

Applied nutritional investigation

Melanocortin-4 receptor polymorphism rs17782313: Association with obesity and eating in the absence of hunger in Chilean children

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ABSTRACT

Objective: The aim of this study was to assess the association between melanocortin-4 receptor (MC4R) rs17782313 alleles with obesity and eating behavior scores in Chilean children.

Methods: A case-control study was conducted with 139 normal-weight and 238 obese children (ages 6–12 y). MC4R rs17782313 genotypes were determined by quantitative-polymerase chain reaction allelic-discrimination assays. Eating behavior scores were evaluated in a subset of participants using the Chilean version of the Child Eating Behavior Questionnaire (CEBQ). Additionally, five normal-weight C-allele carriers of rs17782313 were matched by sex, age, and body mass index (BMI) to five TT homozygous children to carry out the Eating in the Absence of Hunger (EAH) test. **Results:** The frequency of the C-allele of MC4R rs17782313 was higher in the obese group than in the control group, without achieving statistical significance (odds ratio, 1.4; 95% confidence interval, 0.8–2.4; $P = 0.16$). CEBQ scores of “enjoyment of food” were higher ($P = 0.04$) and “satiety responsiveness” were lower ($P = 0.02$) in children with CC genotype than in those with TT genotype matched by sex, age, and BMI. In the EAH test, all five non-obese carriers of the C-allele (three CC and two CT) showed increased sweet snack consumption compared with five matched (by sex-age-BMI) non-carriers after a preload meal, without achieving statistical significance ($P = 0.06$).

Conclusion: MC4R polymorphism rs17782313 may contribute to childhood obesity, affecting enjoyment of food, satiety responsiveness, and possibly eating in the absence of hunger.

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Introduction

Obesity is a multifactorial disease caused by the interaction of genetic and environmental factors operating at molecular, physiological, and societal levels. Worldwide obesity prevalence has more than doubled during the past decades and, according to the International Obesity Task Force, at least 155 million children

are overweight or obese [1,2]. Chile, like other Latin American countries, has suffered a nutritional transition leading to changes in diet and lifestyle. The prevalence of childhood obesity has tripled over the past 15 y, achieving 8.4% obesity among children between the ages of 2 and 5 y and 21.5% among first-grade children [3,4].

Genetics are not only related to phenotypic variation of body mass index (BMI) in adults and children, but they also have an influence in human feeding behavior [5]. Higher correlation coefficients were estimated in monozygotic twins compared with dizygotic twins in eating behavior subscales such as “enjoyment of food” and “satiety responsiveness” [6], indicating the importance of genetics in such behavioral traits. Additionally, a number of rare mutations causing monogenic obesity and common

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polymorphisms related to BMI in the general population have been discovered in genes that play essential roles, mainly in food intake and energy homeostasis, such as the melanocortin-4 receptor gene (*MC4R*) [7].

MC4R is a G protein-coupled receptor encoded by a single exon on chromosome, 18q22 and is critically involved in regulating energy balance [8]. *MC4R* activation by endogenous ligands such as α -melanocyte stimulating hormone in the hypothalamus results in decreased food intake and increased energy expenditure in mice [9–13]. *MC4R*-deficient mice developed an early-onset obesity syndrome characterized by hyperphagia, hyperinsulinemia, hyperleptinemia, hyperglycemia, and increased adiposity [14–16]. On the other hand, rare mutations in the coding sequence of human *MC4R* cause severe childhood obesity [13,15–21]. Recently, a common genetic variant near *MC4R* (rs17782313) located 188 kb downstream of the gene, was associated with increased fat mass, weight, and obesity risks in large epidemiologic studies [22–24]. Additionally, other studies have shown associations between *MC4R* genotypes with a higher intake of total energy and dietary fat, greater long-term weight change in adult women, and eating behavior patterns in adults and children [22,25,26]. The aim of this study was to assess the association between a variant near the *MC4R* gene (rs17782313) with obesity and eating behavior in Chilean children, including eating behavior scores through questionnaires and direct observation of eating in the absence of hunger.

