Population Distributions of APOE, APOH, and APOA4 Polymorphisms and Their Relationships with Quantitative Plasma Lipid Levels among the Evenki Herders of Siberia

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Abstract We examined the distributions of seven polymorphic sites in three apolipoprotein genes (APOE, APOA4, and APOH) and their relationships with quantitative lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) among the Evenki reindeer herders of central Siberia. The polymorphism data reveal several distinctive features that differentiate the Evenki from white populations: the near absence of the *APOE*2* allele, the highest ever recorded frequency of the *APOH*3* allele, the complete absence of the *APOA*2* allele at codon 360, and significantly different frequencies at three other APOA4 polymorphic sites. Our analyses of the relationships of common apolipoprotein polymorphism and plasma lipid levels also revealed interesting results. The well-established positive association between the *APOE*4* allele and LDL cholesterol level reported in white populations was not seen in the Evenki despite a comparable frequency of the *APOE*4* allele. Because the Evenki have significantly lower cholesterol levels than Westernized whites, this difference in allelic effect probably reflects gene-diet interaction, which modulates the effect of APOE polymorphism on LDL cholesterol. At the APOA4 locus the *Hin*cII polymorphism at codon 127 shows a significant impact on plasma triglyceride variation in the Evenki sample: The *Hin*cII − allele was associated with higher triglyceride levels than the *Hin*cII + allele. Our data indicate that both the genetic and the environmental factors conventionally associated with cardiovascular disease risk in Western societies are different in the Evenki.

Apolipoproteins (apo, protein; APO, gene) are the protein moieties of plasma lipoprotein particles that play an important role in the secretion, processing, and catabolism of lipoproteins. Genetic studies carried out mainly in European and American white populations show that common genetic variations in genes coding for apolipoproteins have a significant impact on interindivid-

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ual variation in plasma lipid levels and thus affect the risk for cardiovascular disease in the general population [reviewed by Sing and Moll (1990) and Chan and Dresel (1990)]. However, except for a few studies of Amerindians (Kamboh et al. 1991; Crews et al. 1993) and Eskimos (De Knijff et al. 1992), the distribution of common genetic variations in apolipoprotein genes and their relationships with plasma quantitative traits related to cardiovascular disease risk have not been systematically evaluated in the Arctic area. To fill this gap in this part of the world, we have studied seven polymorphic sites in three apolipoprotein genes (APOE, APOH, and APOA4) among the Evenki reindeer herders of central Siberia in Russia.

APOE is the most studied polymorphic gene involved in lipoprotein metabolism (Davignon et al. 1988). It is a ligand for two cell-surface receptors—low-density lipoprotein (LDL) receptor and LDL-related protein receptor (LRP)—and mediates the uptake of apoE-containing lipoprotein particles by cells. Three common APOE alleles, *APOE*2, APOE*3,* and *APOE*4,* play a significant role in affecting normal plasma cholesterol variation in populations with diverse ethnic backgrounds (Hallman et al. 1991).

Although the precise function of apoA4 is uncertain, it is thought to play an important role in the metabolism of high-density lipoprotein (HDL) and triglyceride-rich lipoprotein particles (Steinmetz et al. 1990; Goldberg et al. 1990). Several polymorphisms in the APOA4 gene have been reported, and some of them have a significant impact on plasma lipid variation (von Eckardstein et al. 1992).

APOH is believed to be involved in triglyceride metabolism, but its precise role is still unknown. The APOH gene exhibits a common polymorphism with the occurrence of three alleles. The effect of APOH polymorphism on plasma lipid levels has yet to be determined unequivocally (Kamboh and Ferrell 1991).

In this article we report the distribution of selected apolipoprotein polymorphisms and their role in determining interindividual variation in total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels among the Evenki people of Siberia.

