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A mitochondrial DNA D-loop polymorphism and obesity in three cohorts of women

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OBJECTIVE: To examine the hypothesis of an association between a mtDNA D-loop *Kpn I* restriction site polymorphism (RSP) at base pair (bp) 16,133 (morph-1) and obesity in women.
DESIGN: Comparisons of carriers and noncarriers of the mutation for BMI (Body Mass Index) levels and of the frequency of the mutation in obese and normal weight women.
SUBJECTS: 567 unrelated adult Caucasian non-diabetic women from the HERITAGE Family Study ($n = 63$; BMI: 15–47 kg/m^2), Québec Family Study (QFS; 77 controls, BMI: 19–26 kg/m^2 and 38 obese, BMI: 27–56 kg/m^2) and Swedish Obese Subjects (SOS) Study (81 controls, BMI: 18–26 kg/m^2 and 308 obese, BMI: 33–58 kg/m^2).
MEASUREMENTS: BMI was calculated from weight and height (kg/m^2). mtDNA was amplified between base pair 15,928 and 16,500 by polymerase chain reaction (PCR) and digested with the restriction endonuclease *Kpn I*.
RESULTS: No significant differences in the age-adjusted BMI for the mtDNA D-loop *Kpn I* RSP at base pair (bp) 16,133 (morph-1) between carriers and non-carriers in the HERITAGE cohort. No significant association was found between BMI and the *Kpn I* RSP carrier status in the SOS and QFS cohorts. The observed frequencies for the *Kpn I* RSP were not significantly ($P > 0.05$) different between the SOS controls and SOS obese irrespective of the degree of severity of obesity (BMI > 40, > 45 or > 50 kg/m^2).
CONCLUSION: We conclude that the mtDNA D-loop *Kpn I* RSP at bp 16,133 (morph-1) is not a determinant of human obesity.

Keywords: body mass index; genetics; HERITAGE Family Study; Québec Family Study; Swedish obese subjects

Introduction

Genetic variation is commonly recognized as one of the causes of the predisposition to become obese.[1,2] Therefore a large number of genetic epidemiology studies have shown that obesity phenotypes are characterized by a significant transmission effect across generations[2] suggesting with some possibly a stronger maternal transmission.[3,4] Mendelian transmission of single gene effects associated with increased body mass or body fat levels and with a stronger effect in females than in males have also been reported.[5,6] At the molecular level, the latter effect, if proven true, could depend on mitochondrial (mt) DNA variation.

There is suggestive evidence supporting the notion that the mt genome may contribute to individual differences in obesity.[7–10] For instance, in twelve pairs of male monozygotic twins, the majority of carriers of a mitochondrial (mt) DNA D-loop *Kpn I* restriction site polymorphism (RSP) at base pair (bp) 16,133 (morph-1) were high weight gainers after 100 d of overfeeding.[8] One study has reported a number of significant associations between mtDNA variants and haplotypes, and measures of obesity in a sample of Hispanics, Anglos and Samoans.[9] Other studies also suggested a possible role of some mtDNA polymorphisms in high BMI[7] and metabolic rate.[10]

mtDNA is maternally inherited and its genes code for 13 of the 67 proteins involved in the respiratory chain and oxidative phosphorylation.[11,12] Moreover, mtDNA encodes two rRNAs and 22 tRNAs. The D-loop region of mtDNA contains the site of replication and transcription and conserved sequences essential for mtDNA expression.[13,14] Since this is a replication region, there is the possibility that mutations in this region could induce alterations in protein content by affecting transcription.[15] Inasmuch as the mtDNA is transcribed from a single promoter region, and that tRNAs, rRNAs, and mRNAs are released from one polycistronic transcript, any event affecting transcription of mtDNA may have a coordinated effect on the expression level of key proteins of the oxidative

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process. Therefore, the mitochondrial genome provides a few candidate genes and regulatory sequences for the study of the complex multifactorial phenotype of obesity due to its contribution to the respiratory chain and oxidative metabolism. The purpose of this study was to further examine the association between the mtDNA D-loop *Kpn I* RSP at 16,133 (morph-1) and obesity, assessed by the body mass index (BMI) in three cohorts of women.

Methods

Subjects

This study is based on data from 565 unrelated adult Caucasian non-diabetic women who were participants in three ongoing studies: HERITAGE Family Study (HER, $n=63$),[16] Québec Family Study (QFS; 77 controls and 38 obese)[17] and Swedish Obese Subjects (SOS) Study (81 controls and 308 obese).[18] The physical characteristics of the subjects are presented in Table 1.

