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Melanocortin-3 receptor gene variants: Association with childhood obesity and eating behavior in Chilean families

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ABSTRACT

Objective: To evaluate the association between melanocortin-3 receptor common genetic polymorphisms with childhood obesity and eating behavior in Chilean families.
Methods: Two hundred twenty-nine obese children (6–12 y old, body mass index >95th percentile of Centers for Disease Control and Prevention/National Center for Health Statistics, 2000) and 270 parents were selected. Genotypes for MC3R genetic markers −239A > G, 17C > A (Thr6Lys), 241 G > A (Val81Ile), +2138InsCAGACC, and microsatellite D20s32e were determined. Eating behavior scores were computed using the Child Eating Behavior Questionnaire and a shorter version of the Three Factor Eating Questionnaire adapted for evaluating eating inclinations in children. Genotype-obesity associations were assessed by the Transmission Disequilibrium Test. Non-parametric tests were used to compare eating behavior scores across study groups.
Results: Allelic frequencies of −239 G, 17A, 241A, and +2138InsCAGACC were estimated as 4.5%, 5.9%, 5.6%, and 17.6%, respectively, in obese children. The Transmission Disequilibrium Test in case–parent trios revealed no significant associations between childhood obesity and genetic markers, including the microsatellite D20s32e. In girls, we found significantly higher scores of the emotional eating subscale in carriers of the +2138InsCAGACC compared with non-carriers ($P = 0.04$). In boys, carriers of 17A and 241A showed lower scores for the emotional eating subscale ($P = 0.01$), whereas carriers of +2138InsCAGACC showed significantly lower scores for the enjoyment of food subscale compared with non-carriers ($P = 0.04$).
Conclusions: There is not sufficient evidence to support the contribution for common melanocortin-3 receptor variants in childhood obesity. However, our results are concordant for a role of melanocortin-3 receptor variants in some dimensions of eating behavior such as emotional eating and enjoyment of food.

Introduction

Obesity is the most prevalent nutritional disease among children and constitutes a major health problem in Western societies. In Chile, the obesity prevalence has tripled over the past 15 y and it is now the most frequent nutritional disease in children [1,2]. It is generally accepted that common multifactorial obesity results from the contribution of multiple genetic and environmental factors affecting energy homeostasis, where a cluster of susceptibility genes interact with the obesogenic environment and therefore predispose to excessive weight gain [3].

The melanocortin system plays an important role in energy homeostasis [4]. Genetic deficiency of the melanocortin-4 receptor gene (MC4R) in mice produces hyperphagia and obesity [5]. In contrast, the phenotype of the Mc3r$^{-/-}$ mouse is characterized by a higher percentage of body fat, reduced lean mass, mild obesity, mild hypophagia, reduced locomotor activity, hyperleptinemia and reduced linear growth compared with the wild-type mice [6]. Furthermore, it has been shown that Mc3r$^{-/-}$ mice show enhanced illness-induced anorexia and weight loss after lipopolysaccharide administration. Likewise, it has been hypothesized that defective MC3R signaling could lead to an enhanced susceptibility to weight loss in human diseases [7].

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Interestingly, mice with genetic deficiency in Mc3r and Mc4r are significantly heavier than mice lacking only Mc4r, indicating a possible non-redundant participation of both melanocortin receptors in obesity and adiposity-related phenotypes [6]. In contrast, rare human MC4R mutations have been related to the most common forms of monogenic obesity [8], and a common polymorphism near MC4R has been consistently associated with multifactorial obesity [9].

The human MC3R gene encodes for a seven-transmembrane G-protein–coupled receptor expressed in hypothalamic nuclei involved in the regulation of feeding behavior [10]. It has been suggested that a heterozygous loss-of-function mutation in MC3R constitutes a special type of human monogenic obesity [11], although this direct cause–effect is still controversial [12]. In multifactorial human obesity, several genome linkage studies have suggested a role of a susceptibility gene for obesity in 20q, which is the chromosomal location of MC3R [13]. A genomewide linkage study focusing on long-term weight gain in the Framingham cohort also found significant linkage for chromosome 20q, indicating MC3R as a candidate gene for obesity [14]. Although there are no evidences for the involvement of common variants near MC3R gene from genomewide association studies (as it occurs for MC4R) [15], there are several association studies that have reported a possible involvement of MC3R variants in childhood obesity traits [16–18].

Research on childhood obesity and nutrient intake has traditionally focused on the evaluation of the amount and type of foods consumed in the usual diet. However, a better understanding of the link between eating behavior and obesity is also of interest. Psychometric tools such as the Child Eating Behavior Questionnaire (CEBQ) and the Three-Factor Eating Questionnaire (TFEQ) can be used to measure eating behavior [19,20]. Because of the special phenotype shown by the Mc3r-deficient mice (increased body fat with mild hypophagia) and the proposed role of MC3R in energy homeostasis, we hypothesized that common MC3R variants are related to human childhood obesity and eating behavior patterns. Therefore, the aim of the present study was to test the association among common genetic variants in the MC3R gene and childhood obesity and eating behavior scores computed in questionnaires specifically designed for children.