Materials and Methods

The Population. This study was conducted on plasma and DNA samples collected from the Evenki, who have settled in the Tunguska region of central Siberia. Most of the blood samples were collected from fasting individuals, and the results of lipid quantitation, including total, LDL, and HDL cholesterol and triglyceride levels, have been described by Leonard, Crawford et al. (1994).

The Evenki are a Tungusic-speaking group whose settlements are distributed over the boreal forest (taiga) region of central Siberia in Russia. The

Table 1. Primer Sequences and Expected Fragment and Restriction Enzyme Sizes for APOE and APOA4 Polymorphisms

Gene	*Polymorphic Site*	*Restriction Enzyme*	*Primer*	*Fragment Size (bp)*
APOE[a]	codons 112 and 158	*Hha*I	5′-GCGGACATGGAGGACGTG-3′ 5′-GGCCTGGTACACTGCCAG-3′	177
APOA4[b]	codon 127	*Hinc*II	5′-GCTGCTGCCCCATGCCAATG-3′	192
	codon 130	*Hae*III	5′-TGCAGGCTGTCGGCGTTCTC-3′	
APOA4[c]	codon 347	*Hin*fI	5′-CGGGTGGAGCCCTACGGGGA-3′	300
	codon 360	*Fnu*4HI	5′-TGGGGCCAGTGCACCAGGGG-3′	
APOA4[b]	exon 3 (noncoding region)	VNTR	5′-AGATGCTGGCCCCTTTGGAG-3′ 5′-GAGGCTAGATTCTCAGCAGC-3′	187/191

a. Kamboh, Aston et al. (1995).
b. This study.
c. Kamboh et al. (1992).

Evenki show reduced growth in stature and body weight compared with the American white normative data (Leonard, Katzmarzyk et al. 1994). Although the Evenki derive a large portion of their diet from reindeer meat and milk, they do not have a high fat intake.

The traditional Evenki also have high levels of energy expenditure, and this is reflected in their low serum lipid levels (Leonard, Crawford et al. 1994). Mean total cholesterol (men, 137 ± 2.8 mg/dl; women, 140 ± 4.5 mg/dl), LDL cholesterol (men, 72 ± 2.5 mg/dl; women, 78 ± 3.4 mg/dl), and triglyceride (men, 87 ± 3.7 mg/dl; women, 83 ± 5.2 mg/dl) levels in the Evenki are significantly lower compared with American whites (Johnson et al. 1993). However, the mean HDL cholesterol level in the Evenki (men, 47 ± 1.3 mg/dl; women, 46 ± 1.9 mg/dl) is comparable to that seen in American whites.

Apolipoprotein Genotyping. With the exception of the APOH polymorphism, which was evaluated by isoelectric focusing and immunoblotting techniques in plasma (Kamboh et al. 1988), all other polymorphisms in the APOE and APOA4 genes were screened using specific polymerase chain reaction (PCR) protocols. The primer sequences, expected fragment sizes, and restriction enzymes used to analyze the APOE and APOA4 polymorphisms are given in Table 1.

Statistical Analysis. Allele frequencies were estimated by allele counting. Hardy-Weinberg equilibrium was tested by a chi-square goodness-of-fit test. To evaluate the effect of each polymorphism on the variation of quantitative variables of lipid and apolipoprotein, we carried out an analysis of covariance

(ANCOVA). The distributions of all the variables were tested for normality. The effects of covariates (age and body mass index) were determined by stepwise regression. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. All statistical analyses were done using the statistical software package SAS.

Quantitative values more than three standard deviations from the mean were excluded as outliers from further analyses. This excluded at most two individuals from the analysis of any one quantitative trait except for HDL cholesterol level in males, where four individuals were excluded. Only the two common APOE genotypes, *APOE*3/*3* and *APOE*3/*4,* were included to estimate the impact of APOE polymorphism on quantitative traits. The number of observations in each of the two remaining classes, *APOE*2/*4* and *APOE*4/*4,* were insufficient to be included in the statistical analyses. Similarly, only the APOH genotypes *APOH*2/*2* and *APOH*2/*3,* the APOA4/*Hin*cII genotypes + + and + −, and the APOA4/VNTR genotypes *ID* and *II* were included in the analyses.