Genotype Determination

Total DNA (nuclear and mitochondrial) was extracted from white blood cells or lymphoblastoid cell lines. The region around the mt DNA D-loop *Kpn I* RSP at bp 16,133 (morph 1) was amplified by adding primers that extended from bp 15,928 to 16,500. The D-loop *Kpn I* RSP at bp 16,133 (morph-1) is observed by the loss of a restriction site at bp 16,133. This allows discrimination between the D-loop *Kpn I* RSP (morph 1) carriers and noncarriers. PCR[19] was performed in a DNA thermal cycler (GeneAmp PCR System 9600, Perkin Elmer Cetus, Norwalk, Connecticut). The primers used were: 5′-TCA AAG CTT ACA CCA GTC TTG TAA-3′ (sense) and 5′-CCT GAA GTA GGA ACC AGA TG-3′ (antisense). The PCR product was digested with the rectriction endonuclease *Kpn I.* The digestion product was separated by electrophoresis on 8% polyacrylamide gels.

Statistical Analysis

BMI was adjusted for age by linear regression procedures. Differences in the age-adjusted BMI between the D-loop *Kpn I* RSP (morph-1) carriers and non carriers were tested by *t*-test and the nonparametric Wilcoxon Rank Sum test. The association between obesity status and the *Kpn I* marker was also tested in SOS and QFS by Chi-square or Fisher's exact test (FET) comparing morph frequencies in obese and control subjects. Statistical analyses were performed with SAS for Windows 6.08 package.

Results

Table 1 presents the prevalence of the D-loop *Kpn I* RSP at bp 16,133 (morph 1) in the HERITAGE, QFS and SOS cohorts. Both the *t*-test and the nonparametric Wilcoxon Rank Sum test revealed no statistically significant differences in age-adjusted BMI (kg/m^2) between the D-loop RSP at bp 16,133 (morph-1) carriers and non carriers in the HERITAGE cohort. The Chi-square statistic test and FET revealed no significant difference between obese and controls for the D-loop *Kpn I* allele in the SOS ($\chi^2=0.78$, $df=1$, $P=0.38$) and QFS (FET $=0.05$, $df=1$, $P=0.83$) cohorts. We further examined the association between the *Kpn I* RSP and the increasing severity of obesity (BMI's >40, >45 and $>50\,kg/m^2$) in the SOS cohort. For each of these BMI classes, the frequencies of the D-loop *Kpn I* marker were not significantly ($P>0.05$) different between the SOS controls and SOS obese.

Discussion

This report tested the hypothesis of an association between a mtDNA D-loop *Kpn I* RSP at 16,133 and obesity, in three cohorts of women exhibiting a wide range of BMI. The D-loop *Kpn I* at bp 16,133 (morph 1) is caused by the loss of a restriction site. The results were consistent across populations and led to reject the hypothesis of an association. Therefore, we failed to replicate the findings of an earlier study which suggested that this mutation may be involved in the etiology of obesity.[8] The mtDNA D-loop is a

Table 1 Descriptive characteristics of the Caucasian women of the HERITAGE Family Study, Québec Family Study (QFS), and Swedish Obese Subjects (SOS) Study cohorts

	HERITAGE (n=63)		*QFS Controls (n=77)*		*QFS Obese (n=38)*		*SOS Controls (n=81)*		*SOS Obese (n=308)*	
	Mean ± sd	*Range*	*Mean ± sd*	*Range*	*Mean ± sd*	*Range*	*Mean ± sd*	*Range*	*Mean ± sd*	*Range*
Age (y)	52 ± 5	42–65	53 ± 5	41–68	53 ± 6	40–68	49 ± 7	37–61	47 ± 6	37–58
Height (cm)	162 ± 6	152–174	159 ± 6	141–171	158 ± 5	147–169	166 ± 5	152–177	164 ± 6	150–179
Body Mass (kg)	72 ± 13	51–116	59 ± 7	43–75	82 ± 17	66–150	62 ± 5	49–77	110 ± 12	82–155
BMI (kg/m^2)	27 ± 5	15–47	23 ± 2	19–26	33 ± 6	27–56	23 ± 2	18–26	41 ± 4	33–58
% carriers of morph 1*	6.3		9.0		7.9		4.9		7.8	

*$\chi^2=1.2$, $df=3$, $P=0.76$.

non-coding region that contains the promoters of the transcription of the heavy and light strands[14] and the origin of replication of the heavy strand.[13]. Although mutations accumulate more rapidly in the D-loop than in any other region of the mt genome, it contains conserved sequences essential for mt gene expression. The present D-loop *Kpn I* RSP is located in a relative hyper variable area, positioned between bp 16,129 and 16,287.[14] It has been reported that variations in such a domain have less influence on the physical and biological functions of the mt genome than mutations in the highly conserved sequence located about 100 bp upstream of the 'oligo (G)' sequence along the light strand.[14]

It is clear that the genetic basis of most obesity cases is not attributable to a single gene.[2,20]. Hence one would expect that genes and mutations contributing to the etiology of the common forms of obesity would each have only minor effects. Here we failed to detect a significant effect in the mtDNA variant over a wide range of BMI's. The failure may also be a reflection of the fact that the maternal effect could be extremely small in human obesity as suggested by the lack of a strong support for a maternal transmission hypothesis in a good number of genetic epidemiology reports. In conclusion, we have shown in three cohorts of women that the mtDNA D-loop *Kpn I* RSP at bp 16,133 is not likely to be an important determinant of BMI.