Methods

Participants and study design: Case–control comparisons

A case–control study was carried out with 139 normal-weight (BMI <85th percentile according to the National Center for Health Statistics/Centers for Disease Control and Prevention [NCHS/CDC] 2000 curve) and 238 obese children (>95th percentile) of both sexes (51.3% girls), with an age range of 6 to 12 y (Table 1). Participants were recruited from different sources: outpatient unit of the pediatrics department at the Institute of Nutrition and Food Technology, University of Chile, outpatient unit of the School of Medicine of the Pontificia Universidad Católica de Chile, and from public schools. All participants received nutritional advice by a nutritional expert and educational brochures and documents promoting healthy eating behaviors and lifestyles. The protocol was approved by the Research Ethics Board of the Pontificia Universidad Católica de

Table 1
Anthropometric measurements and CEBQ scores in normal-weight and obese Chilean children

	Control group (n = 139)	Obese group (n = 238)	P-value
Age (y)	10.4 ± 1.8	9.8 ± 2.2	0.01
Sex (% girls)	48.7	47.2	0.30
BMI (kg/m ²)	16.9 ± 1.9	26.2 ± 4.3	<0.001
BMI z score	0.7 ± 0.6	2.1 ± 0.3	<0.001
Waist circumference (cm)	63.0 (59.7–67.4)	86.0 (78.0–93.0)	<0.001
CEBQ scores	Control group (n = 139)	Obese group (n = 176)*	
Food responsiveness	1.8 (1.2–2.8)	4 (2.6–4.8)	<0.001
Emotional overeating	1.8 (1.0–2.3)	3.0 (2.0–3.8)	<0.001
Enjoyment of food	3.8 (2.8–4.5)	4.5 (3.8–5.0)	<0.001
Desire for drinks	4.0 (2.3–5.0)	4.0 (2.3–5.0)	0.87
Satiety responsiveness	3.2 (2.2–3.8)	2.0 (1.4–2.6)	<0.001
Slowness in eating	2.8 (1.8–4.0)	1.5 (1.0–2.3)	<0.001
Emotional undereating	2.5 (1.5–3.5)	2.3 (1.8–3.0)	0.25
Food fussiness	3.1 (2.0–4.0)	2.8 (2.0–3.6)	0.09

BMI, body mass index; CEBQ, Child Eating Behavior Questionnaire. Values are median and 25th to 75th percentiles for CEBQ scores. P values were calculated by Mann Whitney test

* Obese children with CEBQ scores (n = 176) were not significantly different from those without CEBQ scores (n = 62) in age, sex, or anthropometric variables.

Chile. All written informed consents were obtained from parents or guardians of the children.

Anthropometric measurements

Trained personnel measured each participant for weight, height, and waist circumference (WC) using standard techniques. Weight was measured using a calibrated electronic scale while the children were wearing lightweight clothing. Height was determined without shoes using a calibrated wall stadiometer. WC was measured with a tape at the area between the lowest rib and the upper part of the iliac crest, at the end of the expiration phase, without pressure applied. BMI was calculated as weight (kg) divided by squared height in meters (m²). Weight, height, and BMI values were transformed into z score. Normal-weight children were considered to have a BMI <85th percentile according to the NCHS/CDC 2000 curve for sex and age, whereas obese children were defined as those above the 95th percentile.

Genetic analysis of *MC4R* gene polymorphism rs17782313

Genomic DNA was extracted from whole blood (QIAamp DNA Blood kit) or saliva (DNA Oragene OG-250 kit) samples. The *MC4R* rs17782313 (T>C) was genotyped with the TaqMan Allelic Discrimination Assay (ID c_32667060_10 Applied Biosystems). The polymerase chain reaction amplification was performed in a Stratagene Mx3000P (Agilent Technologies) using the following conditions: 95°C for 10 min and 40 cycles of denaturation at 92°C for 15 sec and annealing/extension at 60°C for 1 min.

Child Eating Behaviour Questionnaire

Child eating behavior was evaluated in the whole group of normal-weight children (n = 139) and in a subsample of obese children (n = 176) using the Child Eating Behavior Questionnaire (CEBQ), which was previously translated, adapted, and validated for Chilean families [27,28]. The CEBQ is composed of 35 items that measure eight subscales of eating behavior, grouped in “food approach” subscales that indicate positive inclinations for eating (enjoyment of food, emotional overeating, food responsiveness, and desire to drink) and “food-avoidant” subscales related to negative inclinations to food intake (emotional undereating, slowness in eating, satiety responsiveness, and food fussiness). Each item was assessed with a Likert-type scale with possible scores from 1 (*never*) to 5 (*always*). CEBQ scores were obtained through face-to-face personal interviews with the mothers of children. Table 2 shows the description of the subscales measured in the CEBQ.