Analysis of variance was performed separately for males and females and for the sexes combined, with sex as a potential covariate, to test the null hypothesis of equality of means of quantitative traits between apolipoprotein genotypes. Initially, for the combined sexes all dependent quantitative variables except for total and LDL cholesterol levels were normalized by taking natural logarithms to ensure that the distribution of the dependent variable was Gaussian. For the analysis of the sexes separately, only triglyceride level required a ln-transformation for both sexes, whereas HDL cholesterol level required a ln-transformation only in females.

Significant covariates for each dependent variable were identified using stepwise regression, with an overall 5% level of significance used to select the most parsimonious set of covariates for each dependent variable. The covariates considered were the linear effects of age, height, weight, and BMI. Each dependent quantitative variable was then adjusted to remove the effects of their respective set of significant covariates. The average excess for each allele was estimated according to the method of Templeton (1987) with some modifications (Kamboh, Aston et al. 1995).

The contribution of the apolipoprotein gene to variation in the dependent quantitative traits was estimated according to the method of Boerwinkle and Sing (1986). The proportion of the total phenotypic variability attributable to the apolipoprotein polymorphism was estimated by $s_G^2/(s_G^2 + s_w^2)$, where s_w^2 is the phenotypic variance among individuals with the same genotype.

Results

Genotype Distributions and Allele Frequencies. Of the six expected APOE genotypes resulting from three alleles, only four were observed

Table 2. Distributions of APOE Genotype and Allele Frequencies in the Evenki

APOE Genotype or Allele	*Number (%)*	*Frequency*
Genotype		
**3/*3*	90 (72.6)	
**3/*4*	29 (23.4)	
**4/*4*	4 (3.2)	
**2/*4*	1 (0.8)	
Total	124	
Allele		
*APOE*2*		0.004
*APOE*3*		0.843
*APOE*4*		0.153

(Table 2). The two common genotypes, *APOE*3/*3* and *APOE*3/*4*, constituted 96% of the total distribution. Only one example of the *APOE*2* allele was observed, giving an estimated allele frequency of 0.004, which is well below the average frequency of 0.08 seen in American and European white populations (Kamboh and Ferrell 1990). However, the frequency of the *APOE*4* allele was comparable to that reported in whites.

The two most common APOH genotypes were *APOH*2/*2* (62.8%) and *APOH*2/*3* (30.1%) (Table 3). The *APOH*1* allele was observed sporadically in combination with the two common alleles. Compared with American and European white populations, the Evenki have a significantly lower frequency of the *APOH*1* allele (0.01 vs. 0.06; $p < 0.001$) and a higher frequency of the *APOH*3* allele (0.20 vs. 0.06; $p < 0.0001$). We further subtyped the *APOH*3* allele based on its reactivity with an apoH monoclonal antibody, 3D11 (Kamboh, Wagenknecht et al. 1995). Thirty-seven of 40

Table 3. Distribution of APOH Genotype and Allele Frequencies in the Evenki

APOH Genotype or Allele	*Number (%)*	*Frequency*
Genotype		
**2/*2*	71 (62.8)	
**2/*3*	34 (30.1)	
**3/*3*	5 (4.4)	
**1/*2*	2 (1.8)	
**1/*3*	1 (0.9)	
Total	113	
Allele		
*APOH*1*		0.013
*APOH*2*		0.788
*APOH*3*		0.199

Table 4. Distributions of Genotype and Allele Frequencies at Five Sites in the APOA4 Gene in the Evenki

