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ORIGINAL ARTICLE

Association of the MC4R rs17782313 polymorphism with plasma ghrelin, leptin, IL6 and TNFα concentrations, food intake and eating behaviors in morbidly obese women

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Abstract

Purpose The rs17782313 variant of the MC4R gene plays an important role in the obesity phenotype. Studies that evaluate environmental factors and genetic variants associated with obesity may represent a great advance in understanding the development of this disease. This work seeks to assess the association of the polymorphism of *MC4R* rs17782313 on plasma parameters, including leptin, ghrelin, tumor necrosis factor (TNFα) and interleukin 6 (IL6), and on the eating behaviors of morbidly obese women.

Methods 70 adult women with BMI between 40 and 60 kg/m^2 were recruited. Laboratory and anthropometric data were recorded. Using a visual analog scale (VAS), the feelings of hunger and satiety were evaluated. The presence or absence of binge eating was evaluated through the Binge Eating Scale (BES) questionnaire. Habitual food intake was analyzed using 3-day dietary records. TaqMan® assays were conducted using real-time PCR to assess genotype polymorphism variants from peripheral blood DNA.

Results This study found that female patients with the MC4R rs17782313 polymorphism had high levels of ghrelin and reduced levels of IL6 in the postprandial period. We observed a higher prevalence of severe binge eating in more than 50% of women with at least one risk allele.

Conclusion Our hypothesis is that the MC4R rs17782313 polymorphism may influence the release of ghrelin, even without being associated with feelings of hunger and satiety. More than half of women with this polymorphism exhibited severe binge eating.

Level of evidence Level III: case–control analytic study.

Keywords rs17782313 · Morbid obesity · Eating behavior · Ghrelin · Leptin

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Introduction

The prevalence of obesity continues to rise worldwide and is one of the most challenging public health problems. Currently, more than 600 million people worldwide are obese [1]. Studies have considered the relationship between the environment and genetic predisposition for the onset of obesity [2, 3]. Accordingly, candidate genes for obesity may influence the control of appetite, energy expenditure, adipocyte differentiation, thermogenic regulation, fuel metabolism and the signaling of some receptors [4–6].

The *MC4R* gene is located at position 21.32 on the long arm (q) of chromosome 18 and consists of one exon with a total length of 1.9 kb [7]. This gene is expressed in adipose tissue, muscle tissue and various regions of the brain, mainly in the hypothalamic nucleus, where it plays a central role in controlling energy balance and food intake [8–10]. In 1997, the involvement of *MC4R* in body weight regulation was described when researchers found that changes in its gene promoted hyperphagia, hyperinsulinemia and obesity in rats [11]. The following year, researchers identified mutations in the *MC4R* gene in humans linked to extreme forms of obesity [12].

Single-nucleotide polymorphism (SNP) is the most prevalent class of genetic variations. The human genome contains more than 10 million SNPs. Approximately, 12 SNPs of the *MC4R* gene are positively associated with an increased body mass index (BMI) and obesity [7].

Among these SNPs, rs17782313 is one of the most studied SNPs of the *MC4R* gene. This SNP has two alleles, C and T, where the T allele is the ancestral allele and the C allele is the risk allele, with a prevalence of 24% in the population [13]. The rs17782313 SNP has been correlated with increased BMI, increased waist circumference [14], insulin resistance (IR) [15], diabetes mellitus type 2 (DM2) and lipid intake [16]. In addition, the presence of the risk allele (C) has been connected with uncontrolled food intake [17].

Therefore, the aim of the present study was to evaluate the association of the *MC4*R rs17782313 polymorphism with plasma concentrations of ghrelin, leptin, interleukin 6 (IL6) and tumor necrosis factor (TNFα) and with eating behaviors in morbidly obese women.

Materials and methods

Study population

Initially, 300 women were screened using GRACO, HUCFF files and medical records and interviews applied by email or phone to women who applied voluntarily through posters, a website and social networks (192 files and 108 interviews). For the present study, women aged between 20 and 48 years, with regular menstrual cycles, with BMI between 40 and 60 kg/m^2 and with a time of diagnosis of obesity equal to or greater than 3 years were considered.

