Polymorphisms in Exon 3 of the Proopiomelanocortin Gene in Relation to Serum Leptin, Salivary Cortisol, and Obesity in Swedish Men

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The objective of the current study was to examine the potential impact of a cryptic trinucleotide repeat polymorphism in exon 3 of proopiomelanocortin (POMC) on serum leptin levels and salivary cortisol, as well as obesity and estimates of insulin, glucose, and lipid metabolism in 284 unrelated Swedish men born in 1944. Moreover, we examined if a single nucleotide polymorphism (SNP) (C→T) in exon 3 was associated with these characteristics. The amplification of the microsatellite locus yielded a 155-bp fragment and a fragment with one additional copy of the 9-bp repeat unit GGCAGCAGC (164 bp). The allelic frequencies were 0.96 and 0.04, respectively. Tests for differences in phenotype showed that subjects with the longer polymerase chain reaction (PCR) repeat product (n = 21) had significantly higher serum leptin concentrations (*P* = .024) compared with subjects with the shorter PCR product (n = 230). Salivary cortisol levels, as well as obesity and its related metabolic perturbations, were the same across the POMC genotypes. In conclusion, a microsatellite polymorphism in exon 3 of POMC is associated with elevated serum leptin levels. This association might reflect variations in melanocortin expression and/or activity, because exon 3 contains, among others, the coding sequences for melanocortins.
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IN 1979, CHRETIEN et al[1] proposed that corticotropin (ACTH) and β-lipotropin were part of a larger precursor. They called it 'pro-opio-melanocortin' (POMC). The primary protein product of POMC is a 285-amino acid precursor that undergoes differential processing to yield at least 8 hormones, depending upon the location of synthesis and the stimulus leading to their production.[2,3] No introns separate the various coding domains. The POMC locus has been mapped to chromosome 2 (2p23).[4]

A recent study has identified a major quantitative trait locus (QTL) influencing serum leptin levels and fat mass located on 2p21, in proximity to the POMC locus.[5] This finding has led to the proposal of an association of POMC with human obesity. However, a mutation screening of the coding region of POMC in 96 extremely obese individuals identified a total of 10 variants, but none that were associated with obesity.[6] One study has identified a genetic defect within POMC, in 2 obese probands, resulting in a distinct form of early-onset human obesity.[7,8] In mice, a complete lack of POMC produces similar characteristics.[9]

The POMC gene harbors several polymorphisms and mutations,[6] including a complex cryptic trinucleotide repeat in exon 3.[10] The aim of the present study was to examine if this polymorphic marker was associated with serum leptin levels and salivary cortisol, as well as obesity and its related metabolic abnormalities, in a random cohort of Swedish men. In addition, we sought to determine if a C→T transition in exon 3 of POMC, resulting in a single nucleotide polymorphism (SNP) in some populations, was associated with the same characteristics.[11]

MATERIAL AND METHODS

For the present study, the subjects (n = 284) were randomly selected from a larger geographically defined total population cohort of men born in Gothenburg, Sweden in 1944. The design has been described elsewhere.[12,13] All subjects gave written informed consent before participating in the study, which was approved by the local ethics committee.

Serum leptin concentrations were determined in duplicate using a human leptin radioimmunoassay (RIA) kit (Linco Research, St Charles, MO). Salivary cortisol was measured repeatedly over a random working day. In addition, an overnight low-dose (0.5 mg) dexamethasone suppression test was performed at home with cortisol analyzed in saliva.[12] Body mass index (BMI, kg/m^2), waist-to-hip ratio (WHR), and abdominal sagittal diameter were measured as described previously.[12,13] A commercial RIA kit was used for the determination of serum insulin. Glucose was determined by the automated glucose analyzer ESAT 6660 from Eppendorf (Hamburg, Germany),[14] and serum lipids were measured with an enzymatic procedure in a Cobas Fara II (Roche Molecular Biochemicals, Mannheim, Germany).[15]

Genotyping was performed on leukocyte DNA and carried out by polymerase chain reaction (PCR) technique. To score the microsatellite polymorphism in exon 3 ($(AAC)_1[(AGC)_2(GGC)_1]$), genomic DNA (150 ng in 10-μL reactions) was amplified by PCR (annealing temperature, 63°C) using primers described previously.[10] The infrared tag IRD800 (LICOR, Lincoln, NE)-labeled DNA fragments were separated by size using automatic infrared DNA sequencers from LICOR. Genotyping of the C→T substitution in exon 3 was performed by PCR restriction fragment length polymorphism analysis using primers described previously.[11] The PCR products were digested overnight at 37°C with 5 U of *Ear*I, and the fragments were separated on a 3% agarose gel.

Statistical differences between genotypes were tested using a 1-way analysis of covariance (ANCOVA) procedure, including genotype as independent factors and BMI and WHR as covariates. To adjust the

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Table 1. Differences in Serum Leptin and Salivary Cortisol Measurements by the Genotype of the Cryptic Trinucleotide Repeat Polymorphism in Exon 3 of the POMC