Polymorphic Site	*Genotype (%)*	*Allele Frequency*
Codon 127 (*Hin*cII)		
	++ = 60 (44.4)	+ = 0.707
	+− = 71 (52.6)	− = 0.293
	−− = 4 (3.0)	
	Total = 135	
Codon 130 (*Hae*III)		
	++ = 132 (98.5)	+ = 0.993
	+− = 2 (1.5)	− = 0.007
	Total = 134	
Codon 347 (*Hin*fI)		
	AA = 122 (96.8)	*A* = 0.984
	AT = 4 (3.2)	*T* = 0.016
	Total = 126	
Codon 360 (*Fnu*4HI)		
	1 − 1 = 131	*1* = 1.00
VNTR (exon 3)		
	DD = 7 (5.4)	*D* = 0.289
	ID = 61 (46.9)	*I* = 0.711
	II = 62 (47.7)	
	Total = 130	

(92.5%) Evenki with the *APOH*3* allele were found to be of the *APOH*3*^{w} subtype, a frequency similar to that found in American whites.

Table 4 shows APOA4 genotype and allele frequency data at five polymorphic sites, including codons 127, 130, 347, and 360 and the 3′ noncoding region in exon 3. With the exception of codon 360, all sites were polymorphic in the Evenki. At codon 127 the frequency of the *Hin*cII − allele was significantly higher in the Evenki than in European whites (0.29 vs. 0.21; $p < 0.05$) (Tenkanen et al. 1992). At codon 130 only two individuals with the variant allele (*Hae*III −) were observed in the Evenki sample compared with its polymorphic occurrence (allele frequency 0.04) in American whites (Kamboh, unpublished data, 1994). Compared with American and European whites, where there is a common polymorphism at codon 347 (Kamboh et al. 1992; Tenkanen et al. 1992), the Evenki are less variable at this site (0.19 vs. 0.02 allele frequency of the *Hin*fI *T* allele). In the noncoding region of exon 3 there is a VNTR (variable number of tandem repeats) polymorphism with either three (deletion) or four (insertion) tetranucleotide repeats (CTGT) with respective allele frequencies of 0.61 and 0.39 in European whites (von Eckardstein et al. 1992). Interestingly, the pattern of this distribution was reversed in the Evenki sample with the common occurrence of the 4-base insertion allele (see Table 4).

Table 5. Mean Adjusted Values of Plasma Lipid Levels (± Standard Error) among Two Common APOE Genotypes in the Evenki

	Men			*Women*			*Combined*		
Lipid Variable	**3/*3* (n = 55)	**3/*4* (n = 18)	p	**3/*3* (n = 31)	**3/*4* (n = 11)	p	**3/*3* (n = 86)	**3/*4* (n = 29)	p
Total cholesterol (mg/dl)	134.9 ± 3.1	142.0 ± 5.6	0.24	142.6 ± 6.6	129.9 ± 10.9	0.12	137.6 ± 3.0	137.7 ± 5.2	0.75
LDL cholesterol (mg/dl)	70.2 ± 2.8	80.2 ± 5.1	0.11[a]	82.4 ± 6.4	65.6 ± 10.7	0.07[b]	75.0 ± 2.8	74.3 ± 4.9	0.69
HDL cholesterol (mg/dl)	44.6 ± 1.1	47.8 ± 1.8	0.12	45.8 ± 2.0	44.7 ± 3.4	0.78	45.0 ± 1.0	46.7 ± 1.7	0.40
Triglycerides (mg/dl)	78.9 ± 3.8	88.2 ± 7.7	0.27	75.5 ± 5.6	69.7 ± 8.5	0.57	78.1 ± 3.3	79.3 ± 5.9	0.99

a. $R^2 \times 100 = 3.4$.
b. $R^2 \times 100 = 7.9$.

Genotype Effects on Lipid Levels. Table 5 shows genotype specific means of four lipid traits among the two most common APOE genotypes (*APOE*3/*3* and *APOE*3/*4*). No significant variability among the two APOE genotypes was observed for any trait. However, a borderline effect ($p \simeq 0.07$) on LDL cholesterol level was noted among women, where the *APOE*3/*4* genotype was associated with lower LDL cholesterol levels compared with the *APOE*3/*3* genotype. The APOE polymorphism explained 8% of the phenotypic variance in LDL cholesterol level in women.

