree and Inuit were not different, both were lower than those in male Hutterites ( $P < 0.01$ ).

Table 1  
Baseline clinical and biochemical characteristics of the three Canadian study samples<sup>a</sup>

	Hutterites		Oji-Cree		Inuit	
	Males	Females	Males	Females	Males	Females
Number	263	331	160	165	81	109
Age (years)	38.0 ± 14.7	37.7 ± 15.3	32.6 ± 13.2	30.4 ± 11.4	35.2 ± 16.6	38.4 ± 14.8
BMI (kg/m <sup>2</sup> )	28.0 ± 3.7	28.0 ± 5.8	26.0 ± 4.5	28.2 ± 5.5	25.8 ± 4.0	27.3 ± 4.7
Cholesterol (mmol/l)						
Total	5.33 ± 1.12	5.15 ± 1.02	4.71 ± 1.00	4.36 ± 0.70	4.74 ± 1.09	5.04 ± 0.97
LDL	3.31 ± 0.94	3.04 ± 0.88	2.85 ± 0.86	2.49 ± 0.60	2.94 ± 0.94	3.03 ± 0.86
HDL	1.24 ± 0.25	1.50 ± 0.34	1.22 ± 0.29	1.29 ± 0.27	1.35 ± 0.37	1.49 ± 0.41
TG (mmol/l)	1.80 ± 1.41	1.39 ± 1.16	1.47 ± 0.73	1.28 ± 0.53	1.00 ± 0.48	1.16 ± 0.62
Apo B (g/l)	1.27 ± 0.32	1.11 ± 0.27	1.16 ± 0.31	1.00 ± 0.22	ND	ND
Apo AI (g/l)	1.40 ± 0.20	1.56 ± 0.26	1.46 ± 0.21	1.52 ± 0.22	ND	ND
Medication (%)	10.6	13.6	5.6	4.2	3.9	9.6

<sup>a</sup> Abbreviations: yrs, years; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; apo, apolipoprotein; ND, not determined.

## 2.2. *CYP7* promoter allele and genotype frequencies

The *CYP7*-278A allele frequency was 0.708, 0.466 and 0.490 in Hutterites, Oji-Cree and Inuit, respectively (Table 2). The *CYP7* genotype frequencies did not deviate significantly from those predicted by the Hardy–Weinberg equation (Table 2).

## 2.3. *CYP7* promoter allele associations with lipoprotein phenotypes

Regression analyses were performed to test for associations of the *CYP7* promoter polymorphism with plasma lipoprotein phenotypes. When lipoprotein associations with *CYP7* promoter genotype were significant, it was always under a recessive model for the *CYP7*-278A allele. Furthermore, the recessive model always accounted for a greater proportion of total variation for each significantly associated plasma lipoprotein trait. Therefore, results from a recessive model are shown in Tables 3–5.

For the Hutterites, strong associations were seen between *CYP7* promoter genotype and both plasma HDL-cholesterol and apo AI ( $P = 0.0027$  and  $0.0001$ , respectively). Furthermore, *CYP7* promoter genotype accounted for 1.2 and 2.6% of the total variation, and 4.6 and 17.9% of the attributable variation, in plasma HDL-cholesterol and apo AI, respectively (Table 3). No other plasma lipoprotein traits were associated with *CYP7* promoter genotype in the Hutterites.

For the Oji-Cree, no associations was seen between *CYP7* promoter genotype and any plasma lipoprotein phenotype (Table 4).

For the Inuit, associations were seen between *CYP7* promoter genotype and plasma total and LDL-cholesterol ( $P = 0.012$  and  $0.015$ , respectively). Furthermore, *CYP7* promoter genotype accounted for 2.8 and 2.9%

of the total variation, and 11.2 and 15.6% of the attributable variation, in plasma total and LDL-cholesterol, respectively (Table 5). The other plasma lipoprotein traits were not associated with *CYP7* promoter genotype in the Inuit.