They were not eligible if they had kidney failure, congestive heart failure or diagnosed dysgeusia; were being treated for cancer; were pregnant or lactating; were diagnosed with hypothyroidism or hyperthyroidism; were using corticosteroids or drugs used to lose weight or that could alter the feelings of hunger and satiety; or previously underwent bariatric surgery.

Volunteers who did not complete all stages or who had any complications previously listed between recruitment and data collection were excluded.

We selected 70 patients between 20 and 48 years of age who had a BMI value between 40 and 60 kg/m^2 and were obese for at least 5 years. This study was approved by the Research Ethics Committee of HUCFF under the protocol CAAE No. 845,537 and registered with ClinicalTrials.gov under the number NCT02598037.

Biochemical and anthropometric variables

The anthropometric data collected were body weight in kilograms (kg), height (cm), BMI, waist circumference (WC) and hip circumference (HC). The waist-to-hip ratio (WHR) was calculated by dividing the WC by the HC [18]. This measurement was performed in duplicate by a single evaluator.

Total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TGs) and glucose were measured by the enzymatic–colorimetric method [19–22]. Low-density lipoprotein (LDL) concentrations were calculated based on Friedewald's equation [23].

Blood samples for measuring plasma levels of acylated ghrelin, leptin, IL6 and TNFα were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and Pefabloc® (a specific protein inhibitor) before and 180 min after standard isocaloric meal intake and were analyzed using Luminex™ xMAP technology and Milliplex kits in the Luminex 200-Xponent/Analyst version 4.2 software.

Dietary records and meal intake

The volunteers were instructed to complete three dietary records on three nonconsecutive days [24], including two typical days (weekdays) and an atypical day (weekend or holiday). The dietary records for each individual volunteer were checked by the researchers. All dietary records were

analyzed using the nutritional assessment software AVANUTRI, version 4.0.

Each meal was prepared individually just before its intake to offer the nutritional value of one-third the resting metabolic rate (RMR) of each volunteer [25]. The meals were prepared with the same ratios of macronutrients (56% carbohydrates, 18% proteins and 26% lipids) at the same volume (350 mL) for each volunteer. To calculate the RMR, Food and Nutrition (FAO) equations from 2001 were used [26].

Assessment of binge eating and visual analog scale

The Binge Eating Scale (BES) was adapted to and validated in the Brazilian Portuguese language [27]. For the classification of periodic binge eating, the following scores were considered: ≤ 17 for no binge eating, 18–26 for moderate binge eating and ≥ 27 for severe binge eating, and the visual analog scale (VAS) was used to evaluate feelings of hunger and satiety [28]. These sensations were assessed using a 10-cm VAS and were applied at intervals of 30 min during the study.

DNA extraction and genotyping

DNA was extracted from whole blood using a commercial DNA extraction kit (Invitrogen™ PureLinkTM™ Genomic DNA). The rs17782313 polymorphism of the *MC4R* gene was genotyped by real-time PCR and detected using the TaqMan® genotyping assay (ThermoFisher®, Carlsbad, CA, USA). Amplification was performed in Step One PlusTM®, and the genotypes were identified using SDS 2.3 software. A negative control (all components excluding DNA) was included.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the distribution of variables. Data with a non-Gaussian distribution are presented as medians and quartiles. The Wilcoxon or Mann–Whitney *U* test was used to analyze paired and unpaired data. Descriptive analysis was conducted for qualitative data, and the Chi-squared test was applied to compare the presence of binge eating between groups.

In the comparison of log-transformed (base 10) levels of proteins in peripheral blood/serum ratios between post- and pre-prandial moments (logFC), the expected mean log-fold change marginal values obtained from multiple linear regression (log-linear) models of fixed effects were used, with the MC4R carrier main effect and the inclusion of age as confounders. Graphical analysis of ordinary least squares fitted model residuals was performed to confirm their randomness.

All of the results were obtained using the statistical software SPSS version 21.0, with P values < 0.05 considered significant.

