

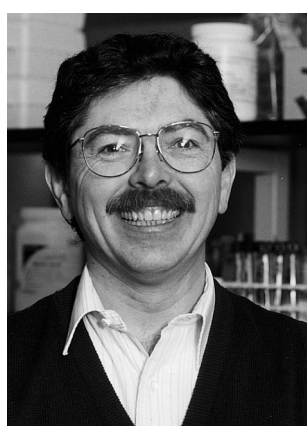
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**Are Canadian Inuit at increased genetic risk for coronary heart disease?**

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**Abstract** The Keewatin Inuit of the Northwest Territories of Canada have a very low age-adjusted mortality rate from coronary heart disease. We hypothesized that this apparent protection from disease has a genetic basis. We determined the prevalence of the disease-associated alleles of five candidate genes for atherosclerosis-related phenotypes. Surprisingly, four of the five alleles studied, namely *AGT* T235, *FABP2* T54, *PON* R192 and *APOE* E4, were significantly more frequent in a sample of 175 Keewatin Inuit than among a representative control sample of whites living in the region. The high frequencies of these disease-associated alleles suggests either that they have no relationship with disease susceptibility in the Inuit, or that some unmeasured genetic and/or environmental factors mitigate disease susceptibility that is associated with these alleles. This highlights the difficulty in extrapolating findings from one population to another. Also, very modest genotype-phenotype associations were observed between *APOE* genotype ( $P=0.016$ ) and plasma low-density lipoprotein cholesterol concentration and between *FABP2* genotype and plasma 2-h postprandial glucose concentration ( $P=0.048$ ). The relationship between *APOE* alleles and plasma low-density lipoprotein cholesterol was the same as has been previously reported in many study samples. However, the relationship between *FABP2* alleles and plasma 2-h postprandial glucose concentrations was the opposite to that reported in other studies. This suggests that differences in environment, such as the type of fatty acid consumed, interacts with functional differences in gene products involved in candidate metabolic pathways to produce phenotypic differences.

**Key words** Angiotensin-converting enzyme · Angiotensinogen · Apolipoproteins · Fatty acid binding protein · Paraonase · Genetic predisposition

**Abbreviations** ANOVA Analysis of variance · BMI Body mass index · CHD Coronary heart disease · HDL High-density lipoprotein · LDL Low-density lipoprotein

## Introduction

Indigenous people from Greenland, Alaska and Canada have lower mortality from coronary heart disease (CHD) than non-native populations [1–3]. This has been attributed both to protective environmental factors, such as consumption of marine-based  $\omega$ -3 fatty acids [4] and to protective genetic factors [3]. However, the prevalence of most genomic variants that are associated with CHD has not been determined in native populations. As North American native groups undergo westernization of their life-style, CHD is expected to become more prevalent, with an attendant increase in both economic and social costs [5]. Assessment of the prevalence of CHD-associated alleles might help to develop a strategy to predict the future incidence of CHD in these people as their environment and life-style change.

There have been numerous reports of associations between CHD-related phenotypes and DNA markers of candidate genes in atherosclerosis. Many of these genomic variants have failed to demonstrate consistent associations in different study samples. However, alleles for some genes are consistently associated with CHD phenotypes, although each has significant exceptions. For example, in most populations, the E4 allele of the gene encoding apolipoprotein E (*APOE*) is associated with elevated plasma concentrations of total and low-density lipoprotein (LDL) cholesterol [6]. Furthermore, the *APOE* E4 allele has been associated with both increased carotid intimal media thickness and CHD [6]. Likewise, homozygosity for the D allele of the gene encoding the angiotensin-converting enzyme (*ACE*) has been associated with CHD-related phenotypes [7–14], with some significant exceptions [15, 16]. The T235 allele of the gene encoding angiotensinogen (*AGT*) has been linked with hypertension in some white [17] and Japanese [18] subjects and hypertension in pregnant women [19] but not with hypertension in other white subjects [20, 21]. The T54 allele of the gene encoding the intestinal fatty acid binding protein (*FABP2*) has been found to be associated with diabetes-related phenotypes in Pima Indians [22] and Mexican-Americans [23]. The R192 allele of the gene encoding paraoxonase (*PON*) has been associated with a pro-atherogenic lipoprotein profile [24] and with increased risk of CHD [25, 26].

Exceptions to the fairly consistent association of the above alleles with CHD-related phenotypes might have been related to genetic differences between the study samples, such as differences in linkage disequilibrium between the marker alleles and causative alleles at the locus. It is also possible that these genomic variants contribute to a background of genetic susceptibility to CHD-related phenotypes, which require additional genetic and/or environmental factors in order to be expressed. These secondary factors might differ between populations. It may be possible to clarify those genetic and/or environmental factors which are generally relevant in contributing to CHD susceptibility by studying such fac-

tors in different human populations whose CHD prevalences vary widely.

