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Association between *MC4R* rs17782313 Polymorphism and Overeating Behaviours

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Abstract

Background/Objectives—Melanocortins play a crucial role in appetite and weight regulation. Although the melanocortin 4 receptor (*MC4R*) gene has been repeatedly linked to obesity and antipsychotic-induced weight gain, the mechanism behind how it leads to this effect in still undetermined. The goal of this study was to conduct an in-depth and sophisticated analysis of *MC4R* polymorphisms, body mass index (BMI), eating behaviour, and depressed mood.

Subjects/Methods—We genotyped 328 individuals of European ancestry on the following *MC4R* markers based on the relevant literature on obesity and antipsychotic-induced weight gain: rs571312, rs17782313, rs489693, rs11872992, and rs8087522. Height and weight were measured, and information on depressed mood and overeating behaviours was obtained during the in-person assessment.

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[3]There was little change to results when controlling for gender ([total indirect effect = 1.55, 95CI = 0.38; 2.95][BDI indirect effect = .25 95CI = −0.31; 1.10][eating indirect effect = 1.30; 95CI = 0.27; 2.75]).

Conflict of Interest

Dr. Kennedy has received honoraria from Eli Lilly and Roche, whereas Dr. Levitan has received honorarium from Astra-Zeneca. Other authors have no financial interests to disclose.

Results—BMI was associated with rs17782313 C allele; however this finding did not survive correction for multiple testing (p=0.018). Although rs17782313 was significantly associated with depressed mood and overeating behaviours, tests of indirect effects indicated that emotional eating and food cravings, rather than depressed mood, uniquely accounted for the effect of this marker and BMI (n=152).

Conclusions—To our knowledge, this is the first study to investigate the link between *MC4R* rs17782313, mood and overeating behaviour, as well as to demonstrate possible mechanisms behind *MC4R*'s influence on body weight. If replicated in a larger sample, these results may have important clinical implications, including potential for the use of MC4R agonists in the treatment of obesity and disordered eating.

Keywords

MC4R; candidate gene; weight gain; eating behaviour; emotional eating; food cravings; depression; obesity

INTRODUCTION

It is estimated that one billion adults worldwide are overweight or obese (1). The causes of obesity are complex, with biological, psychological, and environmental factors contributing to its etiology. Various hormones and neurotransmitter systems have been studied in relation to appetite and weight regulation in the hope of shedding light on the biological risk factors for overeating and obesity (2–4).

Melanocortins are expressed in the hypothalamus and play an important role in regulating appetite. Stimulation of brain melanocortin results in a decrease in food intake and weight; similarly, mutations and defects of the melanocortin 4 receptor (*MC4R*) gene have been implicated in animal models and human studies of weight gain (5–7). Genome-wide association studies (GWAS) and meta-analyses of GWAS suggest that several markers near *MC4R* (e.g., rs17782313, rs571312) are strongly associated with obesity in healthy adults, adolescents, and children (8–13). There have also been small- and large-scale candidate gene studies that replicated the GWAS findings on *MC4R* in various ancestral populations and age groups (14–16). More recently, a GWAS on second-generation antipsychotic-induced weight gain found a peak at *MC4R* on chromosome 18, and an association with rs489693 (17). Similarly, rs8087522, identified as a putative transcription factor-binding site in the promoter region of the *MC4R* gene, has been implicated in weight gain following the prescription of antipsychotic medications (18).

Although it is well documented that common variants near *MC4R* are associated with an increased susceptibility for gaining weight, the mechanisms behind this effect are not fully understood. Animal studies have demonstrated that MC4R deficiency may be responsible for various metabolic and eating-related changes such as increased feeding (19) and high-fat hyperphagia (7). It is not clear, however, whether these models apply to the near *MC4R* region, which is often not covered in animal knockout models. *MC4R* rs17782313, located downstream of the *MC4R* gene, has been studied extensively and replicated consistently in its relation to weight gain (8–16). Thus far, rs17782313 has not been linked to micro- or

macro-nutrient intake (20, 21). In two preliminary studies of obese Chilean children, however, rs17782313 C homozygosity was associated with increased food-enjoyment scores and sweet snack consumption (22, 23). In summary, despite the well-established link between *MC4R* common variants and body weight, no clear mechanism of action has been found to explain how these variants are associated with weight gain in the general population.

