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ORIGINAL ARTICLE

Association of the melanocortin 4 receptor gene rs17782313 polymorphism with rewarding value of food and eating behavior in Chilean children

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Abstract Studies conducted in monozygotic and dizygotic twins have established a strong genetic component in eating behavior. Rare mutations and common variants of the melanocortin 4 receptor (MC4R) gene have been linked to obesity and eating behavior scores. However, few studies have assessed common variants in MC4R gene with the rewarding value of food in children. The objective of the study was to evaluate the association between the MC4R rs17782313 polymorphism with homeostatic and non-homeostatic eating behavior patterns in Chileans children. This is a cross-sectional study in 258 Chilean children (44 % female, 8–14 years old) showing a wide variation in BMI. Anthropometric measurements (weight, height, *Z*-score of BMI and waist circumference) were performed by standard procedures. Eating behavior was assessed using the Eating in Absence of Hunger Questionnaire (EAHQ), the Child Eating Behavior Questionnaire (CEBQ), the Three-Factor Eating Questionnaire (TFEQ), and the Food Reinforcement Value Questionnaire (FRVQ). Genotype of the rs17782313 nearby MC4R was determined by a Taqman assay. Association of the rs17782313 C allele with eating behavior was assessed using non-parametric tests. We found that children carrying the CC genotype have higher scores of food responsiveness (*p* value = 0.02). In obese girls, carriers of the C allele showed lower scores of satiety responsiveness (*p* value = 0.02) and higher scores of uncontrolled eating (*p* value = 0.01). Obese boys carrying the C allele showed lower rewarding value of food in relation to non-carriers. The rs17782313 C allele is associated with eating behavior traits that may predispose obese children to increased energy intake and obesity.

Keywords Melanocortin 4 receptor gene · Children · Polymorphism · Eating behavior

Abbreviations

MC4R	Melanocortin 4 receptor
GWAS	Genome-wide association study
BMI	Body mass index
EAH	Eating in absence of hunger
RVF	Reinforcing value of food
VTA	Ventral tegmental area
MCR	Melanocortin receptors

Introduction

Obesity is a chronic disease characterized by fat accumulation in adipose tissue. In Chile, a middle-income country, childhood obesity has tripled over the last decades and continues to rise. Presently, 25 % of 6-year-old children are obese and 30 % are overweight [15]. As obesity is a complex disease, research is focused in unraveling the genetic and environmental factors causing the disease. Among the multi-level factors that determine the development of this disorder, increased energy intake is likely to play a dominant role in obesity. It is known that eating

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behavior and appetite traits are complex variables that are modulated by many biological and environmental factors [20].

The melanocortin 4 receptor (MC4R, gene ID 4160; 18q22) is a 322-amino acid, seven-transmembrane, G-protein-linked receptor involved in central regulation of energy homeostasis [1]. MC4R is widely expressed in the hypothalamus, brainstem, and other brain regions, where it mediates the anorectic response to the adipocyte-derived hormone leptin and the satiety response to gut hormones such as peptide YY [4]. MC4R is also expressed in dopamine-rich regions of the striatum, and some studies in rodents suggest that melanocortin signaling modulated food reward [17, 5]. Given that MC4R is highly express in the hypothalamus and dopamine-rich regions and dopamine mediates the reinforcing value of food (RVF) [7], it is likely that this receptor is involved in homeostatic and non-homeostatic mechanism of intake. Common variation near MC4R gene was reported as the second association signal for multifactorial obesity in genome-wide association studies (GWAS) [22]. In this sense, the polymorphism rs17782313, mapped 188 kb downstream from MC4R, has been strongly associated with obesity and higher body mass index (BMI) in adults and children [2, 22]. In a previous study in Chilean children, we showed a significant association between MC4R rs17782313 polymorphism with eating behavior traits such as enjoyment of food, satiety responsiveness, and possibly eating in the absence of hunger [24, 32]. Herein, we extend these findings using an independent sample of Chilean children to study both non-homeostatic eating behavior traits related to rewarding properties of food. Then, the aim of this study is to assess the association between the genetic variant rs17782313 and homeostatic and non-homeostatic eating behavior patterns in Chilean children.