Materials and methods

Subjects

A sample of 229 unrelated obese children (body mass index [BMI] >95th percentile of Centers for Disease Control and Prevention/National Center for Health Statistics [CDC/NCHS], 2000; mean age ± standard deviation 9.8 ± 2.1 y; age range 6–12 y; 45% girls) was selected through an open invitation made in public schools and from obese children attended at the Institute of Nutrition and Food Technology (INTA) in Santiago, Chile. In 135 children, we also selected their parents ($n = 270$) to ensemble case–parent trios. The study protocol was approved by the INTA ethics committee of the University of Chile. Signed written informed consents were obtained from all parents or guardians of the children.

Anthropometric measurements

Height and weight were measured in light clothing and without shoes. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured using a non-elastic tape just above the uppermost lateral border of the ilium, at the end of a normal expiration. Obesity was defined as BMI above the 95th percentile of the CDC/NCHS 2000 reference curve [21]. BMI *z*-scores and waist-to-height ratios were calculated in all children.

Genetic analysis of MC3R gene

Blood samples were taken from each participant for the extraction of genomic DNA. The human MC3R gene (gene ID: 4159, 20q13, 1083 bp) is composed of a single exon that encodes for a protein of 360 amino acids (NC_000020.9, NT_011362.9, NM_019888.2, NP_063941.2). The genotypes of the common MC3R single nucleotide polymorphisms −239A > G (rs11575886), 17C > A (Thr6Lys; rs3746619), 241 G > A (Val81Ile; rs3827103), and +2138InsCAGACC were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism techniques. Primers and PCR conditions [16,22] are presented in Table 1. PCR products were digested using *Alw*I, *Hpy*CH4IV, *Bsa*JI, and *Pst*1 restriction enzymes, electrophoresed on 3% agarose gels, and visualized with ethidium bromide using an ultraviolet transilluminator. The TG repeat-rich region of the polymorphic microsatellite marker D20s32e locus was amplified as previously described [23] and PCR products were resolved by capillary electrophoresis in an ABI PRISM 3100 sequence detection system. Alleles were identified according to their PCR product size (115, 117, 119, 121, and 123 bp).

Eating behavior questionnaires

Direct interviews with the mothers were carried out by trained personnel in home visits or in the clinic of INTA (University of Chile) to ascertain eating behavior scores measured using two psychometric questionnaires: the CEBQ and the 19-item TFEQP Parent Version (TFEQP-19) [19,20,24].

The CEBQ consists of 35 items that measures eight subscales of eating behavior: enjoyment of food (four items), emotional overeating (four items), emotional undereating (four items), food fussiness (six items), food responsiveness (five items), slowness in eating (four items), satiety responsiveness (five items), and desire to drink (three items). Each item was answered in a Likert-type scale with possible scores from 1 (never) to 5 (always).

The Chilean TFEQP-19 is an adapted version of the TFEQ R-18 [20]. TFEQP-19 was modified from TFEQ R-18 by our research group to obtain adequate responses from Chilean mothers on their children's eating behavior. In addition, we included a new item in TFEQP-19 compared with the previously published TFEQ R-18. All items were answered on a Likert-type scale with possible scores from 1 (definitely true) to 4 (definitely false). The TFEQP-19 measures three subscales of eating behavior: restrained eating (six items), uncontrolled eating (10 items), and emotional eating (three items).

The CEBQ and TFEQP-19 were adapted to be used in the Chilean population by a procedure involving direct and reverse translations of the original questionnaire by two independent translators and a cultural adaptation to the Spanish language spoken in Chile carried out in a pilot test of 10 child–mother duos [24]. Both adapted questionnaires are available from the corresponding

Table 1
PCR primers and conditions for human MC3R gene polymorphisms

Polymorphism	Primers	PCR product	Annealing temperature	Cycles
−239A > G (rs11575886)*	Forward: 5′-GAGGGAGACAGAAGGAAGACAG-3′ Reverse: 5′-TTCCAGGAGGAGCAGTTTTGATC-3′	86 bp	58 °C	35
Thr6Lys (17 C > A; rs3746619)† Val81Ile (241 G > A; rs3827103)	Forward: 5′-ACCCTCCCCATCCTTTTATTC-3′ Reverse: 5′-AGGGCATTGGACACACTTACC-3′	441 bp	58 °C	30
+2138InsCAGACC	Forward: 5′-CCGCTCAGTGGGTAAATGTAG-3′ Reverse: 5′-TGAGAATCTGAGAAAGTCGTCG-3′	1125/1131 bp	64 °C	30
D20s32e	Forward: 5′-GTTACATGGAGAGATGGATC-3′‡ Reverse: 5′-TGTAGATCATATGAACTCAG-3′	115–123 bp	53 °C	35

MC3R, melanocortin-3 receptor; PCR, polymerase chain reaction
* The underlined nucleotide was introduced to create an artificial restriction site.
† The rs3746619 and rs3827103 were amplified using the same pair of primers.
‡ Forward primer of D20s32e was carboxyfluorescein-aminohexyl amidite-labeled.