MC4R is also known to interact with the serotonin and dopamine pathways, thereby suggesting a possible involvement of MC4R in mood regulation. The relationship between melanocortins and serotonin has been well documented (24), and the effects of the antidepressant d-fenfluramine (a serotonin receptor 2C agonist) are significantly diminished in *Mc4r* knockout mice (25). At the molecular level, it has been recently demonstrated that MC4R signaling may be involved in triggering stress-induced synaptic adaptations in the nucleus accumbens, an area of the brain associated with reward processing and where dopamine is highly expressed (26). Furthermore, blocking MC4R signaling in this region has reversed anhedonia in rodents (26), demonstrating the possible link between dysphoria and melanocortins. Despite the evidence for a possible role of the melanocortin system in mood regulation, to date, no study has investigated whether there are links between *MC4R* genetic variants and depressed mood nor explored how depressed mood, may affect eating behaviour or BMI in the presence of *MC4R* genetic risk variants. One possibility is that MC4R predisposes individuals to weight gain via two related pathways: 1) via overeating behaviours and 2) via depressed mood.

The objectives of the current study were: 1) to examine the relationship between *MC4R* markers and BMI; and 2) to investigate associations between *MC4R* common variants and eating behaviours associated with over-consumption (viz. emotional eating, binge eating, food cravings, and hedonically-driven eating); and 3) to assess overeating behaviours and depressed mood as potential mediators of the predicted association between *MC4R* and body mass index (BMI).

METHODS

Participants

Adults between the ages of 24 and 50 years (230 women and 98 men) were recruited through advertisements placed at universities, hospitals, other public institutions, and local newspapers in the Greater Toronto Area, as well as online sites such as Craigslist. All participants were of European ancestry. Exclusion criteria included: 1) not being fluent in English or having lived in North America for less than five years; 2) being post-menopausal or having a pregnancy within the previous six months for female participants; 3) a current or lifetime DSM-IV-TR diagnosis of any psychotic disorder, alcohol/substance abuse or dependence; or 4) diagnosis of a serious medical/physical illness such as cancer, heart disease, or paralysis. Current or past diagnosis or treatment of other psychiatric disorders did not lead to exclusion from study participation. Prior to the in-person assessment, all participants were screened over the phone by the research coordinator to confirm basic eligibility criteria.

All aspects of this research study were reviewed and approved by the Centre for Addiction and Mental Health Research Ethics Board and conducted in accordance with the Helsinki Declaration as revised in 1989.

Clinical measures

BMI (weight in kilograms/height in metres^2) was calculated from measured height and weight obtained during the in-person screen.

Depressed Mood was assessed using the *Beck Depression Inventory* (BDI) (27). BDI, which consists of 21 items covering the one-week time period before the assessment, is one of the most commonly used scales for the evaluation of mood in clinical and research settings. It captures a variety of depressive symptoms such as dysphoria, changes in eating patterns, sleep disturbances, and anhedonia. Cronbach's α coefficient in the present study was 0.94.

Binge Eating was assessed by the five-item subscale of the *Binge Eating Questionnaire* (BEQ) (28), which includes frequency and severity of symptoms such as loss of control over eating, and negative affect following a binge. Cronbach's α coefficient was 0.85.

Hedonically-Driven Eating was assessed by the *Power of Food Scale* (PFS) (29), a 21-item self-report questionnaire that assesses individual differences in the appetitive responsiveness to food independent of actual consumption (thus differentiating the motivation and appetitive drive for palatable foods from the tendency to overeat). Cronbach's α coefficient was 0.97.