Mean adjusted values of plasma lipid levels among the two common APOH genotypes (*APOH*2/*2* and *APOH*2/*3*) are shown in Table 6. Compared to the *APOH*2/*2* genotype, the *APOH*2/*3* genotype is associated with lower triglyceride levels in men and higher triglyceride levels in women, but in both cases the difference is not statistically significant. All other lipid traits showed comparable values between the two APOH genotypes.

Tables 7 and 8 present the distributions of mean lipid levels among common genotypes of the APOA4/*Hinc*II and VNTR polymorphisms, respectively. Although the VNTR polymorphism showed no effect on any lipid trait, the *Hinc*II polymorphism at codon 127 showed a significant effect on plasma triglyceride level. Individuals with the *Hinc*II + + genotype had a significantly lower triglyceride level compared with the *Hinc*II + − genotype ($p = 0.01$). Although this directional trend was seen in both sexes, it was statistically significant only in women ($p = 0.009$), where this polymorphism explained 15.2% of the phenotypic variance in triglyceride level.

Because of small sample sizes of the less common genotypes in other APOA4 polymorphisms (codons 130 and 347), these samples were not analyzed with respect to plasma lipid levels.

Table 6. Mean Adjusted Values of Plasma Lipid Levels (± Standard Error) among Two Common APOH Genotypes in the Evenki

	Men			*Women*			*Combined*		
Lipid Variable	*2/*2 (n = 40)	*2/*3 (n = 24)	p	*2/*2 (n = 25)	*2/*3 (n = 9)	p	*2/*2 (n = 65)	*2/*3 (n = 33)	p
Total cholesterol (mg/dl)	134.7 ± 3.5	138.9 ± 4.5	0.49	140.6 ± 5.3	140.1 ± 8.8	0.88	136.8 ± 2.9	139.8 ± 4.1	0.62
LDL cholesterol (mg/dl)	71.7 ± 3.2	77.0 ± 4.2	0.36	82.9 ± 4.6	75.9 ± 7.7	0.44	76.0 ± 2.8	77.9 ± 4.0	0.70
HDL cholesterol (mg/dl)	45.2 ± 1.2	44.6 ± 1.5	0.76	45.1 ± 2.2	45.4 ± 3.7	0.94	45.1 ± 1.1	44.8 ± 1.5	0.86
Triglycerides (mg/dl)	86.6 ± 4.8	74.9 ± 5.4	0.12[a]	69.4 ± 5.2	86.9 ± 10.9	0.13[b]	79.5 ± 3.6	78.0 ± 5.1	0.79

a. $R^2 \times 100 = 3.8$.
b. $R^2 \times 100 = 6.7$.

Discussion

We examined the distribution patterns of seven polymorphic sites in three apolipoprotein genes and their relationships with plasma lipid levels in the Evenki reindeer herders of central Siberia. The gene frequency data reveal several interesting features that differentiate the Evenki from American and European white populations. These distinctive features among the Evenki are

Table 7. Mean Adjusted Values of Plasma Lipid Levels (± Standard Error) among Two Common *Hin*cII Genotypes at Codon 127 in the APOA4 Gene in the Evenki

	Men			*Women*			*Combined*		
Lipid Variable	+ + (n = 32)	+ − (n = 47)	p	+ + (n = 20)	+ − (n = 22)	p	+ + (n = 52)	+ − (n = 69)	p
Total cholesterol (mg/dl)	136.5 ± 4.1	136.6 ± 3.4	0.96	138.6 ± 8.0	140.5 ± 7.7	0.37	137.3 ± 3.7	137.9 ± 3.3	0.57
LDL cholesterol (mg/dl)	73.0 ± 3.8	71.3 ± 3.2	0.59	82.8 ± 7.8	75.4 ± 7.8	0.86	77.5 ± 3.5	72.4 ± 3.1	0.51
HDL cholesterol (mg/dl)	44.1 ± 1.4	46.8 ± 1.1	0.14	45.1 ± 2.6	44.2 ± 2.6	0.81	44.5 ± 1.3	46.0 ± 1.1	0.40
Triglycerides (mg/dl)	76.9 ± 4.9	82.9 ± 4.4	0.37	64.0 ± 5.2	86.9 ± 6.7	0.009[a]	72.1 ± 3.7	83.8 ± 3.8	0.01[b]

