Long-Term Weight-Loss in Gastric Bypass Patients Carrying Melanocortin 4 Receptor Variants

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Abstract

Background: The melanocortin 4 receptor (MC4R) critically regulates feeding and satiety. Rare variants in MC4R are predominantly found in obese individuals. Though some rare variants in MC4R discovered in patients have defects in localization, ligand binding and signaling to cAMP, many have no recognized defects.

Subjects/Methods: In our cohort of 1433 obese subjects that underwent Roux-en-Y Gastric Bypass (RYGB) surgery, we found fifteen variants of MC4R. We matched rare variant carriers to patients with the MC4R reference alleles for gender, age, starting BMI and T2D to determine the variant effect on weight-loss post-RYGB. In vitro, we determined expression of mutant receptors by ELISA and western blot, and cAMP production by microscopy.

Results: While carrying a rare MC4R allele is associated with obesity, carriers of rare variants exhibited comparable weight-loss after RYGB to non-carriers. However, subjects carrying three of these variants, V95I, I137T or L250Q, lost less weight after surgery. In vitro, the R305Q mutation caused a defect in cell surface expression while only the I137T and C326R mutations showed impaired cAMP signaling. Despite these apparent differences, there was no correlation between in vitro signaling and pre- or post-surgery clinical phenotype.

Conclusions: These data suggest that subtle differences in receptor signaling conferred by rare MC4R variants combined with additional factors predispone carriers to obesity. In the absence of complete MC4R deficiency, these differences can be overcome by the powerful weight-reducing effects of bariatric surgery. In a complex disorder such as obesity, genetic variants that cause subtle defects that have cumulative effects can be overcome after appropriate clinical intervention.

Introduction

Obesity is a worldwide epidemic that contributes to comorbidities such as diabetes and heart disease [1]. Severe obesity unresponsive to medication and dieting can be effectively treated with bariatric surgery. Roux-en Y gastric bypass (RYGB), vertical sleeve gastrectomy and gastric banding are the most common bariatric surgeries [2]. Importantly, type 2 diabetes (T2D) often remits following RYGB, before significant weight-loss occurs [3,4]. RYGB improves blood glucose levels more rapidly and completely than caloric restricted weight-loss or other common bariatric procedures [2,5].

Regulation of feeding and satiety, essential for maintaining healthy weight, occurs in the hypothalamus. In the fed state, insulin and leptin stimulate neurons expressing proopiomelanocortin (POMC) to release α-melanocyte stimulating hormone (α-MSH) and β-MSH [6]. α-MSH binds and activates melanocortin 4 receptor (MC4R), resulting in an increase in cAMP, inducing the sensation of satiety. Insulin and leptin also inhibit neurons expressing neuropeptide Y (NPY) and agouti-related protein (AgRP). AgRP is a biased agonist of MC4R that stimulates appetite [7]. The balance between the signals from POMC and AgRP neurons critically regulates feeding behavior and energy homeostasis.

The development of obesity, as well as the degree of weight-loss following RYGB surgery can be greatly impacted by genetic variants [8,9]. For example, extreme obesity can be due to mutations in genes such as MC4R [9–12]. Common missense variants in MC4R, occurring in both lean and obese people at equal frequencies, have also been described [8,12–14]. Recently, we reported that having the common MC4R variant I251L leads to better weight-loss and weight maintenance following RYGB [8]. Patients with an I251L allele also resolved their T2D more quickly than patients with two copies of the MC4R reference allele [15]. Numerous rare MC4R variants have also been reported, primarily identified in cohorts of obese individuals [16–18]. While, some of
these rare variants have deleterious effects on MC4R signaling to cAMP (e.g. D90N) [19], binding of agonist (e.g. R18L) [16], or localization (e.g. P299H) [17], many mutations display no known defects. Tao et al. grouped MC4R mutations into five classes: Class I are null mutations; Class II are mutations that cause localization defects; Class III are mutations that cause binding defects; Class IV are mutations that cause cAMP signaling defects; and Class V variants have no known defects [20,21]. Since many MC4R variants have unknown defects, the analysis of each mutation to determine the in vitro signaling defects and their possible correlation with obesity phenotypes is necessary.