The age-adjusted mortality from cardiovascular disease among male and female Canadian Inuit is 0.59 and 0.75, respectively, compared with the rest of Canada [3]. In order to determine whether this healthy aboriginal population has genetic protection from CHD we determined the prevalence of the disease-associated alleles of the *AGT*, *FABP2*, *PON*, *ACE* and *APOE* genes. We also tested for association between these alleles and some intermediate quantitative phenotypes related to CHD.

## Methods and subjects

### Study subjects

The Northwest Territories are located above the 60th parallel of latitude and comprise one-third of the landmass of Canada. In 1986 the population of the Northwest Territories was 52000. Of these, 35% were Inuit (or Eskimos), 15% Dene (or Athapaskan Indians) and 50% predominantly migrants of European origin from other parts of Canada. The traditional Inuit territory extends from the Chukchi Peninsula in northeastern Asiatic Russia, across Alaska and Northern Canada to Greenland. The present study involved residents of eight communities from the Keewatin region of the Northwest Territories, mainly from the eastern coast adjacent to Hudson Bay [27]. A total of 516 randomly selected individuals aged 18–80 participated; of these, 281 reported themselves as being Inuit, 112 of mixed ethnic background, 92 of European background (white) and 31 of an ethnic background other than Inuit, mixed or white. The survey included an interviewer-administered questionnaire and a clinical examination. Plasma samples were obtained with informed consent. The first exclusion criterion was a self-reported ethnic background that was neither Inuit nor white; this left 373 subjects. The second exclusion criterion was an inadequate blood sample for all biochemical and/or genetic determinations; this left 231 subjects. The project was approved by the Institutional Review Boards of the Universities of Manitoba and Toronto.

### Biochemical and genetic analyses

Sufficient DNA and phenotypic information were obtained from 175 Inuit subjects, of whom 91 were women, and from 56 white subjects, of whom 22 were women. Plasma lipids and lipoproteins were determined as described [27, 28]. Concentrations of fasting glucose and glucose 2 h after 75 g glucose load were determined from capillary blood using a reflectance glucometer. Established procedures were used to determine genotypes of *AGT* codon 235 [17], *FABP2* codon 54 [22], *PON* codon 192 [24], the *ACE* intron 16 insertion/deletion (I/D) polymorphism [7] and *APOE* exon 4 [29]. Known standard genotypic controls were run with each genotyping reaction.

### Statistical analysis

SAS (version 6.1) was used for all statistical comparisons [30]. The distributions of biochemical traits, body mass index (BMI), systolic and diastolic blood pressure were significantly non-normal in this dataset. Therefore for parametric statistical analyses each quantitative variable was transformed and subjected to analysis of normality as described [24, 28]. The transformed variables were used for parametric statistical analyses, but the non-transformed values are presented in the tables. Student's *t* test was used to compare mean values of quantitative traits between Inuit and white subjects. An  $\chi^2$  analysis was used to determine whether gen-

otype frequencies deviated from those predicted by the Hardy-Weinberg law;  $\chi^2$  analysis was also used to compare allele frequencies between Inuit and white subjects. Analysis of variance (ANOVA) was performed using the general linear models procedure to determine the sources of variation, with *F* tests computed from the type III sums of squares [30], which is applicable to unbalanced study designs. A total of nine ANOVAs were performed in the Inuit using as dependent variables transformed concentrations of total, LDL and high-density lipoprotein (HDL)=cholesterol, triglycerides, fasting and 2-h postprandial plasma glucose, systolic and diastolic blood pressure and BMI. Independent variables were age, sex and genotypes. To determine the statistical significance of associations in the ANOVA differences between individuals classified by genotype were compared using a *t* test [30].

## Results

### Baseline phenotypic characteristics

Mean values and standard deviations for baseline traits for Inuit and white subjects are shown in Table 1. Significant differences were seen for triglycerides and LDL cholesterol, which were lower in Inuit, and for HDL cholesterol, which was higher in Inuit and genotype frequencies. Allele frequencies are shown in Table 2. Genotype frequencies did not deviate from those predicted by the Hardy-Weinberg law in either the Inuit or the white samples (data not shown). The observed frequencies of all alleles in the sample of white subjects from the Keewatin region were comparable to those observed in over 300

white subjects from Ontario (data not shown) and to those reported in many other studies of these genes in normal control samples of white subjects [7–17, 19–26]. The allele frequencies for all markers tested differed significantly between the Inuit and white subjects of the Keewatin region. Four of the five disease-associated alleles for each marker system, namely *AGT* T235, *FABP2* T54, *PON* R192 and *APOE* E4, were significantly more frequent in the Inuit (Table 2). Only the *ACE* D allele was significantly less frequent in the Inuit.

