financially by the VERUM foundation, the Association Française contre les Myopathies, the Groupement de Recherches et d'Etudes sur les Génomes, and the Association pour le Développement de la Recherche sur les Maladies Génétiques Neurologiques et Psychiatriques. Y.T. received a fellowship from the Hereditary Disease Foundation (USA).

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0002-9297/97/6006-0037$02.00

Am. J. Hum. Genet. 60:1552–1555, 1997

A Common mtDNA Polymorphism Associated with Variation in Plasma Triglyceride Concentration

To the Editor:

Many laboratories are investigating the association between nuclear genomic variation and complex human traits (Lander and Schork 1994). However, the extranu-

clear mitochondrial genome is highly polymorphic (Cann et al. 1987), and this variation also could contribute to the variation in complex traits in energy metabolism (Johns 1996). Rare mutations in mtDNA produce myopathies, encephalopathies, and ocular disorders, which can coexist with lactic acidosis or diabetes mellitus (Luft 1994; Shigenaga et al. 1994; Wallace 1994). We hypothesized that common mtDNA variation was associated with variation in quantitative metabolic phenotypes. We studied variation in the mtDNA displacement D-loop at nucleotide (nt) +16517 in the Oji-Cree from the Sandy Lake Reserve in Ontario and evaluated its relationship to intermediate traits involved in lipoprotein and carbohydrate metabolism. This mtDNA variant is part of all four founding Native American mtDNA haplotypes and is informative in most samples from Native American populations (Torroni et al. 1993).

Sandy Lake, Ontario, is located 2,000 km northwest of Toronto, in the subarctic boreal forest of central Canada (Hanley et al. 1995). This isolated community is accessible only by airplane during most of the year. Most members of the community speak both English and Oji-Cree, a member of the Algonquian family of languages. The ancestors of the contemporary residents lived a nomadic, hunting-gathering subsistence. Within the last 70 years, the lifestyle of these people has become extremely sedentary, and the diet has become high in fat. Five hundred twenty-two community members, 18 years of age and older, were studied. Subjects were classified as having either non–insulin-dependent diabetes mellitus or impaired glucose tolerance, on the basis of World Health Organization criteria (WHO 1985). Blood samples were obtained after an overnight fasting period of 8–12 h. The project was approved by the University of Toronto Ethics Review Committee.

Blood was centrifuged at 2,000 rpm for 30 min, and the plasma was stored at −70°C. Concentrations of fasting glucose and of glucose 2 h after a standard glucose load were determined as described elsewhere (Hanley et al. 1995). Concentrations of fasting plasma lipids and fasting lipoproteins were determined as described elsewhere (Hegele et al. 1995). Concentrations of fasting plasma insulin were determined by radioimmunoassay (Pharmacia). mtDNA nt +16517 *Hae*III genotypes were determined as described elsewhere (Torroni et al. 1993).

SAS (version 6.11; SAS Institute 1987) was used for all statistical comparisons. ANOVA was performed by use of the general linear-models procedure, to determine the sources of variation for fasting plasma total cholesterol, fasting triglycerides, fasting HDL-cholesterol, both fasting and 2-h postprandial glucose, and fasting insulin. *F*-tests were computed from the type III sums of squares. This form of sums of squares is applicable to unbalanced study designs and adjusts the level of significance to account for other independent variables included in the model. Independent variables for each ANOVA were sex, age, the log of the body-mass index, the presence of either diabetes or impaired glucose tolerance, and the mtDNA nt +16517 genotype. Transformation using the natural log for each variable resulted in a distribution that was not significantly different from normal. However, since the results were the same whether transformed or untransformed biochemical variables were used, the results for the untransformed variables are presented in table 1.

Subjects were classified by either the presence or the absence of the *Hae*III site at mtDNA nt +16517 (table 1). The age, sex distribution, body mass, and proportion of diabetic subjects were not different between the genotypes. Plasma concentrations of fasting and 2-h postprandial glucose, fasting insulin, and total and HDL-cholesterol were not different between the genotypes. However, the mean fasting plasma triglyceride concentration differed by ~15%, according to the mtDNA genotype (table 1).