Our sample was stratified into groups according to genotype distribution, where the allele frequencies were 79% for the ancestral allele (T) and 21% for the risk allele (C). Among the additive, recessive and dominant genotype models, the dominant model was used due to the distribution of the alleles in the population, including 4 mutated homozygotes (CC), 22 mutated heterozygotes (TC) and 44 ancestral homozygotes (TT). Hardy–Weinberg equilibrium was calculated and showed that the genotypes were in equilibrium ($\chi^2 = 0.311$, $P = 0.786$).

Results

In the present study, the SNP rs17782313 of the MC4R gene was evaluated in 70 women with severe obesity, and the association of this polymorphism with anthropometric and biochemical indicators was evaluated (Table 1). There were no differences in anthropometric and biochemical measurements between the categorized groups.

Plasma concentrations of ghrelin in the postprandial period were higher than in the pre-prandial period in the group with the polymorphism. For IL6, however, we

Table 1 Anthropometric and biochemical characteristics of the population

Variables	TT (44) (T = ancestral allele)	TC/CC (26) (T = ancestral allele. C = risk allele)	*P* value
Age (years)	35.5 (30.2; 41.7)	36.0 (27.7; 39.5)	0.831
Weight (kg)	125.2 (113.7; 135.6)	116.7 (111.1; 135.2)	0.281
BMI (kg/m^2)	47.5 (42.6; 52.0)	44.9 (42.4; 49.7)	0.314
WC (cm)	122.0 (112.0; 132.0)	119.5 (110.0; 131.7)	0.543
HC (cm)	141.0 (134.2; 147.7)	136.0 (126.9; 146.6)	0.222
WHR	0.87 (0.80; 0.92)	0.87 (0.82; 0.93)	0.523
TC (mg/dL)	169.5 (148.0; 201.7)	174.0 (158.5; 189.3)	0.860
LDL (mg/dL)	104.0 (95.0; 130.0)	108.5 (93.0; 121.0)	0.706
HDL (mg/dL)	43.0 (38.3; 49.0)	46.5 (39.0; 50.3)	0.432
VLDL (mg/dL)	22.0 (15.2; 28.0)	17.5 (14.0; 24.0)	0.278
TG (mg/dL)	110.5 (77.2; 139.5)	88.5 (71.0; 122.0)	0.259
Glucose (mg/dL)	97.5 (92.0; 106.2)	96.5 (88.7; 110.3)	0.870

Data are presented as median values (interquartile range 25–75%) for quantitative traits

BMI body mass index, *WHR* waist-to-hip ratio, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *WC* waist circumference, *HC* hip circumference, *VLDL* very-low-density lipoprotein, *TG* triglyceride

*Mann–Whitney *U* test for the comparison between genotypes, considering a *P* value < 0.05

observed that the plasma concentrations of postprandial IL6 were lower than those of pre-prandial IL6 in the group with the polymorphism. No differences were observed in the other comparisons. The effect of the carrier between the genotypes had no effect on the pre- and postprandial concentrations of ghrelin, leptin, IL6 or TNFa. However, there were changes in the effect for ghrelin and IL6 in those with the polymorphism when comparing the pre- and postprandial ratios (Fig. 1).

For feelings of hunger and satiety, no differences between genotypes were observed (Fig. 2).

Women without the polymorphism ($n=44$) did not present with binge eating, with a mean of 16 points, in contrast to women with the polymorphism ($n=26$), who had an average of 23 points according to the BES classification ($P=0.036$). Regarding the presence of binge eating in this population, Table 3 shows that more than half of women with the polymorphism exhibited severe binge eating. However, the Chi-square test results showed no dependence between the MC4R polymorphism and the presence of binge eating (Fig. 3).

Finally, 3-day dietary records were obtained to analyze whether the SNP rs17782313 influenced dietary intake among morbidly obese subjects. Differences were observed only in the intake of magnesium and manganese between patients with and without the polymorphism. No differences were found in the intake of the other nutritional parameters evaluated (Table 2).

Discussion

The main findings of this study showed increased levels of ghrelin in the postprandial period in patients with the polymorphism but without changes in feelings of hunger and satiety, decreased IL6 in the postprandial period in the group with the polymorphism and a higher prevalence of severe binge eating in more than half of the women carrying the risk allele (C) of rs17782313.