Tables 6–8 show the unadjusted means ± SD of biochemical traits according to genotype, divided by gender, for the three study samples. Table 6 shows that the significant association of *CYP7* genotype with variation in HDL-cholesterol and apo AI in the Hutterites from Table 3 was due to lower mean values of these two traits in subjects with the -278A/A genotype. Table 7 summarizes the means ± SD of biochemical traits in the Oji-Cree, none of which were significantly different between *CYP7* genotypes, as shown in Table 4. Table 8 shows that the significant association of *CYP7* genotype with variation in total and LDL-cholesterol in the Inuit from Table 5 was due to higher mean values of these two traits in subjects with the -278A/A genotype.

## 3. Discussion

In this study of independent samples taken from three Canadian populations, with a wide range of allele

Table 2  
*CYP7*-278A/C allele and genotype frequencies in three Canadian study samples<sup>a</sup>

	Hutterites	Oji-Cree	Inuit
-278A	0.708	0.466	0.490
-278C	0.292	0.534	0.510
-278A/A	0.481	0.215	0.247
-278A/C	0.453	0.502	0.485
-278C/C	0.066	0.283	0.268

<sup>a</sup> Abbreviations: as in Table 1.

Table 3  
Sources of attributable variation for lipoprotein phenotypes in Hutterite study samples<sup>a</sup>

		Partial $r^2$	P-value
Total cholesterol	BMI	0.0085	0.0172
	Age	0.1653	0.0001
	Sex	0.0042	0.0912
	Colony		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype	0.0054	0.0572
LDL-cholesterol	BMI	0.0049	0.0828
	Age	0.0991	0.0001
	Sex	0.0198	0.0006
	Colony	0.0083	0.0242
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
HDL-cholesterol	BMI	0.0898	0.0001
	Age		NS (>0.15)
	Sex	0.1607	0.0001
	Colony		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype	0.0122	0.0027
TG	BMI	0.0657	0.0001
	Age	0.2487	0.0001
	Sex	0.0259	0.0001
	Colony	0.0045	0.0523
	Medication	0.0121	0.0015
	<i>CYP7-278</i> genotype		NS (>0.15)
Apo B	BMI	0.0238	0.0001
	Age	0.2317	0.0001
	Sex	0.0581	0.0001
	Colony		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
Apo A1	BMI	0.0154	0.0019
	Age	0.0057	0.0596
	Sex	0.0977	0.0001
	Colony		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype	0.0259	0.0001

<sup>a</sup> Abbreviations as in Table 1; partial  $r^2$ , partial regression coefficient from regression analysis.

frequencies of the *CYP7* promoter polymorphism, we found inconsistent associations between variation in the *CYP7* promoter and lipoprotein phenotypes. Specifically, in the Hutterites, *CYP7-278A* was associated with reduced plasma HDL-cholesterol and apo AI concentrations. In contrast, in the Oji-Cree, *CYP7-278A* was not significantly associated with any plasma lipoprotein trait. However, in the Inuit, *CYP7-278A* was

associated with elevated plasma total and LDL-cholesterol. Furthermore, there was no consistent relationship between the population mean plasma LDL-cholesterol concentration and the population *CYP7-278A* frequency, since the Hutterites, who had the highest frequency of the -278A allele also had the highest mean plasma LDL-cholesterol. These findings suggest that the common -278A promoter variant of *CYP7*, which had previously been reported to be associated with

Table 4  
Sources of attributable variation for lipoprotein phenotypes in Oji-Cree study samples<sup>a</sup>