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References

1 Bouchard C, Pérusse L, Leblanc C, Tremblay A, Thériault G. Inheritance of the amount and distribution of human body fat. *Int J Obes* 1988; **12**: 205–215.
2 Bouchard C, Pérusse L, Rice T, Rao DC. The genetics of human obesity. In: Bray GA, Bouchard C, James WPT (eds). *Handbook of Obesity.* Dekker Inc: New York, 1998, pp 157–190.
3 Annest JL, Sing CF, Biron P, Mongeau JG. Familial aggregation of blood pressure and weight in adoptive families. III. Analysis of the role of shared genes and shared household environment in explaining family resemblance for height, weight, and selected weight/height indices. *Am J Epidemiol* 1983; **117**: 492–506.
4 Zonta LA, Jayakar SD, Bosisio M, Galante A, Pennetti V. Genetic analysis of human obesity in an Italian sample. *Hum Hered* 1987; **37**: 129–139.
5 Borecki IB, Bonney GE, Rice T, Bouchard C, Rao DC. Influence of genotype-dependent effects on covariates on the outcome of segregation analysis of the body mass index. *Am J Hum Genet* 1993; **53**: 676–687.
6 Comuzzie AG, Blangero J, Mahaney MC, Mitchell BD, Hixson JE, Samollow PB, Stern MP, MacCluer JW. Major gene with sex-specific effects influences fat mass in Mexican Americans. *Genet Epidemiol* 1995; **12**: 475–488.
7 Rowe M, Bremm G, Cooper J, Perry J. Mitochondrial DNA polymorphisms in inherited increased BMI (Abstract). *FASEB J* 1991; **5(4)**: A708.
8 Dionne FT, Truchon J, Turcotte L, Tremblay A, Després JP, Bouchard C. Mitochondrial DNA variants in relation to body fat. In: Ailhaud G, Guy-Grand B. Lafontan M, Ricquier D (eds). *Obesity in Europe 88: Proceedings of the 3rd European Congress on Obesity.* Libbey: London, 1992, pp 369–373.
9 Merriwether DA, Huston SL, McGarvey ST, Ferrell RE. Mitochondrial DNA variation contributes to levels of obesity and adiposity. *Am J Hum Genet* 1995; **57**: A11.
10 Rowe MJ, Willis WT, Norman RA, Ikeme P, Jackman M, Ravussin E. mtDNA type is associated with differences in metabolic rate and substrate oxidation. *Obes Res* 1997; **5** (Suppl 1): S32.
11 Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–465.
12 Wallace DC, Lott MT, Brown MD, Huoponen K, Torroni A. Report of the committee on human mitochondrial DNA. In: Cuticchia AJ (ed). *Human Gene Mapping 1995: a compendium.* Johns Hopkins University Press: Baltimore, MD, 1995, pp 910–954.
13 Clayton DA. Replication of animal mitochondrial DNA. *Cell* 1982; **28**: 693–705.
14 Greenberg BD, Newbold JE, Sugino A. Intraspecific nucleotide sequence variability surrounding the origin of replication in human mitochondrial DNA. *Gene* 1983; **21**: 33–49.
15 Foran DR, Hixon JE, Brown WM. Comparisons of ape and human sequences that regulate mitochondrial DNA transcription and D-loop DNA synthesis. *Nucl Acids Res* 1988; **16**: 5841–5861.
16 Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J. The HERITAGE Family Study. Aims, design, and measurement protocol. *Med Sci Sports Exerc* 1995; **27**: 721–729.
17 Bouchard C. Genetic epidemiology, association, and sib-pair linkage: Results from the Québec Family Study. In: Bray GA, Ryan DH (eds). *Molecular and genetic aspects of obesity.* Louisiana State University Press: Baton Rouge, LA, 1996, pp 470–481.
18 Sjöström L, Larson B, Backman L, Bengtsson C, Bouchard C, Dahlgren S, Hallgren P, Jonsson E, Karlson J, Lapidus L, Lindroos AK, Lindstedt S, Lissner L, Narbro K, Näslund L, Olbe L, Sullivan M, Anders S, Wedel H, Agren G. Swedish obese subjects (SOS): recruitment for an intervention study and selected description of the obese state. *Int J Obes* 1992; **16**: 465–479.
19 Arnheim N, Erlich H. Polymerase chain reaction strategy. *Ann Rev Biochem* 1992; **61**: 131–156.
20 Rosenbaum M, Leibel RL, Hirsh J. Obesity. *New Engl J Med* 1997; **337**: 396–407.