We report fifteen MC4R variants identified in a cohort of 1433 obese patients who underwent RYGB surgery. Of the fifteen variants, two (V103I and E251L) are common alleles occurring at equal frequencies in both lean and obese populations [8]. The thirteen other MC4R variants are rare; twelve were previously reported and one, G34A, is novel. Three of the rare variants were associated with poor weight-loss post-RYGB surgery (V155I, I137T and L250Q). One exhibited reduced cell surface expression (R305Q) and two had reduced cAMP signaling (I137T and C326R). Despite the apparent differences in biochemical characteristics, no correlation was found between in vitro signaling and pre- or post-surgery metabolic phenotypes. Together these data point to the complex involvement of MC4R in obesity, metabolism and weight-loss.

Materials and Methods

Study population, clinical variables and sequencing of MC4R

All procedures and patient information were collected under a protocol approved by the Geisinger IRB. All subjects gave written informed consent for this project. Genomic DNA was isolated from blood collected from patients. The sequence for MC4R was determined for each sample and matched to clinical data obtained in a de-identified manner through a data broker. A cohort of 1433 patients (79.9% female, median age 46 years (range 18–72 years)) who underwent primary Roux-en Y gastric bypass (RYGB) surgery has been previously described [8]. Subjects were categorized as non-diabetic (HbA1c <6.0% and no diabetes medications) or diabetic (HbA1c >6.0% or taking one of four diabetes medications: biguanides, sulfonylureas, insulin, or insulin sensitizing agents). Baseline homocysteate model assessment for insulin resistance (HOMA_R) was calculated as HOMA_R = (fasting plasma insulin × fasting plasma glucose)/22.5. DNA extraction from blood samples and MC4R gene sequencing was performed as described [8]. We genotyped 451 age and gender matched lean subjects in parallel (68.5% female, median age 52 years (range 25–66 years). RYGB patients were followed 12 months prior to surgery and up to 84 months post-surgery. Blood pressure and pre-surgery weight are presented as three month averages. The time to maximum weight-loss and maximum weight-loss for patients with the MC4R reference allele who are non-diabetic, T2D or T2D taking insulin were calculated by plotting the mean body mass index (BMI) and fitted with Hill plot curve as described [8]. Subjects with rare variants were matched with non-carrier patients by Body Mass Index (BMI) (defined as kg/m²) (±1), age- (±5 years), gender-, T2D status-, and whether they were taking insulin. Carrier and non-carrier patients’ BMIs are plotted during the period 12 months prior to- and up to 84- months after RYGB. Three patients (one with L250Q, one with R305Q and one with V253I variant) could not be matched within these parameters, so the BMI range was extended to ±2–3 and/or the age range was extended to ±8 years.

MC4R constructs

Individual mutations were made with the Quickchange site-directed mutagenesis kit (Stratagene, Santa Clara, CA, USA) in a 3x HA tagged MC4R in pcDNA3. The 3x HAMC4R was subcloned into pEF6V5:eGFP-CAAX-2A-mCherry (Addgene plasmid 26901) to create the HAMC4R2aGFP construct. A bungorotoxin binding site (MVRYYESSLPLEYPD) [22] was added to the N terminus of MC4R by PCR amplification and subcloned into pcDNA3.1. All constructs were confirmed by sequencing of the full length clone.

Cell Culture

HEK293 cells (ATCC, Manassas, VA, USA) were cultured in MEM with 10% FBS at 37°C and 5% CO2. For transient transfections, cells were transfected with plasmids described above by Fugene (Roche, Indianapolis, IN, USA) and used two days post-transfection.