		Partial $r^2$	P-value
Total cholesterol	BMI	0.0273	0.0008
	Age	0.1888	0.0001
	Sex	0.0189	0.0060
	Family		NS (>0.15)
	Medication	0.0068	0.0912
	<i>CYP7-278</i> genotype		NS (>0.15)
LDL-cholesterol	BMI	0.0331	0.0003
	Age	0.1755	0.0001
	Sex	0.0480	0.0001
	Family		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
HDL-cholesterol	BMI	0.1100	0.0001
	Age	0.0152	0.0157
	Sex	0.0476	0.0001
	Family		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
TG	BMI	0.1730	0.0001
	Age	0.0106	0.0359
	Sex	0.0489	0.0001
	Family		NS (>0.15)
	Medication	0.0062	0.1066
	<i>CYP7-278</i> genotype		NS (>0.15)
Apo B	BMI	0.0806	0.0001
	Age	0.1681	0.0001
	Sex	0.0849	0.0001
	Family		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
Apo A1	BMI	0.0275	0.0024
	Age	0.0316	0.0013
	Sex	0.0367	0.0004
	Family		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)

<sup>a</sup> Abbreviations as in Table 3.

Table 5  
Sources of attributable variation for lipoprotein phenotypes in Inuit study samples<sup>a</sup>

		Partial $r^2$	P-value
Total cholesterol	BMI		NS (>0.15)
	Age	0.2234	0.0001
	Sex		NS (>0.15)
	Race		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype	0.0277	0.0121
LDL-cholesterol	BMI	0.0122	0.1120
	Age	0.1467	0.0001
	Sex		NS (>0.15)
	Race		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype	0.0294	0.0145
HDL-cholesterol	BMI	0.0638	0.0004
	Age	0.0937	0.0001
	Sex	0.0302	0.0128
	Race	0.0349	0.0065
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)
TG	BMI	0.1194	0.0001
	Age		NS (>0.15)
	Sex		NS (>0.15)
	Race		NS (>0.15)
	Medication		NS (>0.15)
	<i>CYP7-278</i> genotype		NS (>0.15)

<sup>a</sup> Abbreviations as in Table 3.

lower plasma LDL-cholesterol, was at best inconsistently associated with variation in plasma lipoproteins in samples from three independent populations.

Table 6  
Mean ( $\pm$ SD) clinical and biochemical variables for male and female Hutterites by *CYP7-278A/C* genotype<sup>a</sup>

	<i>CYP7-278A/A</i>		<i>CYP7-278A/C</i>		<i>CYP7-278C/C</i>	
	Males	Females	Males	Females	Males	Females
Number	114	172	134	135	15	24
Age (years)	35.9 $\pm$ 14.8	36.9 $\pm$ 15.0	39.2 $\pm$ 14.2	40.3 $\pm$ 15.7	42.8 $\pm$ 16.9	29.5 $\pm$ 11.6
BMI (kg/m <sup>2</sup> )	28.0 $\pm$ 3.6	26.2 $\pm$ 5.7	27.9 $\pm$ 3.8	28.2 $\pm$ 5.9	28.7 $\pm$ 3.3	26.6 $\pm$ 6.1
Cholesterol (mmol/l)						
Total	5.28 $\pm$ 1.19	5.06 $\pm$ 0.99	5.35 $\pm$ 1.07	5.26 $\pm$ 1.03	5.51 $\pm$ 1.00	5.17 $\pm$ 1.10
LDL	3.28 $\pm$ 1.01	3.01 $\pm$ 0.87	3.32 $\pm$ 0.89	3.08 $\pm$ 0.90	3.42 $\pm$ 0.78	3.07 $\pm$ 0.89
HDL	1.24 $\pm$ 0.25	1.46 $\pm$ 0.34	1.24 $\pm$ 0.26	1.55 $\pm$ 0.34	1.20 $\pm$ 0.13	1.56 $\pm$ 0.34
TG (mmol/l)	1.66 $\pm$ 1.21	1.40 $\pm$ 1.39	1.90 $\pm$ 1.59	1.42 $\pm$ 0.88	1.96 $\pm$ 1.1	1.19 $\pm$ 0.73
Apo B (g/l)	1.26 $\pm$ 0.34	1.09 $\pm$ 0.25	1.28 $\pm$ 0.31	1.15 $\pm$ 0.29	1.31 $\pm$ 0.30	1.08 $\pm$ 0.28
Apo A1 (g/l)	1.40 $\pm$ 0.21	1.51 $\pm$ 0.24	1.40 $\pm$ 0.19	1.61 $\pm$ 0.28	1.44 $\pm$ 0.22	1.62 $\pm$ 0.28
Medication (%)	12.2	12.8	8.3	17	20	0

<sup>a</sup> Abbreviations as in Table 3.