Our population included morbidly obese women, 21% of whom had a polymorphism in at least one risk allele (C), which is similar to the average prevalence of 23% (19–28%) found in the population in a genomic association data series [10].

The presence of obesity is well documented in the literature to be associated with an increase in inflammatory cytokines, generating a low-grade chronic inflammatory state [29]. TNFα levels did not differ between genotypes

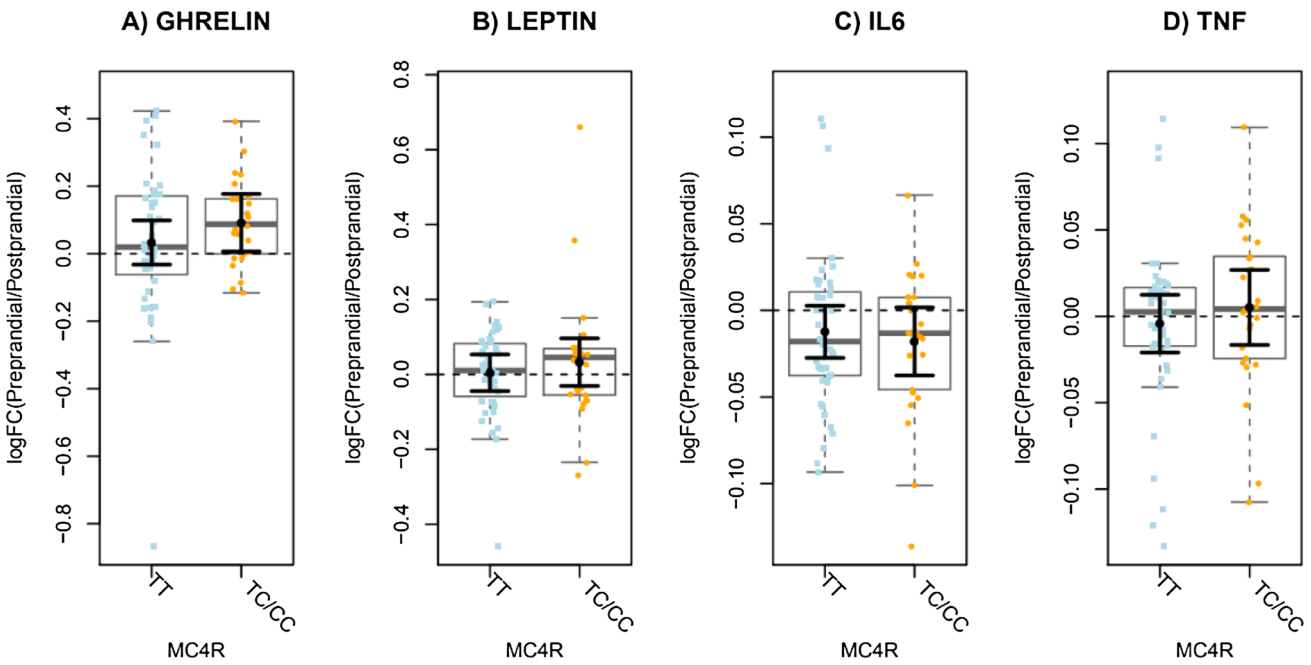

Fig. 1 Proteins in serum pre- and postprandial from MC4R C-carriers (TC/CC) and non-carriers (TT) patients. Proteins were measured in serum by Luminex (GHRELIN, LEPTIN, IL6, and TNFα). The levels obtained from each protein were analyzed on a logarithmic (base 10) fold-change (logFC) between post- and pre-prandial moments and illustrated using box plots to compare MC4R C-carriers (TC/CC) and non-carriers (TT) groups. The small gray dots represent individual logFC, and the box plots represent the interquartile range and the logFC median of the sample (solid gray central line). Larger black dots and vertical bars represent expected mean marginal values estimated by the linear model and its 95% confidence intervals (95% CI). Comparisons of logFC means between groups were performed by contrasts/differences obtained after multivariate models, adjusted by age and fitted by ordinary least squares. $P<0.05$; $^{*}P<0.05$; $^{**}P<0.01$