### Genetic determinants of variation in biochemical traits

The results of the two ANOVAs in the Inuit sample which showed significant genotype-phenotype associations are shown in Table 3. Genetic variation of *APOE* was found to be associated with variation in LDL cholesterol ( $P=0.016$ ). Genetic variation of *FABP2* was associated with variation in plasma 2-h glucose after 75 g glucose challenge ( $P=0.048$ ). None of the other genomic variants was found to be significantly associated with variation in other plasma lipoproteins, blood pressure or BMI in either Inuit or white samples (data not shown).

Homozygotes for the *APOE* E4 allele had the highest mean LDL cholesterol (Table 4), while E4/3 heterozygotes had an intermediate level and E3/3 homozygotes the lowest mean LDL cholesterol. The single Inuit E3/2

**Table 1** Baseline differences between Inuit and white subjects of the Keewatin Region

	Inuit	White	<i>P</i>
Men/women	84/91	34/22	NS
Age (years)	38.1 ±15.7	37.1±12.0	NS
Total cholesterol (mmol/l)	5.06±1.02	5.32±1.05	NS
Triglycerides (mmol/l)	1.01±0.54	1.49±1.06	0.002
LDL cholesterol (mmol/l)	3.14±0.92	3.47±1.05	0.05
HDL cholesterol (mmol/l)	1.47±0.40	1.19±0.34	0.0001
Systolic blood pressure (mmHg)	120.2 ±17.4	118.0 ±11.4	NS
Diastolic blood pressure (mmHg)	75.5 ±10.1	75.9 ±7.87	NS
BMI (kg/m <sup>2</sup> )	26.3 ±4.57	26.0 ±4.72	NS
Current smokers (percentage)	56	70	NS
Diabetes history (percentage)	4	2	NS
Fasting glucose (mmol/l)	6.15±1.15	6.17±1.12	NS
2-h postprandial glucose (mmol/l)	6.88±2.47	6.79±2.12	NS

**Table 2** Allele frequencies in Inuit and white subjects from the Keewatin Region

Gene	Chromosome	Marker	Allele	Inuit (n=175)	White (n=56)	<i>P</i>
<i>AGT</i>	1q42-q44	Codon 235	M235	0.18	0.55	<0.0001
			T235	0.82	0.45	
<i>FABP2</i>	4q	Codon 54	T54	0.35	0.25	<0.05
			A54	0.65	0.75	
<i>PON</i>	7q21-22	Codon 192	Q192	0.30	0.65	<0.0001
			R192	0.70	0.35	
<i>ACE</i>	17q23	Intron 16	Insertion	0.69	0.54	<0.01
			Deletion	0.31	0.46	
<i>APOE</i>	19q13	Exon 4/ <i>Hha</i> I	E2	0.01	0.05	<0.01
			E3	0.76	0.82	
			E4	0.23	0.13	

*AGT*, Angiotensinogen gene; *FABP2*, fatty acid binding protein gene; *PON*, paraoxonase gene; *ACE*, angiotensin-converting enzyme gene; *APOE*, apolipoprotein E gene

**Table 3** ANOVA in Canadian Inuit

Source of variation	df	F-value	P>F
Dependent variable: log plasma LDL cholesterol			
Sex (0.060)	1	3.59	NS
Age	1	9.04	0.0032
Logarithm of BMI	1	7.78	0.006
AGT codon 235 genotype	2	2.08	NS (0.13)
FABP2 codon 54 genotype	2	0.36	NS (0.70)
PON codon 192 genotype	2	0.41	NS (0.66)
ACE intron 16 genotype	2	0.81	NS (0.45)
APOE exon 4 genotype	3	3.58	0.016
Dependent variable: log plasma 2-h postprandial glucose			
Sex	1	15.1	0.0002
Age	1	6.65	0.011
Logarithm of BMI	1	0.02	NS (0.89)
AGT codon 235 genotype	2	0.40	NS (0.67)
FABP2 codon 54 genotype	2	2.98	0.048
PON codon 192 genotype	2	0.37	NS (0.69)
ACE intron 16 genotype	2	0.50	NS (0.61)
APOE exon 4 genotype	3	0.10	NS (0.96)