The disparities in the phenotype associations with the *CYP7* promoter variant between these three study samples are notable, especially when considering the consistency of the phenotype associations with *APOE* variation. Specifically, we have previously shown that plasma LDL-cholesterol was significantly associated with *APOE* restriction isotypes underlying the protein isoforms of apo E in the Hutterites [8], the Oji-Cree [19] and the Inuit [21]. In each sample, the plasma LDL-cholesterol was highest in subjects with the E4 isoform, intermediate in homozygotes for E3 and lowest in subjects with the E2 isoform [8,19,21], despite large inter-population differences in allele frequencies. In each sample, between 3 and 5% of the variation in LDL-cholesterol and/or apo B was associated with the *APOE* genetic variation [8,19,21]. Thus, the disparities in the associations with the *CYP7* promoter variant are remarkable, since the associations with *APOE* are so consistent.

There were some differences in our subjects compared with previously reported study samples. In general, our samples were younger and were more balanced with respect to gender than other samples in association studies of *CYP7* promoter polymorphism. For example, Wang et al. [5] reported significant biochemical associations in samples comprised of 934 and 347 individuals, of whom the second group were ascertained based upon CHD [5]. For this group, CHD was an important ascertainment criterion, although it was not found to be associated with the *CYP7* promoter variant. It is therefore possible that factors related to the expression of CHD were required for the expression of the previously reported associations of *CYP7* promoter genotype with lipoprotein phenotypes [5]. Such an association would be absent in our study samples, since they were young and CHD was virtually absent.

Table 7  
Mean ( $\pm$ SD) clinical and biochemical variables for male and female Oji-Cree by *CYP7-278A/C* genotype<sup>a</sup>

	<i>CYP7-278A/A</i>		<i>CYP7-278A/C</i>		<i>CYP7-278C/C</i>	
	Males	Females	Males	Females	Males	Females
Number	33	37	85	78	42	50
Age (years)	32.5 $\pm$ 12.8	29.6 $\pm$ 9.4	32.3 $\pm$ 13.0	30.6 $\pm$ 10.8	33.3 $\pm$ 14.1	30.8 $\pm$ 13.7
BMI (kg/m <sup>2</sup> )	25.9 $\pm$ 4.4	28.9 $\pm$ 6.1	26.0 $\pm$ 4.6	28.4 $\pm$ 5.6	26.0 $\pm$ 4.5	27.2 $\pm$ 4.9
Cholesterol (mmol/l)						
Total	4.91 $\pm$ 0.95	4.33 $\pm$ 0.67	4.73 $\pm$ 1.0	4.30 $\pm$ 0.61	4.56 $\pm$ 1.04	4.47 $\pm$ 0.85
LDL	3.02 $\pm$ 0.84	2.43 $\pm$ 0.55	2.84 $\pm$ 0.87	2.48 $\pm$ 0.56	2.74 $\pm$ 0.85	2.54 $\pm$ 0.70
HDL	1.16 $\pm$ 0.30	1.34 $\pm$ 0.32	1.24 $\pm$ 0.30	1.26 $\pm$ 0.29	1.22 $\pm$ 0.25	1.29 $\pm$ 0.20
TG (mmol/l)	1.60 $\pm$ 0.81	1.24 $\pm$ 0.51	1.42 $\pm$ 0.67	1.22 $\pm$ 0.43	1.47 $\pm$ 0.78	1.42 $\pm$ 0.67
Apo B (g/l)	1.22 $\pm$ 0.31	0.97 $\pm$ 0.23	1.14 $\pm$ 0.30	0.99 $\pm$ 0.19	1.13 $\pm$ 0.31	1.03 $\pm$ 0.24
Apo A1 (g/l)	1.43 $\pm$ 0.20	1.56 $\pm$ 0.22	1.48 $\pm$ 0.22	1.50 $\pm$ 0.23	1.46 $\pm$ 0.19	1.53 $\pm$ 0.18
Medication (%)	9.1	5.4	7.1	2.6	0	6

<sup>a</sup> Abbreviations as in Table 3.

