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Linkage exclusion analysis of two candidate regions on chromosomes 7 and 11: Leptin and UCP2/UCP3 are not QTLs for obesity in US Caucasians

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Abstract

Leptin (LEP) and the uncoupling proteins 2 and 3 (UCP2/UCP3) are key molecules involved in the regulation of food intake and energy expenditure. However, their contribution to variation of obesity phenotypes in the general population remains controversial. The present study is to investigate whether chromosomal regions 7q and 11q, which contain LEP and UCP2/UCP3, respectively, can be excluded for linkage with obesity phenotypes. The obesity phenotypes include body mass index (BMI), fat mass, and percentage fat mass (PFM), with the latter two measured by dual-energy X-ray absorptiometry. We conducted exclusion linkage analyses using a variance component approach in a sample of 1816 individuals coming from 79 extended Caucasian pedigrees. In this study, we were able to exclude chromosomal region 7q containing LEP as having an effect on fat mass and PFM at effect sizes of 5% or greater, and on BMI at effect sizes of 10% or greater. We were able to exclude chromosomal region 11q containing UCP2/UCP3 as having an effect on fat mass and PFM at effect sizes of 10% or greater, and on BMI at effect sizes of 5% or greater. Our results suggest that the LEP and UCP2/UCP3 genes are unlikely to have a substantial effect on variation in obesity phenotypes in this particular US Caucasian population.

Keywords: Linkage exclusion analysis; Leptin; UCP2/UCP3; Body mass index; Fat mass; Percentage fat mass

Obesity is one of the most serious public health problems in the world [1,2]. As a multifactorial disorder, obesity encompasses various genetic and environmental components manifesting in imbalances in energy intake and expenditure [1]. Energy homeostasis is maintained by signals from feedback loops that regulate food intake, energy expenditure, lipid metabolism, and glucose metabolism [3]. Thus, variation in the genes involved in these pathways could have impact on the development of obesity.

The leptin (LEP) gene, which plays a central role in the central regulation of food intake and energy homeostasis [4], was mapped on chromosomal region 7q31 [5]. LEP mutations in the rodent (*ob*/*ob* mice) could cause extreme obesity [6], but human leptin mutations are rare [7]. The chromosome 7q containing LEP was linked to extreme obesity [8] and obesity phenotypes in Mexican Americans [9]. However, most of the reported whole-genome linkage scans did not find significant linkage at this chromosomal region [10]. Association studies on LEP and obesity have yielded inconsistent results [10].

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Chromosome 11q contains two genes, uncoupling protein 2 and 3 (UCP2/3), which are separated by ~17 kb [11]. UCPs are a family of inner mitochondrial membrane transporters that may release stored energy as heat by dissipating the proton gradient [12]. UCP2 has a wide tissue distribution at varying levels [13]. UCP3 is expressed predominantly in skeletal muscle, a major site of thermogenesis in humans [11]. Earlier studies in Pima Indians reported suggestive linkage on chromosome 11q21–q24 to obesity-related phenotypes [14–16]. However, this region is ~15 cM from 11q13, on which the UCP2/UCP3 genes are located.

Despite the importance of the LEP and UCP2/3 genes in regulation of energy homeostasis, their contribution to variation in obesity phenotypes remains controversial. Pervious genetic studies mainly examined their linkage and/or association with obesity. In this study, for the first time, we used a large sample of US Caucasian pedigrees to test specifically for formal *EXCLUSION* of their linkage to obesity in the context of quantitative trait locus (QTL) analysis.

Materials and methods

Subject. The study subjects came from an expanding database in the Osteoporosis Research Center (ORC) at Creighton University. The sample contains 1816 individuals from 79 extended pedigrees, all Caucasians of European origin. The size of each pedigree varies from 4 to 416 individuals, with a mean of 31.9 ($\pm$SD = 48.9). The exclusion criteria of sampling have been described elsewhere [17]. Individuals who were overweight (BMI > 25 kg/m^2) or obese (BMI > 30 kg/m^2) but permitted under the inclusion/exclusion criteria were included in this study. Every study subject signed an informed-consent document before entering the project.

Genotyping. DNA was genotyped using fluorescently labeled markers as usual [18]. The microsatellite markers genotyped by us are commercially available through Perkin Elmer Applied Biosystems (ABI PRISM Linkage Mapping Sets Version 2.5, Norwalk, CT). The average heterozygosity of the genotyped markers is ~0.76. The PCR was performed on PE 9700 thermocyclers (GeneAmp PCR System 9700, Applied Biosystems, Foster City, CA). PCR cycling conditions followed suggestions in the manual of ABI PRISM Linkage Mapping Sets, Version 2.5. Genotyping was performed using Applied Biosystems automated DNA sequencing systems (Model 3700; Perkin Elmer-ABI, Foster City, CA) running the GENESCAN Version 4.0 and GENOTYPER Version 4.0 software for allele identification and sizing.