a. $R^2 \times 100 = 15.2$.
b. $R^2 \times 100 = 4.9$.

Table 8. Mean Adjusted Lipid Levels (± Standard Error) among Two Common Genotypes of a VNTR Polymorphism in the Noncoding Region of Exon 3 in the APOA4 Gene in the Evenki

	Men			*Women*			*Combined*		
Lipid Variable	*ID* (n = 32)	*II* (n = 38)	p	*ID* (n = 18)	*II* (n = 22)	p	*ID* (n = 50)	*II* (n = 60)	p
Total cholesterol (mg/dl)	132.8 ± 4.1	138.8 ± 3.7	0.31	134.9 ± 8.8	144.8 ± 8.2	0.96	133.9 ± 3.9	140.8 ± 3.6	0.51
LDL cholesterol (mg/dl)	69.1 ± 4.0	75.2 ± 3.6	0.35	72.2 ± 8.8	85.3 ± 8.0	0.88	71.2 ± 3.7	78.0 ± 3.4	0.43
HDL cholesterol (mg/dl)	44.8 ± 1.4	45.6 ± 1.3	0.67	42.6 ± 2.8	45.8 ± 2.5	0.40	44.0 ± 1.4	45.7 ± 1.2	0.36
Triglycerides (mg/dl)	81.2 ± 4.9	79.8 ± 4.4	0.83	73.1 ± 6.7	81.9 ± 6.6	0.36	78.4 ± 4.3	80.4 ± 3.8	0.91

(1) the near absence of the *APOE*2* allele, (2) the highest ever recorded frequency of the *APOH*3* allele, (3) the sporadic detection of the variant alleles at codons 130 and 347 in the APOA4 gene, (4) the complete absence of the *APOA4*2* allele at codon 360, (5) significantly higher frequencies of the APOA4 *Hinc*II − allele at codon 127, and (6) a significantly lower frequency of the APOA4 deletion allele in the VNTR region.

The complete absence of the *APOA4*2* allele, which is a unique marker of white populations, provides further evidence that the Evenki are genetically isolated from other population groups of Russia. The near complete absence of the *APOA4*2* allele in aboriginal populations of America has been noted previously (Kamboh et al. 1991; De Knijff et al. 1992). A relatively high frequency of the *APOH*3,* APOA4 *Hinc*II −, and APOA4 insertion alleles in the Evenki may have been attained through genetic drift.

Our analyses of the relationships between common genetic apolipoprotein polymorphisms and plasma lipid traits among the Evenki revealed interesting results. The well-established association between the *APOE*4* allele and elevated plasma cholesterol levels in several white populations was not observed in the Evenki, despite their having a comparable frequency of the *APOE*4* allele. In fact, among Evenki women the *APOE*4* allele was associated with a significantly lower LDL cholesterol level compared with the *APOE*3* allele. The apparent absence of the positive association of the *APOE*4* allele in this study may be due to significantly lower plasma cholesterol levels in the Evenki compared with American and European whites.

The estimation of allele effects on lipid levels involves both the frequency of each allele in the sample and the grand mean and genotype-specific means of the quantitative variables. In this case, although the frequency of

the *APOE*4* allele was comparable with that in whites, the population mean of plasma cholesterol levels was markedly lower in the Evenki than in those populations in which the effect of the *APOE*4* allele was detected. Alternatively, a specific gene-gene interaction or a unique pattern of linkage disequilibrium present in the Evenki might have confounded the estimation of the allelic effect.