Cell Surface Receptor Imaging

Bungorotoxin Binding Site (BBS) tagged MC4R constructs (BBS-MC4R) were transfected into HEK 293 cells on poly-L-lysine coated glass bottom Fluorodishes (WPI, Sarasota, FL, USA). Cells were rinsed twice with Imaging Low K containing (in mmol/L): 25 HEPES, 114 NaCl, 2.2 KCl, 2 CaCl2, 2 MCl2, 22 NaHCO3, 1.1 NaH2PO4, 2 glucose, pH 7.4, and labeled with 10 μg/mL Bungorotoxin (BTX) conjugated to Texas Red for 10–15 min at 4°C and rinsed again in Low K solution. Live cells were imaged on an inverted Olympus Spinning Disc confocal microscope with IPLab (Beckton-Dickinson, San Jose, CA USA) image acquisition software and processed with ImageJ.

ELISA

Approximately 10,000 HA tagged construct transfected cells were added to wells of a polystyrene coated 96 well plate. The following day, cells were washed with PBS and fixed with either methanol (for total expression) or 4% paraformaldehyde (for surface expression). Cells were then blocked with 1% milk and incubated in peroxidase conjugated anti-HA antibody. The plate was washed with TBS-T three times and then incubated with 100 μL 3,3′,5,5′-Tetramethylbenzidine Liquid Substrate (Sigma, St. Louis, MO, USA) for 30 minutes. 100 μL of 1 mol/L sulfuric acid was added to each well to stop the reaction. Absorbance was then read at 450 nm on a Spectramax 250 plate reader. The absorbance from untransfected cells was first subtracted and then the cell surface labeled signal was plotted as a percentage of total signal (calculated as the non-permeablized signal divided by the permeablized signal ×100) and then normalized to wild-type HAMC4R expression for that experiment. Significant differences from wild-type were determined using one way ANOVA with Dunnet’s post-hoc.

HAMC4R2aGFP Western

HEK293 cells were transiently transfected with HAMC4R2aGFP or mutant HAMC4R2aGFP, where HA-MC4R and GFP are separated by the 18 amino acid 2a peptide sequence from the foot and mouth disease virus [23,24]. HAMC4R2aGFP is transcribed and translated as one gene product. After translation, 2a self-cleaves and separates HA-MC4R and GFP into two proteins [23,24]. Cells expressing HAMC4R2aGFP or mutants were lysed two days post-transfection in lysis buffer (25 mmol/L HEPES, 5 mmol/L MgCl2, 5 mmol/L EDTA and 1% Triton) with protease (Omplete mini) and phosphatase (PhosSTOP) inhibitors (both from Roche). Protein levels were determined by a...
BCA assay (Pierce). The lysates were loaded for equal amounts of GFP protein. Lysates were loaded on a 12% Bis/Tris gel and transferred to a nitrocellulose membrane. After blocking with milk and probing with anti-HA (Roche) or anti-GFP (NeuroMAb, Davis, CA, USA) the membrane was developed using SuperSignal West Pico Chemiluminescent substrate using the Fujifilm LAS-4000 (Pierce, Rockford, IL, USA). Intensities of the HA and GFP bands were quantitated and HA intensity was normalized to the intensity of the corresponding GFP band and normalized to the wild-type ratio for that day (n $\geq 3$).

**cAMP Microscopy Assay**

Cells were transfected with BBS-MC4R constructs and Exchange Protein directly Activated by cAMP (EPAC2)-camps sensor [25], a gift from Drs. Nikolaev and Lohse. Cells were labeled with BTX-Texas Red to identify receptor expressing cells. Images were collected for CFP and Fluorescence Resonance Energy Transfer (FRET) between CFP and YFP. The FRET signal was normalized to the CFP signal for each cell. Baseline cAMP levels were quantitated and then cells were exposed to 100 nmol/L of an MC4R synthetic agonist melanotan II (MTII) and then 100 μmol/L forskolin (for maximum cAMP response).