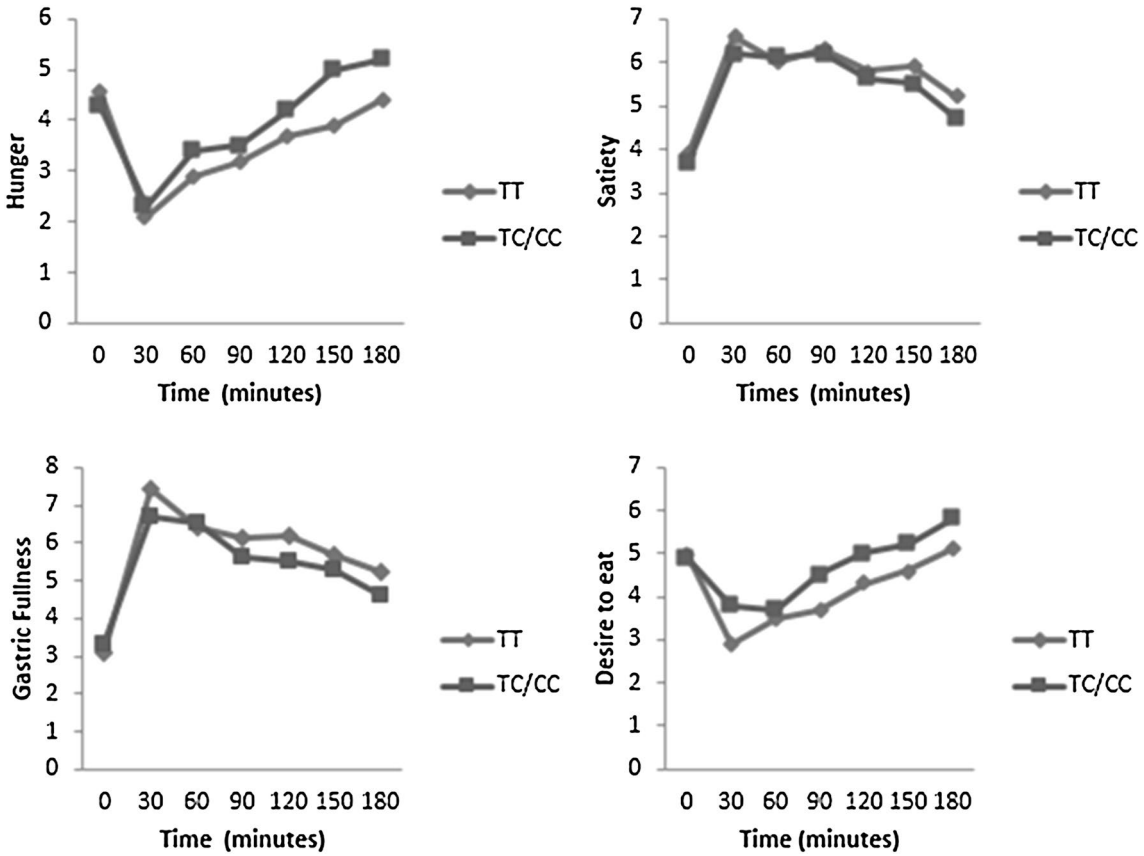

Fig. 2 Feelings of hunger and satiety by genotype in the pre- and postprandial periods. The Mann–Whitney *U* test was used for the comparison between genotypes, considering a *P* value < 0.05