Material and methods

This is a cross-sectional study in which we recruited a convenience sample of 258 children (44.1 % Female), 115 obese, 42 overweight, and 101 normal-weight children (8–14 years old, both genders) according to the WHO international criteria [36]. The inclusion criteria considered being a child (both gender) aged 8–14 years, normal weight, overweight or obese, without taking medications that have impact on body weight, and without treatment for weight loss. We excluded participants with missing phenotypic data and genetics syndromes related to body weight ($n = 5$). Participants were recruited from different schools located in the city of Concepción (Chile). Children were invited to participate by advertisements placed in their schools, community centers and in the San Sebastian University website (Concepción, Chile; www.uss.cl). All children attended laboratory testing at San Sebastian University with their parents and signed assent (children) and informed consent forms (parents). All procedures were approved by the ethics committee of our university. This study was conducted according to the guidelines laid down in the Declaration of Helsinki.

Anthropometry

Height, weight, and waist circumference were measured using a weight scale (Seca 700), with a stadiometer included by standard procedure [12]. BMI was calculated as weight in kilograms divided by the square of height in meters. A child exceeding the 95th percentile of BMI's [www.who.int/childgrowth] were considered obese and between the 85-94th BMI percentiles, overweight. The percentiles and *Z*-scores of height, weight, and BMI were calculated using Epinfo software (http://www.cdc.gov/EpiInfo) [38]. Waist circumference was measured using a non-elastic tape midway between the lowest border of the rib cage and the upper border of the iliac crest, at the end of normal expiration. Hip circumference was measured at the widest part of the hip at the level of the greater trochanter. All measurements were in centimeters (cm) to the nearest. Body composition was assessed after an overnight fast at 9 a.m. in the morning, by foot to foot bioelectrical impedance according to the manufacturer's guidelines with a Tanita TBF-300MA (Tanita Corporation, Tokyo, Japan).

Eating behavior

A trained nutritionist carried out direct interviews with the mothers and their children. Eating behavior was measured using four validated psychometric questionnaires: the Eating in Absence of Hunger Questionnaire (EAHQ), the Child Eating Behavior Questionnaire (CEBQ), the Three-Factor Eating Questionnaire (TFEQ-19), and the Food Reinforcement Value Questionnaire (FRVQ). (a) *Eating in the Absence of Hunger Questionnaire*: This 14-item questionnaire was developed by Tanofsky-Kraff et al. [30] and has been previously validated in Chilean children by Morales et al. [24]. Items were answered by mothers by choosing 1 of the 5 alternatives in a Likert-type scale, with answers ranging from "never (score of 1) through "always" (score of 5)." (b) *Child Eating Behavior Questionnaire*: CEBQ is a 35-item questionnaire [35] previously validated in Chilean children by Santos et al. [28]. This questionnaire evaluates eight subscales of eating behavior: food responsiveness (FR, five items), enjoyment of food (EF, four items), emotional overeating (EOE, four items), desire to drink (DD, three items), slowness in eating (SE, four items), satiety responsiveness (SR, five items), food fussiness (FF, six items), and emotional under-eating (EUE, four items). Each item was answered by mothers in a Likert-type scale with possible scores from 1 to 5, where 1 is complete discordance and 5 the highest accordance of the specific eating behavior statements.

Table 1 Anthropometric measurements in children from Concepción (Chile)