Table 8  
Mean ( $\pm$ SD) clinical and biochemical variables for male and female Hutterites by *CYP7-278A/C* genotype<sup>a</sup>

	<i>CYP7-278A/A</i>		<i>CYP7-278A/C</i>		<i>CYP7-278C/C</i>	
	Males	Females	Males	Females	Males	Females
Number	17	30	41	51	23	28
Age (years)	31.7 $\pm$ 15.4	36.6 $\pm$ 12.1	37.7 $\pm$ 16.7	40.7 $\pm$ 16.6	33.3 $\pm$ 17.4	36.4 $\pm$ 13.9
BMI (kg/m <sup>2</sup> )	26.3 $\pm$ 4.5	26.8 $\pm$ 4.6	25.5 $\pm$ 3.3	28.1 $\pm$ 4.8	25.9 $\pm$ 4.9	26.3 $\pm$ 4.4
Cholesterol (mmol/l)						
Total	4.99 $\pm$ 1.25	5.12 $\pm$ 1.01	4.71 $\pm$ 1.07	5.02 $\pm$ 0.97	4.59 $\pm$ 1.03	5.00 $\pm$ 0.95
LDL	3.15 $\pm$ 1.01	3.15 $\pm$ 0.92	2.89 $\pm$ 0.94	2.96 $\pm$ 0.85	2.86 $\pm$ 0.91	3.00 $\pm$ 0.83
HDL	1.32 $\pm$ 0.41	1.48 $\pm$ 0.38	1.38 $\pm$ 0.39	1.49 $\pm$ 0.47	1.31 $\pm$ 0.33	1.49 $\pm$ 0.33
TG (mmol/l)	1.14 $\pm$ 0.69	1.08 $\pm$ 0.48	0.98 $\pm$ 0.46	1.24 $\pm$ 0.71	0.92 $\pm$ 0.27	1.07 $\pm$ 0.58
Medication (%)	6.3	6.7	4.9	9.8	0	10.7

<sup>a</sup> Abbreviations as in Table 3.

The association between *CYP7* promoter variation and plasma concentrations of HDL-cholesterol and apo AI in Hutterites are consistent with the results of experiments in mice, which indicated that cholesterol 7 $\alpha$ -hydroxylase activity co-segregated with plasma concentrations of HDL-cholesterol [24]. However, the association of *CYP7* promoter genotype and HDL-cholesterol was notably absent from the other study samples. In addition, the association of *CYP7-278A* with higher LDL-cholesterol in the Inuit was exactly opposite to the direction of the association reported by Wang et al. [5]. These observations suggest that variation at or near *CYP7* can affect plasma lipoproteins, although there are marked disparities in the associated phenotype.

Differences in the diet composition could have been a determinant of differences in associations between study samples. Cholesterol 7 $\alpha$ -hydroxylase is a microsomal cytochrome P450 enzyme that catalyzes the first step in bile acid biosynthesis [6]. The expression of

*CYP7* is enhanced when bile acids are depleted and is suppressed when bile acids are present in excess in the diet [6]. Also changes in dietary cholesterol intake in rabbits induces differential regulation of classic and alternative pathways of bile acid synthesis, including induction of 7 $\alpha$ -hydroxylase activity [25]. Therefore, differences in dietary composition can affect expression of *CYP7*. Since the probable mechanistic impact of the *CYP7* promoter variation is to affect the baseline expression of *CYP7*, it is conceivable that there might be interactions between diet and the genomic variation that affects expression of *CYP7*. It is possible that the differences in *CYP7* expression due to a sufficiently large interindividual dietary difference could predominate over a more subtle impact on enzyme expression related to promoter sequence variation. Of course, these differences in expression of enzyme activity would have to affect the end point of plasma lipoprotein concentrations. Because the three groups studied had disparities in the quantity and quality of dietary fat, it is possible

that complex gene–diet interactions modulated the associations of plasma lipoproteins with *CYP7* variation.