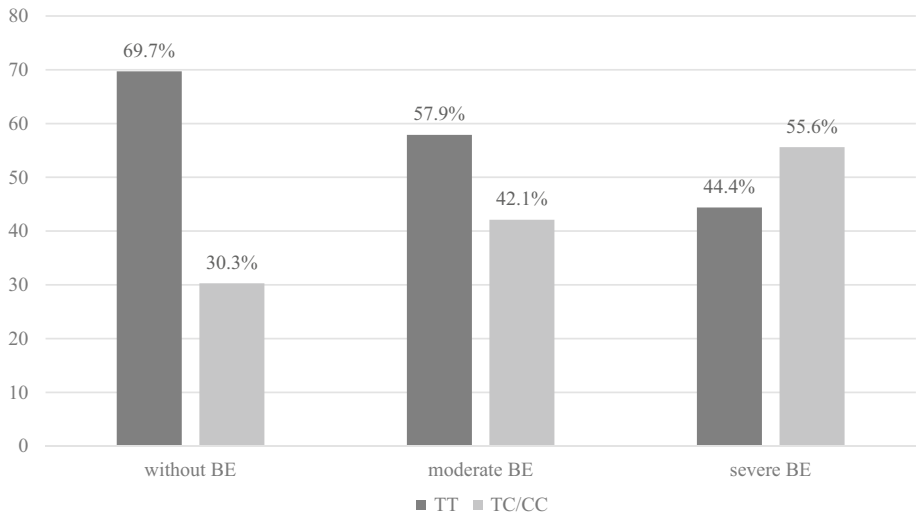

Fig. 3 Prevalence of women with (TT) and without (TC/CC) the polymorphism according to the periodic BES. Chi-square test was used for comparison between allelic groups, considering *P* value < 0.05

or between the pre- and postprandial periods, but postprandial IL6 levels were lower in women with the polymorphism. Geraldo and Alfenas [30] also note that this cytokine was more pronounced with the TT polymorphism both before and after a meal. In addition, few studies in the literature have related IL6 and TNFα to the rs17782313 polymorphism.

No difference was observed in the plasma concentrations of ghrelin and leptin in the two groups in the pre- and postprandial periods. However, it was found that plasma ghrelin

Table 2 Comparisons of energy intake, macronutrient distribution and micronutrient intake variables

Variables	TT (44)	TC/CC (26)	*P* value
Calories	2365.2 (1795.2; 2773.2)	2041.0 (1654.7; 2597.9)	0.146
PTNg/kg of body weight	0.72 (0.57; 0.88)	0.73 (0.56; 0.86)	0.729
Carbohydrates (%)	51.3 (47.0; 56.5)	50.6 (48.3; 54.1)	0.572
Proteins (%)	17.3 (15.1; 19.5)	17.6 (14.9; 20.6)	0.666
Total lipids (%)	31.1 (25.0; 34.4)	33.4 (26.2; 36.2)	0.512
SFA (%)	23.8 (21.0; 28.7)	22.9 (19.7; 26.1)	0.458
MFA (%)	25.2 (17.2; 28.3)	20.9 (18.2; 25.7)	0.164
PFA (%)	17.2 (12.4; 21.4)	16.3 (12.9; 22.3)	0.332
Cholesterol (mg)	281.0 (192.5; 387.6)	251.5 (182.4; 426.1)	0.635
Fiber (g)	13.2 (10.0; 17.5)	11.9 (10.2; 15.5)	0.293
Vitamin A (mcgER)	781.4 (381.2; 1061.4)	635.1 (360.8; 1251.4)	0.455
Vitamin D (mcg)	2.2 (1.3; 4.1)	1.8 (1.0; 2.5)	0.140
Vitamin E (mg)	15.5 (8.4; 25.5)	12.2 (7.4; 20.8)	0.198
Vitamin C (mg)	51.2 (27.6; 135.3)	69.1 (35.6; 122.9)	0.436
Vitamin B1 (mg)	1.3 (1.0; 1.8)	1.2 (0.7; 1.6)	0.166
Vitamin B2 (mg)	1.2 (1.0; 1.8)	1.1 (0.7; 2.1)	0.492
Vitamin B3 (mg)	23.0 (17.9; 26.8)	19.6 (17.5; 23.7)	0.083
Vitamin B5 (mg)	2.7 (2.1; 3.5)	2.7 (2.0; 3.8)	0.805
Vitamin B6 (mg)	1.3 (1.0; 1.7)	1.2 (0.9; 1.7)	0.794
Vitamin B9 (mcg)	128.8 (79.4; 165.0)	100.2 (74.1; 143.8)	0.349
Vitamin B12 (mcg)	3.5 (1.8; 6.0)	2.6 (1.5; 6.3)	0.402
Calcium (mg)	561.8 (335.0; 686.4)	556.4 (332.8; 737.7)	0.805
Phosphorus (mg)	1057.1 (762.0; 1260.1)	1.05.9 (722.5; 1.246.2)	0.601
Magnesium (mg)	176.7 (135.7; 229.9)	135.0 (108.8; 182.5)	0.041
Iron (mg)	14.7 (9.7; 20.2)	11.1 (8.7; 15.6)	0.063
Zinc (mg)	8.2 (5.6; 11.6)	7.2 (5.1, 11.3)	0.216
Copper (mg)	0.9 (0.7; 1.3)	0.6 (0.5; 1.0)	0.084
Iodine (mg)	15.6 (5.7; 37.5)	15.2 (3.2; 29.0)	0.644
Selenium (mg)	67.5 (50.2; 103.7)	58.8 (41.6; 84.7)	0.279
Manganese (mg)	1.2 (0.9; 1.7)	1.2 (0.7; 1.6)	0.046
Potassium (mg)	1781.0 (1305.2; 2321.5)	1291.7 (1059.8; 2037.6)	0.045
Sodium (mg)	2777.7 (1641.4; 2941.2)	2036.1 (1592.0; 2672.5)	0.337