	Genoypes		
	155 bp (n = 230)	164 bp (n = 21)	P*
Serum leptin (μg/L)	5.9 ± 4.1	7.5 ± 5.6	.024
Cortisol level (nmol/L) in the morning	15.0 ± 7.5	14.0 ± 7.1	>.20
Cortisol level (nmol/L) at 11:45 AM	7.1 ± 5.6	8.0 ± 3.5	>.20
Cortisol level (nmol/L) at 30 min after lunch	8.3 ± 8.4	8.2 ± 4.8	>.20
Cortisol level (nmol/L) at 45 min after lunch	7.5 ± 5.6	7.4 ± 4.9	>.20
Cortisol level (nmol/L) at 60 min after lunch	6.8 ± 5.5	6.8 ± 3.9	>.20
Cortisol level (nmol/L) at 5:00 PM	4.8 ± 2.5	4.8 ± 2.4	>.20
Cortisol level (nmol/L) before bedtime	3.4 ± 4.6	2.6 ± 1.4	>.20
Total diurnal cortisol level (nmol/L)	7.4 ± 3.9	7.5 ± 3.1	>.20
Dexamethasone suppression test (nmol/L)	12.2 ± 5.5	10.3 ± 4.4	.183

NOTE. Values are given as mean ± SD.
*Adjusted for BMI and WHR.

significance level ($P < .05$) for multiple comparisons, we used the Scheffe test. All data analyses were performed with STATISTICA for Windows, release 5.1 (StatSoft Inc, Tulsa, OK).

RESULTS AND DISCUSSION

The frequency of allele 155 bp $((AAC)_1(AGC)_2[(AGC)_2(GGC)_1]_2)$ was 0.96 and that of allele 164 bp $((AAC)_1(AGC)_2[(AGC)_2(GGC)_1]_3)$ 0.04. This is in accordance with allelic frequencies observed in other studies in Caucasian populations.[10,16] The observed genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 1.1$, df = 2). In a recent study by Hixson et al,[11] a C→T substitution located in the 3′-untranslated region of exon 3 was found by direct sequencing. This polymorphism could also be typed by digestion with the restriction enzyme *Ear*I.[11] However, no variant individual was found in the present population.

The assessment of cortisol in saliva is noninvasive and stress free, as well as laboratory independent.[12,17] The concentration of cortisol in saliva represents the unbound ("free") hormone fraction and reflects accurately the free fraction of cortisol in plasma.[17] Because leptin, cortisol, insulin, and the metabolic variables are highly dependent on BMI and WHR,[18] these measurements were adjusted for the influence of the anthropometric factors.

The results in Table 1 show that heterozygotes with 3 copies of the 9-bp repeat unit GGCAGCAGC (164 bp) had significantly ($P = .024$) higher serum leptin levels compared with homozygotes with the shorter PCR product (155 bp). Salivary cortisol levels were virtually identical across the POMC genotypes. A recent study by Hixon et al[11] has shown that POMC haplotypes using a polymorphic *Rsa*1 site in the 5′-flanking region and a C→T polymorphism were associated with normal variation in leptin levels. However, the polymorphism in exon 3 was not by itself associated with serum leptin concentrations, and only limited variability was found in the population.[11] Similar findings have recently been reported in a Danish cohort.[19] One reason for the disparity between our results and those of previous studies[11,19] could be differences in the genetic background, as the allele frequencies seem to diverge. For instance, in the Danish cohort,[19] the frequency of allele 164 bp was lower compared with our study. Another reason could be that the microsatellite polymorphism serves only as a marker for an as yet unidentified functional variant, with different degrees of linkage disequilibrium among these populations.

Table 2 presents the differences in anthropometric and metabolic measurements by genotype of the microsatellite polymorphism in exon 3. There were no significant differences between the genotype groups with respect to estimates of obesity (BMI), body fat distribution (WHR and abdominal sagittal diameter), and related metabolic abnormalities. This confirms previous findings to the effect that DNA sequence variation in the coding regions of POMC is rarely associated with obesity.[6] Rare mutation in exon 2 (C3804A) of the POMC had been found to be associated with early-onset severe obesity and adrenal insufficiency.[7] However, we did not detect a single case with the C3804A mutation by restriction-endonuclease digestion analysis with *Sph*I in the present sample.

Exon 3 contains the coding sequences for the various polypeptide products of POMC, including melanocortins, which act in the brain to reduce food intake and are mediators of leptin action.[20] In addition, animal studies suggest that leptin stimulates arcuate nucleus POMC expression via a pathway involving leptin receptors.[21] Therefore, one might speculate that the observed association of the POMC 9-bp microsatellite polymorphism with serum leptin levels reflects variations in melanocortin expression and/or activity.

In summary, we have shown that a cryptic trinucleotide repeat polymorphism in exon 3 of POMC is associated with elevated serum leptin levels. However, the results from the present population of middle-aged Swedish men indicate that the microsatellite marker is not associated with either cortisol or obesity and its related metabolic abnormalities.

Table 2. Differences in Anthropometric and Metabolic Measurements by the Genotype of the Cryptic Trinucleotide Repeat Polymorphism in Exon 3 of the POMC

	Genoypes		
	155 bp (n = 230)	164 bp (n = 21)	P*
BMI (kg/m^2)	26.0 ± 3.7	26.5 ± 5.0	>.20
WHR	0.94 ± 0.1	0.95 ± 0.1	>.20
Abdominal sagittal diameter (cm)	22.5 ± 3.5	22.6 ± 4.5	>.20
Insulin (mU/L)	12.2 ± 11.0	13.8 ± 7.5	>.20
Glucose (mmol/L)	4.5 ± 0.9	4.4 ± 0.5	>.20
Triglycerides (mmol/L)	1.8 ± 1.1	1.6 ± 0.7	>.20
Cholesterol (mmol/L)	6.2 ± 1.1	6.2 ± 1.1	>.20
High-density lipoprotein cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3	>.20
Low-density lipoprotein cholesterol (mmol/L)	4.1 ± 1.0	4.2 ± 1.1	>.20

NOTE. Values are given as mean ± SD.
*Adjusted for BMI and WHR.

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