Differences in genetic backgrounds of the study samples could have been another determinant of differences in associations between study samples. From animal experiments, it is clear that the genetic background is a crucial determinant of large differences in quantitative phenotypes resulting from a single genetic mutation [26]. Thus, the different genetic backgrounds of the populations from which our study samples were taken could have affected the association between *CYP7* promoter variation and plasma lipoprotein phenotype. Furthermore, experiments performed in mice indicated that there was redundancy in the pathways of bile acid biosynthesis, which resulted in some compensation even after a complete loss of activity due to targeted disruption of murine *CYP7* [27]. Assuming such redundancy also exists in humans, then there could be differences with respect to the ability to express alternative pathways that are related to the genetic background. These differences might alter the sensitivity of some subpopulations to the impact of a monogenic change that affects a specific biosynthetic pathway, such as the *CYP7* promoter polymorphism.

Thus, we have observed no association of *CYP7* promoter genotype with plasma lipoproteins in Oji-Cree, and two different genotype-phenotype associations in the other two study samples. We could not confirm the previously reported association of *CYP7*-278A with lower plasma LDL-cholesterol in any of our study samples. These disparities suggest that population-specific factors, such as between-population differences in linkage disequilibrium with functionally relevant variations, differences in genetic background, differences in gene–gene or gene–environment interactions, and/or differences in specific environmental factors, such as diet, might be very important determinants of genotype–phenotype associations in diverse ethnic groups.

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### References

- [1] Goldstein JL, Brown MS. The clinical investigator: bewitched, bothered, and bewildered but still beloved. *J Clin Invest* 1997;99:2803–12.
- [2] Mehrabian M, Lusis AJ. Genetic markers for atherosclerosis and related risk factors. In: Lusis AJ, Rotter JI, Sparkes RS, editors. *Molecular Genetics of Coronary Artery Disease: Candidate Genes and Processes in Atherosclerosis*. Basel: Karger, 1992:363–418.
- [3] Hegele RA. Candidate genes, small effects, and the prediction of atherosclerosis. *Crit Rev Clin Lab Sci* 1997;34:343–67.
- [4] Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 1994;265:2037–48.
- [5] Wang J, Freeman DJ, Grundy SM, Levine DM, Guerra R, Cohen JC. Linkage between cholesterol 7 $\alpha$ -hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. *J Clin Invest* 1998;101:1283–91.
- [6] Bjorkheim I, Reihner E, Angelin B, Ewerth S, Akerlund JE, Einarsson K. On the possible use of the serum level of 7  $\alpha$ -hydroxysterol as a marker for increased activity of cholesterol 7  $\alpha$ -hydroxylase in humans. *J Lipid Res* 1987;28:889–94.
- [7] Hegele RA, Brunt JH, Connelly PW. A polymorphism of the angiotensinogen gene is associated with variation in blood pressure in a genetic isolate. *Circulation* 1994;90:2207–12.
- [8] Hegele RA, Evans AJ, Tu L, Ip G, Brunt JH, Connelly PW. A gene–gender interaction affecting lipoproteins in a genetic isolate. *Arterioscler Thromb* 1994;14:671–8.
- [9] 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.
- [10] Hegele RA, Brunt JH, Connelly PW. Multiple genetic determinants of variation of plasma lipoproteins in Alberta Hutterites. *Arterioscler Thromb Vasc Biol* 1995;15:861–71.
- [11] Hegele RA, Brunt JH, Connelly PW. Genetic variation on chromosome 1 associated with variation in body fat distribution in men. *Circulation* 1995;92:1089–93.
- [12] Hegele RA, Breckenridge WC, Brunt JH, Connelly PW. Genetic variation in factor VII associated with variation in plasma lipoprotein(a) concentration. *Arterioscler Thromb Vasc Biol* 1997;17:1701–6.
- [13] Hegele RA, Brunt JH, Connelly PW. Genetic and biochemical factors associated with variation in blood pressure in a genetic isolate. *Hypertension* 1996;27:308–12.
- [14] Harris SB, Gittelsohn J, Hanley AJG, Barnie A, Wolever TMS, Gao J, Logan A, Zinman B. The prevalence of NIDDM and associated risk factors in native Canadians. *Diabetes Care* 1997;20:185–97.
- [15] Hegele RA, Harris SB, Hanley AJ, Sadikian S, Connelly PW, Zinman B. Genetic variation of intestinal fatty acid-binding protein associated with variation in body mass in aboriginal Canadians. *J Clin Endocrinol Metab* 1996;81:4334–7.
- [16] Hegele RA, Connelly PW, Scherer SW, Hanley AJ, Harris SB, Tsui LC, Zinman B. Paraoxonase-2 gene (PON2) G148 variant associated with elevated fasting plasma glucose in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1997;82:3373–7.