Emotional Eating was assessed by two separate questionnaires: i) the same-named subscale of the *Dutch Eating Behaviour Questionnaire* (DEBQ) (30), which reflects the degree to which eating behaviour is triggered by emotional states such as worry rather than by hunger (e.g., "Do you have the desire to eat when somebody lets you down?"); and ii) the *Eating Behaviour Patterns Questionnaire* (EBPQ) (31). Similar to the DEBQ, the EBPQ emotional-eating subscale captures eating driven by emotional states as opposed to internal hunger cues (e.g., "When I am in a bad mood, I eat whatever I feel like eating."). Cronbach's α coefficients for the two scales were 0.96 and 0.90, respectively.

Food Cravings were assessed by the *Food Craving Questionnaire - Trait* (FCQ-T) (32). This 39-item questionnaire derives from the evidence that food cravings can be expressed both physiologically and psychologically. The nine factor-analytically derived scales measure cravings experienced as, or associated with positive reinforcement, negative reinforcement, cue-dependent eating, feelings of hunger, preoccupation with food, intentions to eat, lack of control, negative affect, and guilty feelings. Cronbach's α coefficient was 0.98.

Snacking on Sweets was assessed with another subscale of the EBPQ, which reflects the extent to which the individual prefers foods with high-sugar content (e.g., "To me, cookies are an ideal snack food."). Other subscales of EBPQ were not included in our analysis, because they are not relevant to overeating. Cronbach's α coefficient was 0.77.

Laboratory Methods

Blood samples for genetic analysis were collected on the day of clinical assessment. Blood lymphocyte DNA was extracted using the high-salt method (33). The four *MC4R* single nucleotide polymorphisms (SNPs) genotyped as a part of this study (Table 1) were selected based on the literature on obesity and antipsychotic weight gain (8, 17, 18) and these markers and their context sequence information for the ABI assays (available on demand) are as follows: GTTTAAAGCAGGAGAGATTGTATCC[C/T]GATGGAAATGACAAGAAAAGCTTCA for rs17782313 (ABI assay ID: C__32667060_10), TCTTAATTCTGTTGTCATTAGTTCC[A/C]G TTTGTTAAATGTTTACAGCGTGGC for rs489693 (ABI assay ID: C___3058718_10), TAAGAACCCAGCCAGTAGTGGTTCA[A/G]TTAAAATACCTGAAAAACAGAGAGG for rs8087522 (ABI assay ID: C__29004626_10), and ATTATGAATGGGTTCAAAAGGGGTT [A/G]ATTTTAATGCACTAATATCCCATAT for rs11872992 (ABI assay ID: C___2747891_10). Due to having been associated with BMI in a number of important publications (10), we also included *MC4R* rs571312 in our study. However, because it is well documented that rs571312 is in high linkage disequilibrium (LD) with rs17782313, we decided to analyze this SNP in a *post hoc* manner, separate from the other four SNPs. The context sequence information for the ABI assay (available on demand) for rs571312 is AAATTTGAGT TGCAGCTTTAAGTGA[A/C]CTATGACCCGATGTTTGTGTAAAAT (ABI assay ID: C____797201_10). Polymerase chain reactions of 10μl volume using 20 ng genomic DNA were performed using Assays-on-Demand by Applied Biosystems Inc. (ABI; Carlsbad, CA) under the following conditions: 95°C for 10 min, followed by 60 cycles of 92°C 15 s, 60°C 1 min. Determination of alleles was performed using the ABI 7500 Sequence Detection System with the Allelic Discrimination software. Genotyping of the DNA was performed at the Neurogenetics Laboratory at the Centre for Addiction and Mental Health in Toronto, Canada, with laboratory staff blind to psychiatric diagnosis. For quality control purposes, 10% of the samples were re-genotyped, and ambiguous genotypes were excluded from the analysis.

Statistical Methods

Hardy-Weinberg equilibrium and LD were assessed using a chi-squared test through Haploview version 4.0. The relationships between *MC4R* genotypes and BMI measures were assessed using linear regression in SPSS 15.0 (genotype coded as 0 for TT, 1 for CT and 2 for CC), whereas UNPHASED version 3.1.5 was used to perform allelic analysis. For the quantitative analyses involving BMI, we corrected for multiple testing using Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD; 34), determining the effective number of independent marker loci to be 3, thus setting the significance threshold α at 0.017.