Data are presented as median values (interquartile range 25–75%) for quantitative traits

PTNg/kg body weight proteins per kilogram of body weight, *%* percentage, *mg* milligrams, *g* grams, *mcgER* micrograms (mcg) of retinol equivalents (ER), *SFA* saturated fatty acids, *MFA* monounsaturated fatty acids, *PFA* polyunsaturated fatty acids. Population data were expressed as the median (interquartile). The Wilcoxon or Mann–Whitney *U* test was used for comparisons between genotypes considering a P value < 0.05

levels increased in women with the polymorphism in the postprandial period, which did not occur in women without the polymorphism. No study in the literature has evaluated the association of MC4R rs17782313 with the plasma concentrations of this hormone.

Ghrelin levels increased postprandially in women with the polymorphism, and the feelings of hunger and satiety assessed by the BES remained unchanged. It is well characterized in the literature that the decrease in ghrelin inhibits appetite and that leptin stimulates satiety; on the other hand, although these hormones play fundamental roles in these sensations, it is worth remembering that several gastrointestinal hormones, as well as the lateral hypothalamus, the nucleus arcuate and the middle region of the ventromedial hypothalamus, are crucial to balancing our feelings of hunger and satiety [31].

The leptin and ghrelin hormones exert reciprocal regulatory effects on the expression of inflammatory cytokines, where leptin promotes proinflammatory effects and ghrelin acts as an anti-inflammatory factor by inhibiting the expression of proinflammatory cytokines (TNFα and IL6) [32].

Leptin regulates metabolism and is involved in inflammatory responses. Increased leptin production facilitates the secretion of proinflammatory cytokines, such as TNFα, IL1 and IL6, which in turn promote their release from adipose tissue [33].

Authors have suggested that ghrelin is involved in transcriptional regulation and in the mRNA expression of proinflammatory cytokines, inhibiting the production of TNFα and IL6 through the growth hormone-releasing pathway [34]. On the other hand, researchers have investigated the effects of proinflammatory cytokines on the expression of ghrelin and have suggested that IL6 and TNFα indirectly inhibit ghrelin gene expression and increase insulin concentrations [35]. In contrast, another study evaluating these effects found no effect of inflammatory cytokines on ghrelin expression [36].

In our population, there was no difference in leptin or ghrelin between the groups with and without polymorphisms. A study of 77 women with obesity that aimed to assess the relationship of MC4R rs17782313 with plasma concentrations of ghrelin and leptin also found no significant correlation of this gene with these hormones [37].

The *MC4R* gene is regulated by neurons of the arcuate nucleus of the hypothalamus (ARC), which transmit signals involved in the overall balance between food intake and energy expenditure [9, 38]. The *MC4R* rs17782313 polymorphism has been found in pro-opiomelanocortin (POMC) and has been shown to reduce POMC expression and to be associated with morbid obesity, since POMC can change regulatory mechanisms that promote satiety [39].