AGT, Angiotensinogen gene; FABP2, fatty acid binding protein gene; PON, paraoxonase gene; ACE, angiotensin-converting enzyme gene; APOE, apolipoprotein E gene

**Table 4** Biochemical traits in Canadian Inuit classed by genotypes of APOE

	n	LDL cholesterol (mmol/l)	P	
			vs. E4/3	vs. E3/3
E4/4	8	3.54±1.05	0.050	0.015
E4/3	63	3.22±0.85	–	0.050
E3/3	103	3.07±0.92	0.050	–

**Table 5** Biochemical traits in Canadian Inuit classed by genotypes of FABP2

	n	Glucose 2 h postprandial (mmol/l)	P	
			vs T/A	vs A/A
T/T	21	5.70±1.58	0.034	0.021
T/A	80	6.96±2.63	–	NS (0.87)
A/A	74	7.11±2.42	NS (0.87)	–

subject had a plasma LDL cholesterol of 1.85 mmol/l. Pairwise comparisons showed that mean LDL cholesterol in E4/4 homozygotes was significantly higher than in E4/3 heterozygotes and E3/3 homozygotes ( $P=0.050$  and  $0.015$ , respectively). These observations are consistent with an autosomal codominant LDL cholesterol raising influence of the E4 allele. While not statistically significant, a similar trend was seen for mean total cholesterol among the APOE genotypic classes (data not shown).

Homozygotes for the FABP2 T54 allele had significantly lower mean plasma 2-h glucose after 75 g glucose challenge than both heterozygotes and homozygotes for the A54 allele ( $P=0.034$  and  $0.021$ , respectively; Table 5).

These observations are consistent with a recessive lowering of mean plasma 2-h glucose following 75 g glucose challenge in homozygotes for the FABP2 T54 allele compared with subjects with the other two genotypes.

## Discussion

When compared with whites living in the region, the Keewatin Inuit have a significantly increased prevalence of: (a) AGT T235, which has been associated with hypertension; (b) FABP2 T54, which has been associated with insulin resistance; (c) PON R192, which has been associated with CHD; and (d) APOE E4, which has been associated with elevated plasma LDL cholesterol and CHD. Only the ACE D allele, which has been associated with CHD and related phenotypes, is significantly less frequent in the Keewatin Inuit. While the number of white subjects in this study was small, the allele frequencies were similar to those previously reported in European populations [7–17, 19–26] and in a reference Canadian white sample (data not shown).

The higher frequency in Keewatin Inuit of four of the five disease-associated alleles suggests that this group may be genetically predisposed to CHD. However, the present incidence of CHD and the related intermediate phenotypes in the Keewatin Inuit is low [3]. The low CHD incidence is especially striking when considering the high prevalence of cigarette smoking [3]. One explanation for this paradox is that a potentially deleterious influence of the alleles studied is overridden by traditional Inuit life-style and diet. Were this true, westernization of the Inuit life-style could increase expression of disease phenotypes. Alternatively, these genomic variants may have no association with CHD in the Inuit, and other unmeasured genomic variants determine CHD susceptibility.

The Keewatin Inuit had significantly lower plasma concentrations of triglycerides and LDL cholesterol and significantly higher HDL cholesterol than the regional control white sample and the general population of Canada [31]. A similar difference has been observed between Greenland Inuit and Danish whites and was attributed to dietary differences, particularly fatty acid composition [32]. The composition of the diet of the Keewatin Inuit may also be a significant factor explaining the biochemical phenotype and the CHD prevalence since the majority still consume arctic fish on a daily basis [27].

Among populations reported to date, only African Americans have a prevalence of the AGT T235 allele as high as the Keewatin Inuit [33]. It has been proposed that genetic variation of AGT, for which T235 allele is a marker, may modulate blood pressure and lead to hypertension in the presence of secondary factors [33]. The variability of the association between T235 and blood pressure [17–21, 33] may be due to population differences in linkage disequilibrium between T235 and the actual functional genomic variant. The Keewatin Inuit were normotensive, with similar blood pressure determi-

nations as the white control sample. This suggests that other factors affect blood pressure in the Inuit.

The prevalence of the *FABP2* T54 allele in the Keewatin Inuit is higher than that observed in Pima Indians and North American whites [22]. The protein product of *FABP2* is a member of a family of intracellular lipid binding proteins [22]. Its expression is abundant in enterocytes and is induced by fat feeding [34], suggesting that it plays a role in absorption and intracellular transport of dietary long-chain fatty acids. The T54 form has greater affinity for long-chain fatty acids than does the A54 form [22].