Tests of mediation (also known as tests of indirect effects) are a common statistical approach in psychological science to test hypothesized processes (such as eating behaviours) linking distal variables (such as genetic vulnerability) and more proximal outcomes (such as weight). Originally based on the work of Baron and Kenny (35), tests of mediation assess whether putative mediating variables account for the association between a predictor variable and the outcome variable. More recent approaches assess whether the cross-product

of path coefficient linking the predictor variable and the mediating variable (path a) and the path coefficient linking the mediating variable and the outcome variable (path b) is significantly different from zero. This approach uses bootstrapping to create 95% confidence intervals around the "true" value of this cross-product (referred to as the indirect effect). If zero is not within this confidence interval then the indirect (i.e., mediating) effect if significant. This approach can be extended to assess multiple mediators simultaneously. Multiple mediation analyses assess the significance of the indirect pathways via putative mediators as well as the overall mediation model. In order to test simultaneously whether the overeating behaviours and depressed mood accounted for the association between *MC4R* (coded for the number of risk alleles present carried by an individual) and BMI, a multiple mediation model was tested according to the procedures described by Preacher and Hayes (36). As shown in Figure 1, path *c* refers to the *total effect* of *MC4R* on BMI in the absence of the mediators. *Specific indirect effects* refer to the influence of path *a* x path *b* via each *specific* mediator (a_1 x b_1 is the indirect effect of *MC4R* on BMI via depressed mood; a_2 x b_2 is the indirect effect of *MC4R* on BMI via overeating behaviours). Multiple mediation analysis also allows statistical control over the correlations between the mediators. The *total indirect effect* reflects the summation of the *specific indirect effects*. Path c' refers to the *direct effect* of *MC4R* on BMI when also controlling for the mediators.

Bias-corrected bootstrap confidence intervals (n=5000, confidence intervals set at 95%) were used to assess the significance of indirect effects. The SPSS "INDIRECT" macro developed to accompany the paper by Preacher and Hayes (36) was used to test the significance of the overall total indirect effect, as well as the significance of the specific individual mediators. The absence of zero in the confidence interval indicates significant indirect (i.e., mediated) effects.

RESULTS

Sample Description

Table 2 summarizes the genetic baseline characteristics of the participants included in the study. Minor allele frequencies for the *MC4R* loci in our sample were as follows: 23.3% for rs571312, 24.1% for rs17782313 (n=322), 31.9% for rs489693 (n=324), 30.3% for rs8087522 (n=322), and 15.3% for rs11872992 (n=323). The average rate for successful genotyping across the SNPs was over 98%, and all markers were in Hardy-Weinberg equilibrium. LD plot for the study sample is shown in Figure 2.

The Association between *MC4R* Loci and BMI

Table 3 summarizes the relationship between the *MC4R* common variants and BMI. In accord with previous studies (8–13), carriers of the rs17782313 minor allele had a higher BMI compared to carriers of the major allele. After correction for multiple testing, however, this finding was a strong trend that closely approached statistical significance (p=0.018). Genotypic analysis revealed a dominant effect for the C allele, but this finding also fell short of statistical significance after correcting for multiple testing (p=0.028). The other three *MC4R* markers were not associated with BMI in our sample.

MC4R rs571312 was analyzed separately from the other SNPs in a *post hoc* manner because it was in high LD with rs17782313. The association of rs571312 with BMI also did not reach significance following correction (p=0.054 for allelic analysis; n=321; results not shown).