	All children (n = 258) Mean ± SD	Girls (n = 114) Mean ± SD	Boys (n = 144) Mean ± SD	p value
Age (years)	11.4 ± 1.6	11. 3 ± 1.6	11.5 ± 1.6	0.40
Weight (kg)	52.2 ± 15.3	51.2 ± 14.1	52.9 ± 16.2	0.62
Z-score for height	0.3 ± 0.9	0.2 ± 0.9	0.36 ± 0.8	0.28
Z-score for weight	1.1 ± 1.0	1.0 ± 1.02	1.19 ± 1.06	0.34
Z-score for BMI	1.2 ± 1.0	1.1 ± 1.0	1.2 ± 1.01	0.46
BMI (kg/m^2)	23.1 ± 4.8	23.1 ± 4.8	23.0 ± 4.9	0.36
Waist to height ratio	0.53 ± 0.07	0.53 ± 0.08	0.5 ± 0.07	0.72
Waist circumference (cm)	79.0 ± 12.1	78.4 ± 11.5	79.4 ± 12.6	0.68
Body fat mass (%)	29.6 ± 10.3	32.8 ± 9.2	27.8 ± 10.5	0.001

*Significant differences between girls and boys were analyzed with the non-parametric Mann-Whitney test

(c) *Three-Factor Eating Questionnaire*: 19 items were answered by mothers on a Likert-type scale with possible scores from 1 (definitely true) to 4 (definitely false) that measures three subscales of eating behavior**:** cognitive restrained (CR, six items), uncontrolled eating (UE, ten items) and emotional eating (EA, three items) [6].

Standardized scores for each subscale were calculated as the average of summing individual raw scores divided by the number of items in each subscale. All subscales showed an internal consistency higher than 0.6 as measured with the Cronbach alpha statistic (range, 0.60–0.88), indicating moderate to strong reliability of the instruments in the sample obtained [3].

(d) *Food Reinforcement Value Questionnaire*: The FRVQ was developed by Goldfield et al. [10] and further validated by Hill et al. [13]. It is a psychometric tool that evaluates the reinforcement value of food based on the report of children in relation to their feeding behavior. This questionnaire includes 12 items related to the effort that children are willing to make to obtain a specific reinforcer. Briefly, children rank their preferences (mini cookies/sticker) and then they indicate their preferences to click in a hand-held joystick to receive either their most preferred food reward (mini cookies) or a non-food reward (small sticker). The schedule of reinforcement began at an equal fixed ratio (FR) of 20 presses to receive either the food or non-food reinforcer [e.g., "Would you prefer to press the button 20 times for a biscuit or 20 times for a sticker ?"]. For each subsequent question, the number of clicker presses required for the food increased on a fixed ratio progressive schedule of reinforcement of 20 presses, resulting in a maximum FR of 20 or 240 presses required for non-food or food options respectively, at the last question. At the end of the test, a questionnaire item number was selected at random by picking a number out of a box, and the child was required to carry out the number of clicker presses selected for that question. Children were told that they would receive all the rewards (food or non-food items) at the end of the testing session. The reinforcing value of food was defined as the total number of clicker presses for food choices.

Table 2 Genotype-allelic frequencies and Hardy-Weinberg equilibrium in rs17782313 (MC4R) in children from Concepción (Chile)

	Genotypic frequencies (%)			Allelic frequencies (%)		p value HWE
	TT	TC	CC	T	C	
rs17782313	198	58	2	454	62	0.55
(n = 258)	(76.4)	(22.4)	(0.78)	(87.9)	(12.0)	
Normal weight	87	14	0	188	14	1.0
(n = 101)	(86.6)	(13.8)		(93)	(6.9)	
Overweight/obese	111	44	2	266	48	0.53
(n = 157)	(70.7)	(28.0)	(1)	(84.7)	(15.2)	
Girls	87	26	1	200	28	1.0
(n = 114)	(76.3)	(22.8)	(0.88)	(0.87)	(0.12)	
Boys	111	32	1	254	34	0.69
(n = 144)	(77.0)	(22.2)	(0.69)	(0.88)	(0.11)	

HWE Hardy-Weinberg equilibrium

Table 3 Eating behavior scores by MC4R genotype (c-allele carriers vs non-carriers of rs17782313) in girls from Concepción (Chile) by nutritional status