In order to determine whether this association was independent of the presence of diabetes, a separate ANOVA was performed post hoc in 332 nondiabetic subjects. This showed a significant association between the mtDNA genotype and plasma triglycerides ($P = .0060$). A third ANOVA performed post hoc determined that the plasma triglyceride concentration was not associated with an interaction between diabetes and the mtDNA genotype ($P = .84$).

Plasma triglyceride variation has genetic and environmental determinants; estimates of heritability are within a range of 10%–65% (Boomsma et al. 1996). Plasma triglyceride variation has been associated with variation in such nuclear genes as *LPL, APOC3,* and *APOC2* (Dammerman and Breslow 1995). Our findings suggest that extranuclear mtDNA variation also could determine variation in plasma triglycerides. Thus, mtDNA variation could be a possible source of variation in studies of complex metabolic phenotypes.

The fatty acids derived from plasma triglycerides are precursors of acyl CoA, which is used in the mitochondrial β-oxidation cycle of fatty-acid metabolism (Luft 1994; Shigenaga et al. 1994; Wallace 1994). Genetic variation affecting mitochondrial function thus could affect utilization of fatty acids in β-oxidation. Differences in cellular utilization may alter the demand for fatty acids from triglycerides and, in turn, may affect the plasma triglyceride concentration. Alternatively, genetic variation affecting hepatic β-oxidation may alter the availability of hepatic free fatty acids that otherwise would be channeled to the synthesis and secretion of circulating triglyceride-containing lipoproteins.

The D-loop polymorphism also may have determined directly the variation in plasma triglycerides. Since both

Table 1

Phenotypic Characteristics (Least-Square Mean ± Standard Error) of Sandy Lake Oji-Cree, Classified by mtDNA *Hae*III Genotype at Mitochondrial nt +16517

	*Hae*III Site Absent	*Hae*III Site Present	*P* difference[a]
Total no./no. of females	229/133	293/160	
Age (years)	35.3 ± .97	36.3 ± .85	NS (.44)
Presence of diabetes or impaired glucose tolerance	37.1%	33.1%	NS (.40)
Body-mass index (kg/m^2)	28.4 ± .35	27.8 ± .31	NS (.20)
Cholesterol (mmol/l)	4.77 ± .064	4.79 ± .061	NS (.80)
Triglycerides (mmol/l)	1.63 ± .054	1.83 ± .061	.0015
HDL-cholesterol (mmol/l)	1.22 ± .022	1.20 ± .020	NS (.24)
Glucose (mmol/l):			
Fasting	8.24 ± .17	8.32 ± .16	NS (.65)
2-h postprandial	11.4 ± .22	11.3 ± .21	NS (.93)
Fasting plasma insulin (pmol/l)	147.8 ± 7.93	161.8 ± 7.44	NS (.12)

[a] Probability of a greater *F*-value between genotypic classes. NS = not significant, with nominal $P = < .05$.

strands of mammalian mtDNA are transcribed from promoters situated in the D-loop region, this variant could have affected mitochondrial function through an effect on gene expression. Alternatively, one or other form of the mtDNA genotype at nt +16517, in the Oji-Cree, might have marked some mitochondrial lineages that harbor other mutations that affect mitochondrial function. While it also is possible that the admixture of non-Amerindian mtDNA haplotypes might have explained the association, it must be noted that other alleles that are prevalent in Europeans, such as the M235 allele of angiotensinogen and the T54 allele of the intestinal fatty-acid binding protein, are markedly less prevalent in the Oji-Cree (R. A. Hegele, B. Zinman, A. J. G. Hanley, S. Harris, and P. W. Connelly, unpublished data), providing indirect evidence against significant admixture.

The possibility that mtDNA variation could influence complex human traits is important for several reasons. First, some mtDNA mutations may select for individuals with advantageous metabolic phenotypes. mtDNA has a mutation rate that is 10 × that of nuclear DNA (Luft 1994; Shigenaga et al. 1994; Wallace 1994). This is explained partly by inefficient mtDNA repair mechanisms, the absence of protective histones, and the constant exposure of mtDNA to oxygen free radicals during oxidative phosphorylation. The rapid rate of mutation in mtDNA may quickly produce advantageous phenotypes during periods of change in energy supply or in requirements such as those associated with migration or with climatic shifts. Although genetic drift in the female germ line fixes neutral mutations in the maternal lineage (Jenuth et al. 1996), selection may affect the persistence of some mtDNA changes. Second, genetic variation affecting *trans*-acting nuclear factors can alter the mtDNA sequence and affect phenotypes (Suomalainen et al. 1995). Thus, associations between some nuclear genomic regions and quantitative traits may be mediated through the effect of mtDNA variation on mitochondrial function. Third, heteroplasmy of mtDNA in some individuals may complicate the simple relationship between mtDNA variation and quantitative-trait variation. Finally, mtDNA variation may contribute to the disparities between studies of the nuclear genetic determinants of complex quantitative traits.