Associations among rs17782313, Overeating Behaviours, and Depressed Mood

The rs17782313 C allele was significantly associated with elevated scores on the DEBQ Emotional Eating subscale (r=0.17, p=0.004), the EBPQ Emotional Eating subscale (r=0.15, p=0.01), and the FCQ-T (r=0.20, p=0.013). None of the other *MC4R* markers were related to scores on any of the other overeating measures. The C allele of the rs17782313 polymorphism was also significantly associated with an increase in depressed mood, as assessed using the BDI (r=0.18, p=0.005). BDI and BMI were highly correlated (r=0.33, p<0.001); similarly, BDI was significantly associated with the DEBQ Emotional Eating subscale (r=0.54, p<0.001), the EBPQ Emotional Eating subscale (r=0.50, p<0.001), and the FCQ-T (r=0.54, p<0.001). Due to the significant associations of rs17782313 with overeating behaviour and depressed mood, mediation analyses were carried out to test the indirect effect of the *MC4R* marker on BMI, with depressed mood, and the overeating measures significantly associated with this marker (viz. the two emotional-eating variables and food cravings) as the mediators.

Mediation Analyses

Only 152 participants completed all measures used in the mediation analyses. The three overeating variables were highly correlated and appear to be measuring a shared construct. One strategy for managing highly correlated variables is to calculate a factor score to capture their shared variance. This approach resolves the problems associated with multi-collinearity and increases scale reliability (37). Thus, an exploratory factor analysis was carried out to create a single composite score of the emotional-eating and craving variables.[1] A single factor accounted for 86% of variance in the three overeating scales and all three loaded strongly on the factor (loadings ranging between 0.925 and 0.931).

As shown in Figure 1, the *total indirect effect* was statistically significant (unstandardized coefficient = 1.61; 95% CI: 0.52 – 3.03). However, only the overeating factor produced a significant *specific* indirect effect (Unstandardized coefficient = 1.31; 95% CI: 0.38 – 2.69) after controlling for depressed mood. While depressed mood was significantly associated with *MC4R* and BMI, the *specific* indirect effect was not significant (unstandardized coefficient = 0.31; 95% CI: −0.21 – 1.25). Therefore, whilst *MC4R* rs17782313 is associated with both depressed mood and emotional eating/cravings, only the latter shows a unique indirect effect to BMI (when controlling for depressed mood).[2]

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[1]Data for this study were collected over a period of six years, and the FCQ was added as a measure about halfway through the data collection. Our exploratory analyses showed that the group with the FCQ scores was on average had a higher BMI than the group without the FCQ scores. The reason for this difference was that as the study progressed, the recruitment focus shifted to including individuals who were overweight, as this group gave us a greater spread of scores on our measures of overeating. To account for the missing data on the Food Cravings and emotional eating scales used in our study, data were imputed using Expectation Maximization Imputation and the model tested again (n = 322). Equivalent results were found with the imputed data with the total indirect effect (1.43; 95% CI: 0.57–2.29) and the specific indirect effect via eating (1.14; 95% CI: 0.44 – 1.93) significantly different from zero. The specific indirect effect via depressed mood remained non-significant (.28; 95% CI: −0.03 –0.85).

DISCUSSION

To date, little is known about the mechanisms by which *MC4R* common variants contribute to high BMI, and only a few studies have investigated the role of eating behaviour in this association. Furthermore, despite the interconnections among the melanocortinergic, dopaminergic and serotonergic systems, *MC4R*-linked obesity has not been studied in relation to depressed mood. This project is the first to propose a possible mechanism to explain the relationship between *MC4R* rs17782313 and BMI. Specifically, we hypothesized two pathways by which *MC4R* may increase body weight: 1) via depressed mood and 2) via overeating behaviours. As discussed in the Results section, the rs17782313 polymorphism was associated with both depressed mood and emotional eating/cravings. However, when accounting for the shared variance between emotional eating and depressed mood, our analysis revealed that eating behavior alone (more specifically emotional eating and food cravings) accounted for the association between rs17782313 and BMI in our sample.