	Normal weight			Overweight			Obese		
	Non-carrier (n = 39) mean ± SD	C carrier (n = 8) mean ± SD	p	Non-carrier (n = 11) mean ± SD	C carrier (n = 7) mean ± SD	p	Non-carrier (n = 37) mean ± SD	C carrier (n = 12) mean ± SD	p
EAHQ									
Negative affect	9.4 ± 3.9	8.5 ± 2.0	0.84	7.6 ± 2.1	7.1 ± 1.9	0.64	10.0 ± 3.8	10.2 ± 6.7	0.2
External eating	9.23 ± 2.9	9.3 ± 3.4	0.98	8.7 ± 3.9	11.0 ± 3.1	0.12	10.2 ± 3.5	12.1 ± 4.6	0.1
Fatigue/boredom	7.0 ± 4.1	5.1 ± 0.9	0.98	6.0 ± 2.0	7.5 ± 3.46	0.3	7.2 ± 3.3	8.3 ± 4.2	0.45
CEBQ									
Food responsiveness	2.4 ± 1.0	2.3 ± 1.1	0.7	2.7 ± 1.3	3.1 ± 1.1	0.49	3.4 ± 1.1	4.0 ± 0.86	0.08
Emotional overeating	2.3 ± 0.9	1.7 ± 0.74	0.07	2.4 ± 1.2	2.0 ± 0.7	0.68	2.8 ± 1.0	3.2 ± 1.1	0.19
Enjoyment to food	3.3 ± 0.91	2.8 ± 0.8	0.15	3.4 ± 1.0	3.8 ± 0.83	0.49	3.9 ± 0.91	4.2 ± 0.9	0.3
Desire to drink	3.3 ± 1.2	3.1 ± 1.5	0.65	3.5 ± 1.1	4.0 ± 1.1	0.36	3.8 ± 1.0	4.2 ± 0.9	0.3
Satiety responsiveness	3.1 ± 0.9	3.1 ± 0.9	0.76	2.7 ± 0.9	2.8 ± 0.63	0.81	2.7 ± 0.7	2.1 ± 0.8	0.02
Slowness in eating	3.0 ± 1.1	3.6 ± 0.8	0.32	2.2 ± 0.7	2.6 ± 0.6	0.31	2.3 ± 0.97	2.1 ± 1.0	0.46
Emotional under-eating	2.6 ± 0.87	2.5 ± 1.1	0.79	2.39 ± 0.7	2.8 ± 0.81	0.29	2.8 ± 0.9	2.6 ± 0.8	0.93
Food fussiness	3.3 ± 0.91	3.25 ± 0.7	0.96	3.0 ± 0.9	3.1 ± 0.88	1.0	2.8 ± 0.9	2.8 ± 1.1	0.88
TFEQ									
Cognitive restrained	2.1 ± 0.8	1.83 ± 0.6	0.4	2.4 ± 0.8	2.3 ± 0.45	0.6	2.3 ± 0.6	1.8 ± 0.7	0.2
Uncontrolled eating	2.1 ± 0.6	1.9 ± 0.6	0.3	2.3 ± 0.8	2.4 ± 0.6	0.3	2.6 ± 0.6	3.1 ± 0.7	0.01
Emotional eating	1.5 ± 0.5	1.2 ± 0.3	0.06	1.7 ± 0.7	1.7 ± 0.6	0.7	2.0 ± 0.8	2.3 ± 1.0	0.3
FRVQ									
Food choice (%)	35.2 ± 34.2	38.3 ± 30.8	0.07	30.3 ± 29.4	22.6 ± 19.7	0.71	35.5 ± 32.7	40.7 ± 35.7	0.85

EAHQ Eating in Absence of Hunger Questionnaire, *CEBQ* Child Eating Behavior Questionnaire, *TFEQ* Three-Factor Eating Questionnaire, *FRVQ* Food Reinforcement Value Questionnaire;

*Significant differences by carrier status were analyzed with the non-parametric Mann-Whitney test

Collection of biologic samples

Blood samples were drawn by a pediatric nurse by a vacuum system using standard protocols in tubes without additives for biochemical analysis and with EDTA-K3 as an anticoagulant. Collected sample with EDTA was used for the extraction of nucleic acids (DNA) from leukocytes.