- [17] Hegele RA, Connelly PW, Scherer SW, Hanley AJ, Harris SB, Tsui LC, Zinman B. Paraoxonase-2 G148 variant in an aboriginal Canadian girl with non-insulin-dependent diabetes. *Lancet* 1997;350:785.
- [18] Hegele RA, Zinman B, Hanley AJ, Harris S, Connelly PW. A common mtDNA polymorphism associated with variation in plasma triglyceride concentration. *Am J Hum Genet* 1997;60:1552–5.
- [19] Hegele RA, Connelly PW, Hanley AJ, Sun F, Harris SB, Zinman B. Common genomic variants associated with variation in plasma lipoproteins in young aboriginal Canadians. *Arterioscler Thromb Vasc Biol* 1997;17:1060–6.
- [20] Hegele RA, Harris SB, Hanley AJ, Sun F, Connelly PW, Zinman B. Angiotensinogen gene variation associated with variation in blood pressure in aboriginal Canadians. *Hypertension* 1997;29:1073–7.
- [21] Hegele RA, Young TK, Connelly PW. Are Canadian Inuit at increased genetic risk for coronary heart disease? *J Mol Med* 1997;75:364–70.
- [22] Hegele RA, Tully C, Young TK, Connelly PW. V677 mutation of methylene-tetrahydrofolate reductase and cardiovascular disease in Canadian Inuit. *Lancet* 1997;349:1221–2.
- [23] SAS/STAT Guide for personal computers. SAS Institute, Cary, NC, 1987.
- [24] Machleder D, Ivandic B, Welch C, Castellani L, Reue K, Lusis AJ. Complex control of HDL levels in mice in response to an atherogenic diet. Coordinate regulation of HDL levels and bile acid metabolism. *J Clin Invest* 1997;99:1406–19.
- [25] Xu G, Salen G, Shefer S, Tint GS, Nyugen LB, Chen TS, Greenblatt D. Increasing dietary cholesterol induces different regulation of classic and alternative bile acid synthesis. *J Clin Invest* 1999;103:89–95.
- [26] Dansky HM, Charlton SA, Sikes JL, Heath SC, Simantov R, Levin LF, Shu P, Moore KJ, Breslow JL, Smith JD. Genetic background determines the extent of atherosclerosis in Apo E-deficient mice. *Arterioscler Thromb Vasc Biol* 1999;19:1960–8.
- [27] Ishibashi S, Schwarz M, Frykman PK, Herz J, Russell DW. Disruption of cholesterol 7 $\alpha$ -hydroxylase gene in mice. *J Biol Chem* 1996;271:18017–23.