Eating in the Absence of Hunger Test

Eating in the absence of hunger (EAH) was measured in our study in normal-weight children by recording the amount of snacks eaten after the consumption of a meal until achieving satiety [29]. In our study, we decided to restrict the EAH test to only normal-weight children because the nature of the test may possibly mislead to unhealthy behaviors (eating sweet snacks after a meal), which are not appropriate for obese children. Then, we recontacted families with normal-weight children having CC or CT genotypes of *MC4R* rs17782313, and finally five normal-weight children carriers of the C-allele of *MC4R* rs17782313 (3 CC and 2 CT genotypes) agreed to participate in a laboratory EAH test. For this study, we also selected five normal-weight children with the TT genotype matched by sex, age, and BMI to the five C-allele carriers. Children arrived at our laboratory with their mothers 3 h after the lunch (16:30). Once in the laboratory, children were offered a standard meal consisting of a sandwich with chicken and cheese, yogurt, and a fruit juice. We instructed children to consume the meal in 30 min and requested mothers to behave as they normally do during a meal at home. Children consumed the standard meal, and additional food was provided until the child felt satiated, as evaluated by visual analog scales before and after the meal. Ten minutes after finishing the meal, the EAH test started. Child–parent pairs were allocated in separate rooms, where children received a puzzle book and a preweighed pack containing five different, individually wrapped sweet snacks (148.6 g; 742 kcal): a chocolate cookie, one small-size milk chocolate bar, one snack-size sweet and chocolate cake, one chocolate bar with nuts, and one coconut chocolate bar. Children were allowed to play and eat sweet snacks as much as they wished. After 15 min, the session was completed, packs with remaining snacks were collected and children and their parents left the laboratory. All snacks were weighed before and after the session. The amount of food (grams and kcal) was calculated according to the nutritional labels of each snack.

Statistical analysis

The association between *MC4R* rs17782313 genotypes and obesity status was determined by logistic regression (odds ratios and 95% confidence intervals). The Hardy-Weinberg equilibrium was assessed in both cases and controls. As eating

Table 2
Subscales measured in the Child Eating Behaviour Questionnaire

Subscale	Items	Description
Enjoyment of food	4	Condition positively associated with the sensation of hunger, desire to eat, and food gratification
Food responsiveness	5	Reflects different aspects of excessive responsiveness to external food cues: smell, taste, or appearance.
Emotional overeating	4	Measures an increase in eating in response to a range of emotions, such as anger, loneliness, or anxiety.
Desire to drink	3	Reflects the desire of children to have drinks with them (in Chilean social context, usually sugar-sweetened drinks).
Satiety responsiveness	5	Represents the ability of a child to reduce food intake after eating to regulate their energy intake.
Food fussiness	6	Related with a rejection of substantial amounts of new or familiar foods, narrowing the range of the variety of consumed foods.
Emotional undereating	4	Measures the decrease in eating in response to a range of emotions.
Slowness in eating	4	Higher scores are associated with a reduction in eating rate.

behavior scores showed deviations from a normal distribution, summary statistics of CEBQ scores were shown as median and 25 to 75 percentiles. The comparison between groups was performed with Mann-Whitney tests (independent groups) or Wilcoxon matched-pairs signed rank test (matched comparisons in the EAH test). Summary statistics of continuous variables were represented by mean \pm SD and compared through Student's *t* tests. Categorical variables are expressed as percentages. Sample size was calculated to achieve a sufficient statistical power (0.8) to detect odds ratios (OR) >2 in the case-control study with a confidence interval (CI) of 95%. All statistical tests were performed with STATA 11.0 and GraphPad Prism 5.04.

Results

The baseline characteristics of the participants are summarized in Table 1. The proportion of girls was similar in both obese and non-obese groups. In agreement with a previous report using partially the same data [28], higher CEBQ scores of “food approach” subscales and lower CEBQ scores for “food-avoidant” were found in obese rather than in normal-weight participants (Table 1).

Genotype and allele frequencies for *MC4R* rs17782313 were found to be in agreement to Hardy-Weinberg equilibrium in both cases ($P = 0.94$) and controls ($P = 0.33$). The frequency of C-allele carriers in *MC4R* rs17782313 was higher in the obese group than in controls, without achieving significant differences (OR, 1.4; 95% CI, 0.8–2.4; $P = 0.16$) (Table 3).