Genetic analysis of MC4R

DNA from blood samples was extracted with the QIAGEN QIAamp DNA blood mini kit #51104 according to the manufacturer's instructions. The genotype of the common variant of MC4R rs17782313 (T > C) [C, risk allele] was determined with a predesigned Taqman assay ID c_32667060_10 (Applied Biosystems) that allows for genotype discrimination using the real time thermocycler ABI-Stepone.

Statistical methods

Summary statistics for quantitative variables are shown as means ± standard deviations. Genotype and allele frequencies were estimated and Hardy-Weinberg equilibrium was evaluated based on a goodness-of-fit χ^2 test. The non-parametric statistical Mann-Whitney test was used to assess associations between study variables across study groups. The sample size was calculated considering a significant level of 5 % and a desire power of 80 %. We assume a difference of 0.7 units between the case and control groups in the score of "external stimuli" of the EAHQ and standard deviations of 1.8 units.

Results

Anthropometric measures

Summary statistics for anthropometric measurements are listed in Table 1. We recruited 258 children in the whole sample (40 % normal weight, 16 % overweight, 44 % obese). As

Table 4 Eating behavior scores by MC4R genotype (c-allele carriers vs non-carriers of rs17782313) in boys from Concepción (Chile) by nutritional status

	Normal weight			Overweight			Obese		
	Non-carrier (n = 48) mean ± SD	C carrier (n = 6) mean ± SD	p	Non-carrier (n = 18) mean ± SD	C carrier (n = 6) mean ± SD	p	Non-carrier (n = 45) mean ± SD	C carrier (n = 21) mean ± SD	p
EAHQ									
Negative affect	8.7 ± 3.5	6.8 ± 0.9	0.27	10.0 ± 4.2	7.3 ± 1.7	0.18	9.5 ± 4.9	10.3 ± 4.0	0.18
External eating	9.5 ± 2.8	8.3 ± 2.2	0.29	10.3 ± 2.9	9.6 ± 2.7	0.63	11.0 ± 3.1	10.5 ± 1.9	0.51
Fatigue/boredom	6.6 ± 3.4	5.1 ± 1.6	0.47	8.0 ± 4.2	5.5 ± 1.0	0.45	8.1 ± 4.3	7.1 ± 2.8	0.6
CEBQ									
Food responsiveness	2.3 ± 0.9	3.2 ± 1.3	0.09	3.3 ± 1.2	2.6 ± 1.5	0.26	3.2 ± 1.2	3.3 ± 1.4	0.58
Emotional overeating	2.0 ± 0.8	1.8 ± 0.8	0.6	2.5 ± 0.9	1.9 ± 0.5	0.11	2.7 ± 1.1	2.7 ± 1.1	0.91
Enjoyment to food	3.2 ± 0.8	3.5 ± 1.0	0.5	3.9 ± 0.9	3.3 ± 1.5	0.4	4.1 ± 0.8	3.8 ± 1.1	0.36
Desire to drink	3.5 ± 1.2	3.9 ± 0.8	0.4	3.8 ± 1.1	3.5 ± 1.4	0.68	4.0 ± 1.0	3.6 ± 1.4	0.53
Satiety responsiveness	2.9 ± 1.0	3.0 ± 1.1	0.8	2.4 ± 0.9	2.6 ± 1.2	0.76	2.2 ± 0.6	2.1 ± 0.78	0.59
Slowness in eating	2.5 ± 1.2	2.6 ± 1.3	0.7	1.9 ± 0.9	2.5 ± 1.5	0.2	2.0 ± 0.8	1.8 ± 0.95	0.4
Emotional under-eating	2.4 ± 0.8	2.5 ± 0.4	0.5	2.5 ± 0.7	2.3 ± 0.4	0.63	2.5 ± 0.9	2.4 ± 1.0	0.93
Food fussiness	3.3 ± 1.0	2.8 ± 1.2	0.2	3.3 ± 0.89	2.6 ± 1.1	0.17	3.2 ± 0.7	3.1 ± 1.1	1.0
TFEQ									
Cognitive restrained	2.0 ± 0.6	1.7 ± 0.7	0.3	2.1 ± 0.9	2.4 ± 1.1	0.3	1.8 ± 0.7	1.7 ± 0.8	0.67
Uncontrolled eating	2.1 ± 0.6	2.1 ± 0.5	0.85	2.7 ± 0.7	2.0 ± 0.8	0.12	2.6 ± 0.7	2.6 ± 0.8	0.87
Emotional eating	1.4 ± 0.5	1.4 ± 0.5	0.96	1.9 ± 0.8	1.5 ± 0.8	0.3	2.0 ± 0.8	1.7 ± 0.8	0.21
FRVQ									
Food choice (%)	54.6 ± 36.7	50.0 ± 47.4	0.75	58.8 ± 38.6	58.3 ± 32.0	0.97	50.5 ± 34.1	27.3 ± 31.9	0.007