However, the most plausible explanation of this different allelic effect between the Evenki and whites is a gene-diet interaction that modulates the effect of the APOE polymorphism. Despite consuming a diet that contains a large amount of animal meat, the Evenki do not have a high saturated fat intake, which contrasts with industrialized Western countries (Leonard, Crawford et al. 1994). In addition to low fat intake, the Evenki also have high levels of energy expenditure (Leonard, Katzmarzyk et al. 1994), which explains why they have low cholesterol levels despite having a similar frequency of the atherogenic *APOE*4* allele.

Although the APOE polymorphism has been found to influence normal plasma cholesterol variation in populations with diverse ethnic backgrounds, exceptions have been noted in Malays from Singapore (Utermann 1987), Mayan Indians from Mexico (Kamboh et al. 1991), and Eskimos from Greenland (De Knijff et al. 1992), further confirming that gene-environment (diet) interaction plays an important role in determining plasma lipid levels.

Although the APOH polymorphism did not reveal a significant impact on any lipid traits, a gender-specific effect on triglyceride level was suggested. Among men the *APOH*3* allele was associated with a lower triglyceride level compared with the common *APOH*2* allele. However, in women the effect of the *APOH*3* allele was in the opposite direction. The elevating effect of the *APOH*3* allele on triglyceride level in women also has been described in an African sample (Sepehrnia et al. 1989). However, this effect of the *APOH*3* allele has not been found to be consistent between studies (Eichner et al. 1989; Kaprio et al. 1991; Saha et al. 1993; Cassader et al. 1994) and therefore may be a chance observation in this study.

Among the five APOA4 polymorphisms studied only two (*Hin*cII and VNTR) were polymorphic enough to be studied in relation to lipid levels. Similar to a previous report in European whites (von Eckardstein et al. 1992), the VNTR polymorphism in the Evenki was not associated with quantitative variation in lipid levels. However, the *Hin*cII polymorphism at codon 127 was found to have a significant impact on triglyceride variation ($p = 0.01$). Both men and women with the *Hin*cII $-$ allele had higher triglyceride levels, but the effect was more pronounced in women, where this polymorphism explained about 15% of the variation in triglyceride level. The absence of the *Hin*cII site at codon 127 is associated with the replacement of the commonly occurring asparagine by serine, and therefore this alternation in the primary structure of the apoA4 protein may have a direct impact on triglyceride metabolism. Although the precise function of apoA4 in lipid metabolism

is uncertain, it appears to be involved in the metabolism of HDL and triglyceride-rich lipoprotein particles. ApoA4 enhances lipoprotein lipase (LPL) activity in the presence of apoC2, which is an essential cofactor for LPL activity (Goldberg et al. 1990). (LPL is an enzyme required for hydrolysis of core triglycerides of triglyceride-rich lipoprotein particles.) ApoA4 is thought to be required for the efficient release of apoC2 from HDL to triglyceride-rich lipoprotein particles, and therefore structural changes in the apoA4 molecule may play an important role in regulating the postprandial metabolism of lipoproteins. In this case the *Hinc*II − allele (serine) may be less effective in transferring apoC2 from HDL compared with the *Hinc*II + allele (asparagine), thus affecting LPL activity, which results in a high plasma triglyceride level in individuals carrying the *Hinc*II − allele. It is noteworthy that not all samples collected in this investigation were taken from fasting subjects, and this may have helped to detect the effect of this polymorphism on triglyceride variation, which otherwise could have been obscured in non-fasting subjects.

In summary, this is the first study among the Evenki that investigated the extent of distributions of apolipoprotein gene polymorphisms and their impact on plasma quantitative risk factors for cardiovascular disease. We conclude that both the genetic and the environmental factors conventionally associated with cardiovascular disease risk in Western societies differ in the Evenki.

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