As differences in CEBQ scores vary importantly with age, sex, and obesity status, we carried out a matched analysis derived from the case-control study to assess the association between CEBQ scores and *MC4R* rs17782313 variant. In this analysis, each of the six CC homozygous subjects for rs17782313 with complete available data of CEBQ scores (three obese and three normal-weight children) was matched to 10 TT homozygous of the same sex, age, and BMI. This matched comparison (in total, 6 children with CC genotype versus 60 children with TT genotype) revealed that children with the CC genotype showed significantly higher “Enjoyment Food” (EF) scores ($P = 0.04$) than children with the TT genotype and lower “Satiety Responsiveness” (SR) scores ($P = 0.02$). Specifically, percentiles 25, 50, and 75 in EF for children with the TT genotype were 3.0, 4.3, and 4.8, whereas the

Table 3
Genotype frequencies of *MC4R* rs17782313 polymorphism in obese and normal-weight Chilean children

<i>MC4R</i> rs17782313 genotypes	Control group (n = 139)	Obese group (n = 238)	OR (95% CI)	<i>P</i> -value
TT	110 (79.1)	173 (72.7)	Reference	-
CT	26 (18.7)	60 (25.2)	1.5 (0.9–2.6)	0.15
CC	3 (2.2)	5 (2.1)	1.1 (0.2–7.0)	0.94
C-allele carriers	29 (20.9)	65 (27.3)	1.4 (0.8–2.4)	0.16

CI, confidence interval; OR, odds ratio
Values are absolute number (%). 95% CI; exact method

same percentiles for the CC genotype were 3.8, 4.8 and 5. On the other hand, percentiles 25, 50, and 75 in SR for children with the TT genotype were 1.8, 2.4, and 3.0, whereas the same percentiles for the CC genotype were 1.0, 1.7, and 2.4.

In the EAH test, all five normal-weight carriers of the C-allele (three CC and two CT genotypes) showed increased sweet snack consumption compared with five matched (by sex, age, and BMI) non-carriers after a preload meal (two-tailed $P = 0.06$; one-tailed $P = 0.03$; Wilcoxon matched-pairs signed rank test) (Table 4 and Fig. 1).

Discussion

Childhood obesity has reached epidemic proportions, increasing metabolic health risk during childhood, adolescence, and adulthood [30]. The *MC4R* gene is a key element in hypothalamic control of energy homeostasis, mainly regulating feeding behavior [31–33]. Mutations of the *MC4R* gene are the most frequent cause of severe obesity in humans [13,15]. In this study, we evaluated the effects of the common rs17782313 near of the *MC4R* locus in childhood obesity and eating behavior scores.

Genome-wide association scans have identified common polymorphisms in the *FTO* gene and 188 kb downstream of the *MC4R* gene (the region that includes rs17782313), that are associated with BMI and increased risk for obesity [23,34–36]. In French adults, *MC4R* was found associated with obesity and fat mass in combination with *FTO* [36]. Therefore, the rs17782313 polymorphism located close to *MC4R* may be involved in the development of obesity by influencing the level, location, or timing of gene expression or through linkage disequilibrium with polymorphisms or mutations of the *MC4R* gene. In our study, we did not find a significant association between the rs17782313 polymorphism and childhood obesity, although we did find a similar difference in C-allele carrier frequency in normal-weight versus obese children compared with other studies [23,34,35]. The lack of significant association found in our study is likely the result of a modest gene effect and the reduced statistical power is likely due to the small sample size [37]. In this

Table 4
Snack intake in the EAH test in normal-weight Chilean children according to *MC4R*-rs17782313 genotypes

	<i>MC4R</i> rs17782313 (T>C)		<i>P</i> -value
	C-allele carriers (n = 5)	Non-carriers (n = 5)	
Sweet snacks (g)	81.1 \pm 32.9	31.1 \pm 30.6	0.06
Sweet snacks (kcal)	410.7 \pm 179.1	161.2 \pm 155.8	0.06

EAH, Eating in the absence of hunger

Consumption of sweet snacks is shown as mean \pm SD. *P*-values computed through Wilcoxon matched-pairs signed rank test

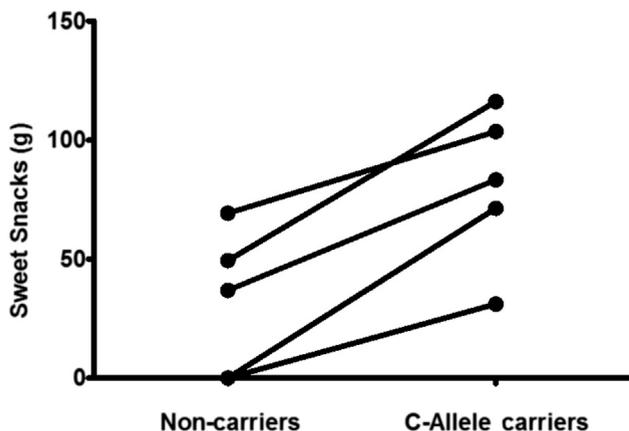


Fig. 1. Intake of sweet snacks in five rs17782313 C-allele-carriers and five matched non-carriers after the “Eating in the Absence of Hunger” Test.

context, no significant association between rs17782313 with susceptibility to obesity has been found in other populations, such as black children and adolescents [38].