EAHQ Eating in Absence of Hunger Questionnaire, *CEBQ* Child Eating Behavior Questionnaire, *FRVQ* Food Reinforcement Value Questionnaire, *TFEQ* Three-Factor Eating Questionnaire

**Significant differences by carrier status were analyzed with the non-parametric Mann-Whitney test

expected, girls had higher levels of total body fat in relation to boys (32.8 ± 9.2, 27.8 ± 10.5 %). We found significant differences in all anthropometric variables by weight status ($p < 0.001$) (data not shown). In this sample, the gender-specific maturational stage distribution was Tanner 1 (18.4 and 33.3 %), Tanner 2 (25.4 and 23.6 %), Tanner 3 (12.2 and 23.6 %), Tanner 4 (2.6 and 12.5 %), and Tanner 5 (41.2 and 6.9 %) in girls and boys, respectively. This distribution was significantly different in girls by genotype ($p \leq 0.05$) but not in boys.

MC4R rs17782313 and obesity-related variables

Genotype and allele frequencies for the genetic variant of the rs17782313 of MC4R estimated in Chilean children are shown in Table 2. The genotype distribution was TT = 76.4 %, TC = 22.4, and CC = 0.78 %. The genotype distribution of study sample did not deviate significantly from the Hardy-Weinberg equilibrium (p value = 0.55). Allele frequencies were estimated as 88 % for the T allele and 12 % for the C allele. We found significant differences in genotype frequencies by nutritional status (p value 0.01). When data was analyzed by carrier status, we found significant differences comparing C-allele carriers versus non-carriers, with higher values in C-allele carriers for the BMI Z-score (1.5 ± 0.9, 1.1 ± 1.0 p value = 0.005) and total body fat (32.2 ± 10.3, 28.8 ± 10.2 % p value = 0.01).

MC4R rs17782313 and eating behavior variables

Because boys and girls differ for eating behavior patterns, data were stratified by gender and nutritional status. Tables 3 and 4 show summary statistics for eating behavior scores calculated from the EAHQ, CEBQ, TFEQ, and RVFQ by carrier status of the alternative allele. In obese girls, we found significant lower scores of the SR subscale compared to non-carriers and higher scores of the UE subscale in carriers of the C allele in rs17782313 compared to non-carriers (Table 3). In obese boys, carriers of the C allele showed significantly lower reinforcing value of food in relation to the non-carriers (Table 4).

Discussion

The discovery of about new loci associated with BMI and obesity in humans, originating from GWAS, has confirmed the association between BMI with variants near MC4R gene [37]. Previous studies have shown in subjects that each copy of the rs17782313 C allele was associated with a difference in BMI of 0.22 kg/m^2. Furthermore, the rs17782313 exerts a strong influence on fat mass and weight, with an allelic overtransmission (56 %) of the C allele in case-parent trios, indicating higher risk for obesity in adulthood and with an 8 % per allele increase in the odds of being overweight and 12 % of being obese [22, 19]. In our study, the minor allele frequency in the entire sample was lower (12 %) than other populations [32, 14].