A genetic database management system (GenoDB) [19] was used to manage the phenotype and genotype data for linkage analyses. PedCheck [20] was employed for checking confirmation to Mendelian inheritance pattern at all the marker loci and for checking the relationships of family members within pedigrees.

Measurements. Weight and height are measured following standardized protocols for the collection of anthropometric measures. BMI is defined by the weight/height^2 ratio in the units of kilograms/meters^2. Fat mass was measured by dual energy X-ray absorptiometry (DXA) with a Hologic 2000+ or 4500 scanner (Hologic, Bedford, MA, USA). Percentage fat mass (PFM) was calculated as the ratio of fat mass to total body mass (i.e., the sum of fat mass + bone mass + lean mass, all measured by DXA). The precision of BMI measurement as reflected by the coefficient of variation (CV) was 0.2%. The CVs for fat mass and PFM were 2.2% and 2.1%, respectively, for measurements obtained on the Hologic 2000+ scanner, and were 1.2% and 1.1%, respectively, for measurements on the Hologic 4500 scanner. Members of the same pedigree were usually measured on the same type of machine.

Statistical analyses. Using the program package SOLAR [21], we performed multipoint linkage exclusion analyses on chromosomes 7q and 11q. SOLAR employs a variance component model to calculate LOD-scores. Prior to linkage exclusion analyses, effects of age, sex, age * sex, and age^2 were evaluated in polygenic models as covariates, with the significant covariates ($P \leqslant 0.05$) incorporated in the model. The estimated heritabilities ($\pm$SE) of BMI, fat mass, and PFM were 0.42 ($\pm$0.04), 0.42 ($\pm$0.05), and 0.41 ($\pm$0.05), respectively, after adjusting for significant covariates.

In multipoint linkage exclusion analysis, we compared models with fixed QTL effect sizes of 5%, 10%, 15%, and 20% with a model allowing for no QTL effect. The linkage was evaluated at 1-cM interval across the two chromosomal regions. The likelihood ratio test was employed in comparison of the competing models, producing a test statistic asymptotically distributed as a χ^2, with degrees of freedom equal to the difference in the number of independent parameters being estimated in the two models.

Simulations. To establish the criteria for linkage exclusion, we performed simulation analysis using SOALR. Simulation was based on the observed data and pedigree structure of the study sample. We assumed that the simulated population was random and the genotype data were in Hardy–Weinberg equilibrium. Quantitative traits and genotype data were simulated for 2000 replicates of the data set. The power to exclude a region as a QTL was calculated as the proportion of replicates for which we obtained an LOD score less than a certain criterion [22]. Thus, the exclusion power is referred to as the probability that we can correctly exclude a tested candidate chromosomal region when it is not a true QTL at a certain effect size. Besides exclusion power, type I error rate was also taken into account. The type I error here was defined as the probability that a candidate region is excluded for linkage while it actually is a true QTL at a certain effect size.

Since linkage was evaluated at 1-cM interval across the two chromosomal regions, multiple testing should be taken into account to control type I error. The number of testing on 7q31–35 and 11q13–24 was ~37 and ~74, respectively. To be stringent, we adopted Bonferroni correction to correct multiple testing. At the overall significance level (α) of 0.05, the pointwise significance levels were 0.0014 and 0.0007 (i.e., 0.05/37 and 0.05/74), respectively.

In Fig. 1, we show the exclusion power and corresponding type I error at effect size of 5% (and 10%) under a range of LOD scores. Under an ideal situation (that is the statistical power of 0.874 and type

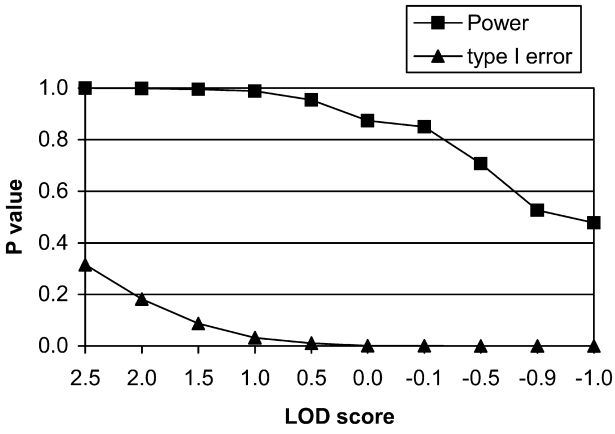

Fig. 1. The statistical power and type I error rate to exclude a gene or region with a fixed effect size of 5% corresponding to the LOD scores varying from −1 to 2.5.