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0002-9297/97/6006-0038$02.00

Am. J. Hum. Genet. 60:1555–1558, 1997

Up-Regulation of the Brain and Purkinje-Cell Forms of Dystrophin Transcripts, in Becker Muscular Dystrophy

To the Editor:

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic disorders caused by mutations in the DMD gene. Clinical pictures in BMD patients are more heterogeneous than those in DMD patients; some patients with BMD exhibit dilated cardiomyopathy in the absence of overt skeletal muscle atrophy or weakness. We previously reported two atypical BMD patients from two unrelated families, who showed severe dilated cardiomyopathy in their teens without obvious skeletal muscle atrophy or weakness (Yoshida et al. 1993). Both patients showed slight calf pseudohypertrophy, and one of them had exertional myalgia. Molecular genetic analysis revealed a deletion ranging from the first muscle exon to the first muscle intron, in the DMD gene in these patients (Yoshida et al. 1993).

Meanwhile, the term "X-linked dilated cardiomyopathy" ("XLDCM," "XLCM," or "XLDC"), originally proposed by Berko and Swift (1987), now is assigned to a clinical phenotype of dystrophinopathy that is characterized by severe dilated cardiomyopathy without apparent skeletal myopathy (Muntoni et al. 1993; Towbin et al. 1993). To date, five mutations in the DMD gene have been reported in this phenotype (Muntoni et al. 1993; Oldfors et al. 1994; Towbin et al. 1994; Franz et al. 1995; Milasin et al. 1996), and a correlation between the XLDCM phenotype and the locations of mutations in the DMD gene has been controversial. Among these mutations, two were identified at the 5′ end of the DMD gene; one mutation was a deletion that removed the muscle promoter, the first muscle exon, and part of the first muscle intron (Muntoni et al. 1993), and the other was a point mutation in the 5′ splice site of the first muscle intron (Milasin et al. 1996). Together with our report (Yoshida et al. 1993), these data indicate that the mutations at the 5′ end of the DMD gene are associated with a distinct phenotype of dystrophinopathy with preferential involvement of cardiac muscle. Further examinations in XLDCM patients revealed that the expression of the brain and Purkinje-cell forms of dystrophin transcripts was up-regulated in the skeletal muscle, whereas there was a lack of the muscle form of dystrophin transcripts (Muntoni et al. 1995; Milasin et al. 1996), suggesting that compensatory transcripts from the brain and Purkinje-cell promoters may maintain adequate levels of dystrophin in the skeletal muscle of XLDCM patients (Muntoni et al. 1995; Milasin et al. 1996). We also analyzed the expression of each form of dystrophin transcripts in one atypical BMD patient (patient 1) with a deletion affecting the first muscle exon and the first muscle intron, in the DMD gene, and also in two BMD patients, with typical skeletal muscle involvement, having a deletion of either exons 45–47 (patient 2) or exons 45–48 (patient 3).

Patient 1, a 24-year-old man, showed slight calf pseudohypertrophy and exertional cramping myalgia in the legs but not muscle atrophy or weakness. He was free of cardiac symptoms; however, an electrocardiogram (ECG) showed an increased R/S ratio in leads V_1 and V_2 and prominent Q waves in leads II, III, and aV_F. An echocardiogram (UCG) revealed diffuse hypokinesis of the left ventricle (LV), with dilatation (LV end–diastolic dimension [LVDd] of 64 mm, LV end–systolic dimension [LVDs] of 52 mm, and fractional shortening [FS] of 19%). Especially the posterior wall of the LV was markedly thin and akinetic. The LV ejection fraction (EF; normal >60%) was depressed severely (26%). Mild mitral valve regurgitation also was observed. A ^{201}Tl