Studies conducted in monozygotic (MZ) and dizygotic (DZ) twins have established there is a strong genetic influence in obesity-related anthropometric phenotypes, eating attitudes, quantity and rate of consumption, macronutrient preference, frequency of eating, and many eating behavior traits such as satiety response, responsiveness to food cues, and enjoyment of food [9, 18, 20, 21, 26, 31, 34]. Herein, we have found that obese girls who carry the C allele of rs17782313 showed lower satiety responsiveness (*homeostatic pathway*) and higher uncontrolled eating than obese girls who do not carry the C allele. This is the third study that evaluate the association of MC4R genetic variants with eating behavior in Chilean children, and our outcomes are consistent with the results of Valladares et al. (2009) and Ho-Urriola et al. (2014) in Chilean obese children [32, 14]. In addition, in the study by Ho-Urriola et al. (2014), children with the CC genotype showed increase sweet snack consumption after a controlled preload condition compared with non-carriers. Moreover, Stutzmann et al. [29] found in Finnish and in French adolescents that the rs17782313 C allele was associated with higher percentage of snaking behavior and Lv et al. (2015) showed higher preference for salty flavor [23]. In adults, Qi et al. (2008) using the FFQ (Food Frequency Questionnaire) reported that homozygous CC had higher total energy intake in comparison to non-carriers [25]. Consistent with our results, the C allele has been associated with higher uncontrolled eating, with higher energy intake, and with overeating behavior in adults [33, 16, 39].

In obese boys, we have found that carriers of the C allele have lower rewarding value of food (% of food choice) compared to non-carriers. The rewarding value of food is a quality that refers to how much an individual is willing to work to obtain palatable food [11]. Individual differences in the reinforcing efficacy of food may relate to differences in eating and energy intake [7]. For example, Epstein et al. (2004) have shown that subjects who found food highly reinforcing consumed more energy in an ad libitum situation than those who are low in food reinforcement [8]. The RVF and the pleasure one which originates from eating and other appetitive behaviors (smoking, drinking, drug abuse) are associated to activity of the dopaminergic system [7]. We do not have a clear explanation for our results in obese boys that showed that C carriers have reduced RVF, but these could be connected with the fact that this variant may affect gene function participating in MC4R expression and translation regulation, and with recent evidence, that indicate that α-melanocyte-stimulating hormone (αMSH) and agouti-related protein (AgRP) can also act on dopamine pathways to affect food intake and food reward. Studies have shown that injection on MCR agonist and antagonist directly into de mesocorticolimbic system, such as the amygdala and VTA, directly alters feeding [27].

There are some limitations in our research: derived from its limited sample size, the cross-sectional nature of this study, and the inherent uncertainty related to the measurement of subjective eating behavior in humans through questionnaires. It is also worth noting that our study shows nominal p values not corrected by multiple comparisons. The methodological limitations of our study are balanced by several strengths: (i) Children's weight and height were measured directly and not parentally reported. (ii) All eating behavior measures were obtained by personal interviews conducted face-to-face by trained personnel. (iii) To our knowledge, the current study is the first to examine the relationship between MC4R and eating behavior measures related to the rewarding value of food in children. It is important to note that our study represents an independent replication of previous Chilean studies [32, 14], showing genotype-behavior associations that roughly persist in different samples of children from different cities in Chile (Santiago and Concepción). It is also interesting to note that all significant associations are restricted to the obese group, possibly indicating that eating behavior variables may operate mainly in at-risk children.

In summary, our results are concordant for a role of rs17782313 polymorphism nearby MC4R with some dimensions of eating behavior. In obese girls, carriers of the C allele of rs17782313 showed lower satiety responsiveness and higher uncontrolled eating scores compared to non-carriers. In obese boys, carriers of the C allele of rs17782313 showed a lower rewarding value of food compared to non-carriers.

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Compliance with ethical standards All children attended laboratory testing at San Sebastian University with their parents and signed assent (children) and informed consent forms (parents). All procedures were approved by the ethics committee of our university. This study was conducted according to the guidelines laid down in the Declaration of Helsinki.

Competing interests The authors declare that they have no competing interests.

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