All participants in our study belong to the Chilean population, which constitutes a melting pot of individuals from different parts of the world, including Europeans originating from different countries, mainly Spain, and Amerindians sharing genetic ancestry with the Asian population [39,40]. In Asian populations such as in China, the association between *MC4R* variants with obesity in childhood and adolescence yielded mixed results [41,42], whereas a genome-wide association meta-analysis of population-based cohorts, including Koreans, detected significant association between *MC4R*-rs17782313, an effect size similar to that observed in Europeans [43]. Significant associations with BMI and obesity risk also were observed in two studies in Chinese individuals from Shanghai [44] and Hong Kong [45]. In another study carried out with an Asian population, the C-allele rs17782313-SNP was associated with obesity and obesity-related phenotypes [46].

CEBQ scores previously have been associated with body weight and adiposity in children between the ages of 3 and 13 y [47–49]. Twin studies have shown that CEBQ scores have a substantial genetic influence, meaning that CEBQ is an adequate psychometric tool for evaluating the effect of genetic variants in children’s eating behavior [50]. In a matched analysis from our case-control study, we found that CEBQ scores of “satiety responsiveness” were lower and “enjoyment of food” was higher in obese participants with CC genotype compared with matched TT children. It has been suggested that obese people may have impaired sensitivity to internal satiety signals and response to external food cues [37,51,52]. In this context, *MC4R* represents a strong candidate gene for explaining genetic influences on childhood eating behavior, given that *MC4R* mutations are a known cause of severe obesity. In agreement with our study, the CC genotype of the rs17782313 polymorphism near *MC4R* also has been associated with increased prevalence of consuming large amounts of food in children from European populations [25]. A related effect also was reported in women with the CC genotype of rs17782313 having a higher intake of total energy, total fat, and protein in their diets compared with women with the TT genotype [22]. On the other hand, animal models support the hypothesis of a role for *MC4R* in energy intake and dietary high-fat/high-sucrose preference [53]. Moreover, the influence of human genetic variation in *MC4R* is concordant with the known

biological function of *MC4R* in regulating food intake [11,15,53]. On the other hand, there are few published studies that have evaluated genetic influences in the EAH behavior in children, which is a trait that previously has been associated with obesity, especially in boys [52]. In our study, normal-weight C-allele carriers of rs17782313 consumed more grams of sweet snacks after a standardized meal compared with age-gender matched non-carriers, although without achieving statistical significance.

We consider that our study has some strengths and limitations. The sample is culturally representative of Chilean children, with a relatively homogeneous socioeconomic background. Additionally, weight and height were directly measured and not parentally reported, and interviews were conducted face to face with the families with trained personnel. In the EAH study, children–mother pairs were tested in separate rooms, without the possible distorting influence of other children in the room that may affect the amount of sweet snacks eaten. We also selected individually wrapped sweet snacks that are easily handled in order to more accurately measure the grams consumed during the EAH test. On the other hand, limitations in our research also must be considered. In addition to the assumed small sample sizes of the case-control and EAH studies, there also is an inherent uncertainty related to the subjective way in which eating behavior is measured through questionnaires. Regarding the EAH test, it has been reported that the presence of parents may act as an inhibitory influence on the number of snacks eaten by children [54]. However, parents in our study were specifically instructed to not interfere with children behavior during the EAH test.

In summary, we evaluated the effect of the rs17782313 near the *MC4R* locus in childhood obesity and eating behavior scores in Chilean children. We did not achieve a statistically significant association between this polymorphism and childhood obesity, although we did find an increased frequency of the C-allele in obese versus non-obese children, as previously described. In a matched analysis from our case-control study, we found that CEBQ scores of “enjoyment of food” were higher and “satiety responsiveness” scores were lower in children with the CC genotype compared with children with the TT genotype, matched by sex, age, and BMI. We also found that normal-weight C-allele carriers of rs17782313 consumed more grams of sweet snacks after a standardized meal compared with age, sex, and BMI matched non-carriers during the EAH test, without achieving statistical significance.

Conclusion

Our results provide evidence supporting the idea that the *MC4R* polymorphism rs17782313 appears to contribute to childhood obesity by affecting satiety responsiveness, enjoyment of food, and possibly eating in the absence of hunger.

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