To our knowledge, the indirect effect of *MC4R* rs17782313 on BMI via emotional eating and food cravings is a novel pathway that has neither been proposed nor demonstrated before. The result of our mediation analysis is especially intriguing in that a causal link between depression/anhedonia and overeating has been well documented. One of the diagnostic criteria for major depressive disorder is a change in eating behaviour, and individuals suffering from atypical depression or seasonal affective disorder often present with overeating (38). Furthermore, patients with atypical depression—especially women—are more likely to be obese and present with higher BMI, waist-to-hip ratio, and percent fat mass compared to those with melancholic depression (39, 40). Although *MC4R* rs17782313 was associated with depressed mood in our study, current evidence does not suggest a specific indirect role for depressed mood in increased BMI in individuals carrying the risk allele. Considering the well-documented link between overeating and disinhibition (41–45) and in line with the recent findings published by Horstmann and colleagues (46) regarding the genotype-related gray matter volume differences in the prefrontal cortex, our results suggest a possible role for disinhibition and impulsivity rather than anhedonia with regards to *MC4R*-linked weight gain.

It is also important to mention that in accord with previous findings (8–13), we detected a positive trend between rs17782313 and BMI. None of the markers associated with antipsychotic-induced weight gain (rs8087522 and rs489693) were related to BMI in our sample, suggesting that a different genetic mechanism may be responsible for medication-induced changes in appetite. As we discussed, it was proposed that *MC4R* variants may affect body mass via more proximal factors such as overeating. In particular, our preliminary results suggest a potential pathway to high BMI via emotionally-driven eating and the presence of the *MC4R* rs17782313 risk allele. Although our significant pathway did not involve depressed mood, our results suggest that the C allele of the rs17782313 polymorphism may play a role in the susceptibility to depression. We are unaware of any studies linking depression to *MC4R* variants in humans; however, recent animal studies have

[2]The same models were also tested using the individual eating scales as the mediator of *MC4R* and BMI and showed equivalent results as the factor score.

shown that disruption of MC4R negates the effects of antidepressant medications (25) and that MC4R may be associated with anhedonia (26). The functionality of the near-*MC4R* region (which includes rs17782313) is currently not well understood, and preclinical studies are needed to investigate the biological mechanism underlying the links between *MC4R* common variants and depressed mood.

In summary, while the rs17782313 was the only polymorphism associated with BMI, it was also the only SNP related to overeating behaviours. Specifically, rs17782313 C allele was associated with increased emotional eating and food cravings. This particular polymorphism has been previously associated with increased food enjoyment and snacking on sweets (22, 23). In our study, however, the Snacking-on-Sweets measure was not linked to *MC4R* variants, which meshes with a recent report showing that *Mc4r* knockout mice increase their overall level of food consumption without an increased preference for high-fat or high-sugar foods (47). A recently published imaging study has also provided preliminary evidence for *MC4R*'s involvement with emotional eating (46), also in line with the findings of the present study.

Despite our novel findings, it is important to acknowledge some limitations of the current study. First, information on eating behaviours was obtained entirely from self-report measures. Therefore, since we did not include more objective measures from laboratory or observational paradigms, we must acknowledge the possibility of response bias. We also did not have complete data on the eating measures for all individuals included in the genetic analyses. In addition, the majority of our sample was women, and there have been numerous studies reporting sex differences for the effect of rs17782313 on various measures of food intake and body composition (20, 46, 48). However, when controlling for sex in the mediation analyses, there was minimal change to our results, suggesting that the association we identified may not be sex-biased. Finally, we anticipate that these preliminary findings will assist other researchers with hypothesis generation for future studies with larger samples.

If replicated, our preliminary results have a number of possible clinical implications. First, *MC4R*'s association with emotional eating and food cravings in our sample may suggest an MC4R-dopamine system interaction, especially considering that dopamine is highly expressed in the brain's reward pathways and the involvement of dopaminergic genes in overeating has been widely reported (49–52). Our group has also demonstrated a link between markers of the dopamine receptor D2 (*DRD2*) gene and emotional eating in binge eating disorder (53). Administration of agouti related protein, a melanocortin inverse agonist, is known to activate the dopaminergic neurons in the midbrain, to increase dopamine turnover in the prefrontal cortex, and to attenuate sucrose-seeking behaviour in rats (54), highlighting the possible link between MC4R, dopamine, and eating behaviour. Furthermore, MC4R signaling of dopamine 1 receptor neurons has been shown to play a role in procedural memory learning (55), as well as in stress-induced synaptic adaptations in the nucleus accumbens (26), thus suggesting an interaction between the dopamine (specifically DRD1) and the melanocortin systems in mood regulation.