Keewatin Inuit homozygous for *FABP2* T54 had lower plasma glucose 2 h after an oral glucose load than subjects with the other genotypes. In marked contrast, Pima Indians homozygous for T54 had a less favourable plasma insulin and glucose response to feeding than subjects who were either heterozygous or homozygous for A54 [22]. The disparity may be due to the fact that Keewatin Inuit, unlike Pima Indians, have a baseline diet that is rich in marine-based polyunsaturated fatty acids [27]. Adaptation to the type of dietary fat is an important determinant of the rate of clearance of a test fat meal [35]. The type of fat and duration of intake might interact with *FABP2* polymorphism to produce variation in response to dietary components. Since fatty acids and glucose compete as oxidative fuel sources in muscle, both the availability and type of circulating free fatty acids may affect peripheral glucose uptake and thus plasma glucose concentration.

The prevalence of the *PON* 192R allele in the Inuit is the highest yet reported in a human population [24–26]. The high prevalence of the gene encoding the high activity variant of serum paraoxonase was predicted by phenotypic characterization in another study of Inuit [36]. Genomic variation in *PON* affecting paraoxonase structure may not underlie the associations with CHD. It is possible that the *PON* codon 192 alleles are in linkage disequilibrium with the actual functional determinant of the phenotype, which may be within a flanking region or within a neighbouring gene.

The prevalence of the *ACE* D allele in the Keewatin Inuit is among the lowest reported in a human population [7–16]. This lower frequency may be one feature of this study sample that is consistent with the lower prevalence of CHD. However, there are significant inconsistencies among other studies using this genotype system as a marker of cardiovascular disease in diverse samples [7–16]. These discrepancies could be due to differences between samples with respect to linkage disequilibrium between this intronic marker and the actual functional variant at the *ACE* locus.

The unique distribution of *APOE* alleles in the Keewatin Inuit is the same as in Greenland Inuit [37] and in American Indians [6] but different from all other study samples [6]. Also, E4 in the Inuit had the same relationship with plasma LDL cholesterol as E4 in whites [6]. However, study of 133 Greenland Inuit found no association between the *APOE* polymorphism and plasma lipoproteins [37]. It is possible that a subtle effect of the E4

allele was more readily detected in our larger Inuit sample. Furthermore, when the frequency of the E2 allele is low, samples of at least 600 individuals are required to detect modest associations between *APOE* genotype and plasma lipoproteins [24, 28]. Since the E2 allele has been associated with increased longevity [6], it might also be interesting to evaluate lifespan in this population. Furthermore, since the E4 allele is associated with Alzheimer disease [6], it would be interesting to determine the incidence of dementia in the Inuit.

The distinctive allele frequencies might have resulted from founder effects involving the ancestors of the contemporary Keewatin Inuit. Others have invoked this explanation for the distinctive distribution of alleles of serological markers in the Inuit [38]. The Keewatin Inuit, also referred to as Central Inuit, are descendants of the Thule, who moved eastward into the central arctic approximately nine centuries ago, supplanting an earlier population of the Dorset culture [39]. All North American Inuit ultimately descended from migrants crossing the Beringia land bridge, exposed at various times during the last glaciation of the Pleistocene Age [40]. The Keewatin Inuit arrived about 5000 years ago and were among the most recent arrivals. Despite insights into the peopling of the Americas that derive from mitochondrial DNA analyses, the timing and number of waves of immigration remain controversial [41].

The unusual frequencies of some of these alleles might also have arisen as the result of selection pressure. It is possible that some of the “disease-associated” alleles have imparted a survival benefit to carriers. For example, heterozygosity for the R192 allele of *PON* and higher plasma paraoxonase activity could have protected against an environmental or dietary toxin [24]. It is also possible that infectious diseases such as tuberculosis have produced the changes in allele frequencies, especially if the alleles studied were in linkage disequilibrium with “resistance” genes in the Keewatin Inuit. It would also be important to determine the haplotypes for each of the alleles studied to assess whether differences in allele frequency are related to the presence of different haplotypes.

In summary, genomic variants of some candidate genes for CHD and related phenotypes occur with significantly higher frequency in the Keewatin Inuit than in a sample of whites living in the same region. The paradox arising from this observation, given the very low incidence of CHD in the Inuit, may have one of two possible explanations. First, if the alleles studied are associated with a functional impact on CHD risk, it would be necessary to invoke other, unmeasured genetic and/or environmental factors which would attenuate this increased risk. Alternatively, these alleles may have no relationship with CHD risk in the Inuit, in contrast with the positive associations seen in some other populations. This study highlights the difficulty in extrapolating findings from one human population to another. We plan to follow the Inuit prospectively to determine the role of specific genetic factors in CHD susceptibility in this community.

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