**Results**

We sequenced the MC4R gene of 1433 patients who underwent primary RYGB. A total of eighty patients had fifteen distinct MC4R variants. Two of these variants, V103I and I251L, are common and occur in 1% of both lean and obese populations [26]. The frequencies of V103I and I251L carriers in the RYGB cohort and 451 lean subjects were similar [8]. The other thirteen variants S4F (11C$\rightarrow$T), G34A (101G$\rightarrow$C), H76R (227A$\rightarrow$G), V95I (283G$\rightarrow$A), T112M (335C$\rightarrow$T), I137T (410T$\rightarrow$C), F202L (606C$\rightarrow$A), L207V (619C$\rightarrow$G), L250Q (749T$\rightarrow$A), V253I (757G$\rightarrow$A), S295P (883T$\rightarrow$C), R305Q (914G$\rightarrow$A), C326R (976T$\rightarrow$C) are considered rare, and occurred in just eighteen patients. Twelve of these variants have been reported previously [11,13,14,18,21,27–35]. Of the previously reported variants, S4F, H76R, V95I, I137T, L250Q, and C326R [13,14,18,23–35] have been reported in both obese and lean populations. The G34A variant has not been previously reported. Notably, no rare variants in MC4R were found in our lean cohort.

The MC4R variant of each patient and their corresponding body mass index (BMI), T2D status pre- and post-surgery, hemoglobin A1c (HbA1c) (glycated hemoglobin, 3 month blood glucose value), HOMA$_{IR}$ (homeostatic model assessment for
insulin resistance) values, and pre-surgery blood pressure are detailed in Table 1. The pre-surgery BMIs of patients with rare variants were not different from patients with the MC4R reference allele [15], due to the RYGB selection criteria and the multiple genetic and environmental factors that can contribute to obesity. Of the 18 rare variant carriers, 8 were assessed for binge eating and none were diagnosed. Resting energy expenditure (REE) data was collected for 55% of all RYGB patients, among these the REE of rare variant carriers and non-carriers were not significantly different (data not shown). Following RYGB, most patients lose weight rapidly, reach a maximum weight-loss nadir at ~10 months and regain some weight in the following months [8]. All patients with MC4R variants lost weight following RYGB (Table 1). Patients who achieved maximum weight-loss quickly, lost less weight long-term (Table 1; V95I, I137T, R305Q). To assess whether rare alleles of MC4R are associated with weight-loss outcomes following RYGB, we matched each patient carrying a rare MC4R variant with patients with the reference sequence for gender, age, starting BMI, and T2D status (non-diabetic, T2D, T2D-taking insulin) (Figures 1 and 2). The post-RYGB weight-loss of patients carrying rare MC4R variants reported in obese and lean cohorts (T112M, F202L, L207V, V231I, S295P and R305Q) were indistinguishable from matched controls (Figure 1). Similarly, the weight-loss following RYGB of patients with MC4R variants G34A, H76R and C326R, found in obese only populations, were similar to matched controls (Figure 2). Insufficient data were available to assess the S4F mutant. Patients carrying the V95I, I137T and L250Q variants did not achieve the same weight-loss compared to control patients (Figure 2). Interestingly, these variants have only been reported in obese individuals [13,14,31]. We also assessed overall weight-loss by patients using either loss of initial BMI (%BMI) or percent excess body weight loss (%EBWL), where excess body weight is defined as weight above BMI of 25. Using either one of these criteria, patients carrying V95I, I137T and L250Q did not achieve the same weight loss as others. However, the overall odds of achieving the same RYGB results, %BMI loss or %EBWL, as defined above, were not different for patients carrying rare variants or non-carriers.