The serotonin system also plays a pivotal role in the regulation of eating and mood, and the interaction between MC4R and serotonergic system on energy balance has been well documented (56, 57). As a result of this, a possible genetic interaction between the serotonin system genes and *MC4R* may also be responsible for our findings in eating behaviour, highlighting the possibility of utilizing serotonin agonists in the treatment of MC4R-linked obesity. For instance, MC4R agonists (such as BIM-22493, BIM-22511) (58) may be helpful in the treatment of a subgroup of obese individuals with increased emotional eating and food cravings.

In conclusion, our results suggest that increased emotional eating and food cravings may account for the association between *MC4R* rs17782313 and BMI. Furthermore, although rs17782313 was also linked to depressed mood in our sample, the effect of this polymorphism on BMI was not via depression. If replicated, this proposed mechanism has the potential to have significant impact on our understanding of MC4R-linked weight gain and obesity.

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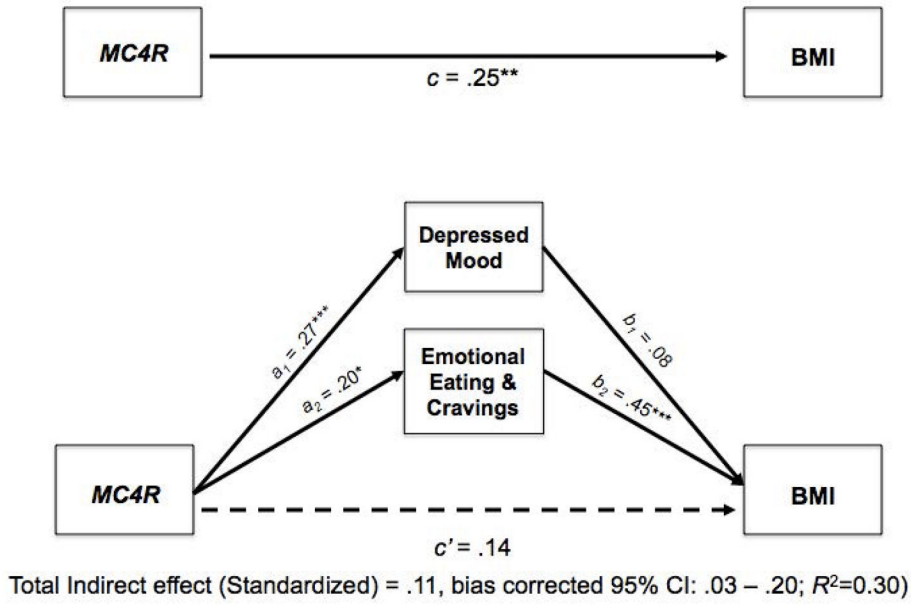

Figure 1.

Multiple mediation model of the relationship between MC4R, depressed mood, emotional eating/cravings, and BMI.

Note. Standardized coefficients are presented and tested for significance with 95% confidence intervals calculated using the bias-corrected bootstrap method (5000 samples). a = standardized IV to Med coefficient, b = standardized Med to DV coefficient, c = standardized total effect (IV to DV), c′ = standardized direct effect. Subscripts refer to specific indirect paths.