Our study showed that although there is no dependence between the MC4R polymorphism and the presence of binge eating, the BES showed that more than half of the women presenting with severe binge eating had the polymorphism. A similar pattern was found in a European population, where the association of the MC4R rs17782313-C allele was observed with a higher prevalence of pinches. The authors describe that mutations and a monogenic context in this gene lead to hyperphagia and that the effects of the polymorphism on gene expression or activity can somehow lead to changes in eating behavior [40]. As a result, a possible genetic interaction between the genes of the reward system and MC4R may also be responsible for increased binge eating and subsequent altered eating behavior, which may explain the association between MC4R rs17782313 and an increase in BMI [41].

It is important to note that the measurement of eating behavior through self-reported questionnaires remains somewhat subjective, leading to the detection of inconsistent associations between genetic variants and eating behavior in everyday life, since environmental factors can affect eating behavior despite a genetic predisposition or susceptibility. One study aimed to assess whether there was a difference in energy intake in 34 overweight/obese women with and without binge eating disorder. The results of this study indicated that there were no differences between groups [42].

A review evaluating eating behaviors showed that mutations in MC4R appear to promote negative eating behaviors but do not cause obesity; however, more evidence on this subject is needed [43]. Consistent with these results, in a study with adult individuals, it was found that the presence of the risk allele (C) was associated with excess and greater uncontrolled intake of food and energy [16].

Although no differences were found with respect to energy intake and macronutrients among the patients with or without the polymorphism, evidence of consistent associations between SNPs and total energy, carbohydrate or fat intake is still not well clarified, according to the literature [44]. We observed increased consumption of total and saturated fats and decreased consumption of fiber according to reference values for the obese population [45].

Regarding micronutrient intake, the results showed high consumption of sodium and low consumption of vitamins A, D, E, C, B5, B6 and B9, calcium, copper, iodine, iron, magnesium, manganese, potassium and zinc in the studied population compared with the corresponding reference values [46, 47]. The intake of magnesium and potassium was higher in women with the polymorphism; however, this increased intake may be explained by increased reported consumption of food sources containing these minerals by this group, which would not be directly related to the presence of the MC4R polymorphism.

Quantifying food intake in the population is a difficult task, since there is no gold standard method for assessing food and nutrient intake, and the methods used are subject to variations and measurement errors. In addition, underreporting of the total energy intake is a common and well-known source of measurement error in dietary assessment, and evidence suggests that this bias is particularly significant in obese individuals [48].

In this study, we used a well-characterized cohort of participants. In addition, we analyzed the role of the studied polymorphism in plasma concentrations of hormones involved in controlling appetite, eating behavior and feelings of hunger and satiety. However, there were some limitations: (1) the sample cohort had a low educational level, which may influence the understanding of certain responses; (2) only one SNP was evaluated in this study, and clarifying whether this gene has a direct relationship with eating behavior is not possible; and (3) gene expression in individuals with the risk allele *MC4R* rs17782313 was not evaluated.

What is already known about this subject?

The MC4R gene has been considered a candidate for an increased risk of developing obesity because of its relationship with the secretion of important hormones involved in the regulation of food intake.

What does our study add?

A few studies address the influence of genetics on eating behavior, and this study showed that there were more women exhibiting severe binge eating in the MC4R polymorphism group.

Conclusions

The risk allele (C) was present in 21% of morbidly obese women. Despite the increase in plasma concentrations of ghrelin in the postprandial period, there were no changes in feelings of hunger and satiety in women with a risk allele. A decrease in IL6 was observed in women with the studied polymorphism.

An interesting finding was the higher prevalence of binge eating, as well as its greater severity, in women with the polymorphism.

Due to the association of the MC4R gene rs17782313 polymorphism with ghrelin release and a higher prevalence of binge eating, new studies should evaluate the relationship between this gene and compulsive behavior in individuals in all BMI ranges.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Availability of data and material The authors do not have permission to share data.

Ethical approval This study was approved by the Ethics and Research Committee of the University Hospital Clementino Fraga Filho.

Consent to participate All patients provided their written informed consent prior to enrollment in this study.

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