* p < .05

** p < .01

*** p <.001

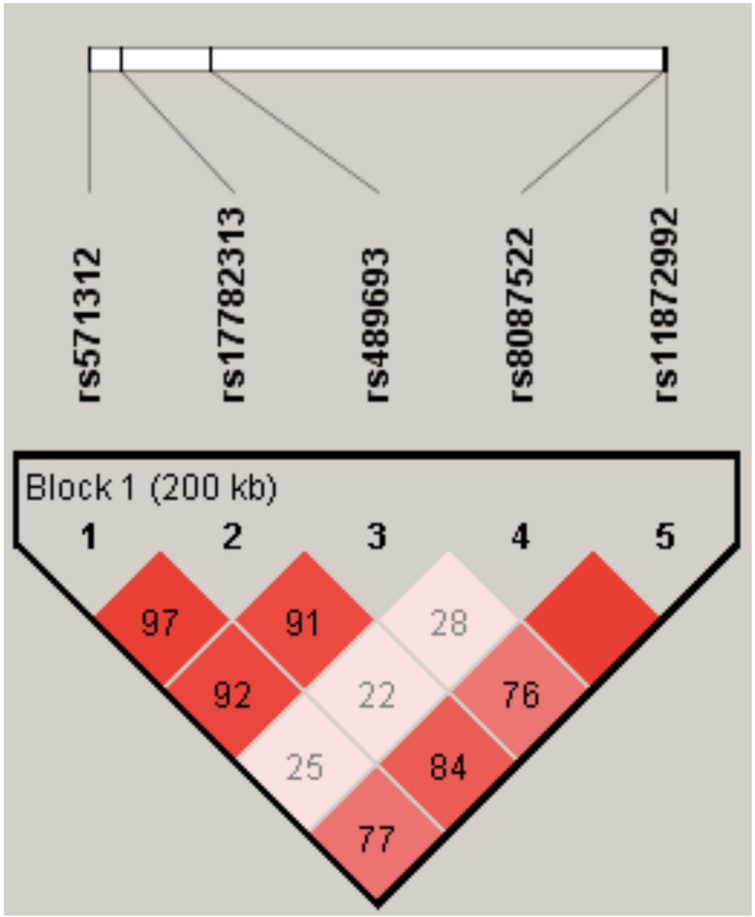

Figure 2.
Linkage disequilibrium plot for the five *MC4R* markers.

Table 1

MC4R marker information

***MC4R* marker**	**Region**	**Nucleotide substitution**	**Minor allele**
rs17782313	Near *MC4R*	C/T	C
rs489693	Near *MC4R*	A/C	A
rs8087522	*MC4R* promoter	A/G	A
rs11872992	*MC4R* promoter	A/G	A

Table 2

Means and standard deviations (SD) for all quantitative variables in the study

	Mean	SD	N
Age (years)	33.9	7.0	328
BMI (kg/m^2)	31.8	9.5	327
BDI	10.6	9.6	250
BEQ	2.1	1.9	281
PFS	58.2	21.4	284
DEBQ_Emot	3.0	1.1	283
EBPQ_Emot	36.0	8.4	284
EBPQ_Sweet	17.5	4.9	284
FCQ-T	123.3	40.7	155

Note. BMI=Body Mass Index; BDI=Beck Depression Inventory; BEQ=Binge Eating Questionnaire; PFS = Power of Food Scale; DEBQ_Emot=Dutch Eating Behavior Questionnaire Emotional Eating subscale; EBPQ_Emot=Eating Behavior Patterns Questionnaire Emotional Eating subscale; EBPQ_Sweet=Eating Behavior Patterns Questionnaire Snacking on Sweets subscale; FCQ-T= Food Craving Questionnaire – Trait.

Table 3

Relationship between the *MC4R* markers and BMI

***MC4R* marker**	**Genotype**	***n***	**BMI (*M* ± *SD*)**	***p* (genotype)**	***p* (allele)**
rs17782313	CC	23	35.6 ± 10.0	0.028*	0.018*
	CT	109	32.5 ± 10.2		
	TT	191	31.0 ± 8.9		
rs489693	AA	35	33.5 ± 11.2	0.271	0.315
	AC	137	31.9 ± 9.7		
	CC	153	31.5 ± 9.0		
rs8087522	AA	26	29.5 ± 10.3	0.328	0.649
	AG	143	33.0 ± 10.4		
	GG	154	31.1 ± 8.3		
rs11872992	AA	7	29.9 ± 6.2	0.619	0.782
	AG	86	31.8 ± 8.1		
	GG	231	31.8 ± 10.1		

*statistical trend after correction for multiple testing (α=0.017)