To better understand the potential molecular mechanisms associated with the MC4R mutations, we measured expression and canonical signaling in vitro. For each of the fifteen MC4R variants in the RYGB cohort, we analyzed the cell surface localization by ELISA and imaging of cell surface labeled receptors. Approximately 40% of total MC4R was located at cell surface and all mutants showed similar percent cell surface expression. Only the R305Q mutation displayed a small but significant decrease in cell surface expression compared to the wild-type receptor (Figure 3A). We examined the cell surface expression of these variants by imaging a bungarotoxin binding sequence (BBS) tagged-MC4R. Labeling of BBS-MC4R and mutants with membrane impermeant bungarotoxin-Texas Red [22] showed that all mutant receptors were effectively expressed on the cell surface (Figure 3B). Labeled receptors were present in the cytoplasm of cells expressing the mutants or wild-type receptor, indicating some receptor internalization.

In order to determine if there were differences in total expression of the MC4R protein in vitro, we assayed expression by western blot analysis. We employed an HA-MC4R2aGFP construct where HA-MC4R2aGFP is transcribed and translated as one gene product, but the 2a peptide self-cleaves co-translationally.
Table 1. Clinical variables for Roux-en Y gastric bypass patients with rare MC4R variants.

| DNA change | Protein change | Gender (F/M) | Pre-surgery BMI (mean ± SEM) | Time to lowest BMI (months) | Lowest Recorded BMI (mean ± SEM) | Last Recorded BMI (mean ± SEM) | Pre-surgery HbA1c (%) | Pre-surgery HOMA IR (Arbitrary Units) | Pre-surgery Blood Pressure SBP/DBP (mmHg) | Pre-surgery Type 2 Diabetes Status | Type 2 Diabetes Remission @ 6 Months Post Surgery (remitted/total) |
|------------|----------------|---------------|-----------------------------|----------------------------|---------------------------------|---------------------------------|----------------------|----------------------------------|------------------------------------------|----------------------------------------|------------------------------------------|
| 11C>T      | MC4R 599/125   | F 59.5       | 32.9±0.1 | 1.3 No data No data 15.4 19.5 143 6 11/78 | Yes No Yes Yes | 101G>A T S4F F 42.9 | 5.2 21 25 23/18 5.6 13.1 20.6 135 6 10/79 | 1.7 No | 141/17/78 12 12 | 73/3 175 | 73/3 175 |
| 101G>A T S4F | F 42.9 | 5.2 21 25 23/18 5.6 13.1 20.6 135 6 10/79 | 1.7 No | 141/17/78 12 12 | 73/3 175 | 73/3 175 |

Type 2 Diabetes patients are separated into non-diabetics, type 2 diabetics (T2D) and T2Ds taking insulin. Pre-surgery BMIs and blood pressure are reported as three month averages. No data were collected after surgery for the S4F (denoted "F"). *The total number of patients differ in the six month remission data because those patients that did not have enough follow-up data collected were excluded from analysis (T2D-25 patients, T2D insulin dependent-9 patients).

Non-carrier patients are separated into non-diabetics, type 2 diabetics (T2D) and T2Ds taking insulin. Pre-surgery BMIs and blood pressure are reported as three month averages. No data were collected after surgery for the S4F (denoted "F"). *The total number of patients differ in the six month remission data because those patients that did not have enough follow-up data collected were excluded from analysis (T2D-25 patients, T2D insulin dependent-9 patients).
creating equal numbers of two distinct proteins HA-MC4R and GFP. To account for different transfection efficiencies, we loaded equal amounts of GFP protein and assayed for expression of HA-MC4R. Any differences in MC4R expression must be due to changes in protein lifespan of the mutants, since GFP was initially transcribed and translated at a 1:1 ratio with MC4R. We found that the expression of the mutant receptors was not different from the wild-type receptor (Figure 3C).

We next assayed the ability of the MC4R mutants to signal to the cAMP pathway using a FRET based sensor (Epac2-camps) [25]. We expressed BBS-MC4R and mutants, selected cells expressing similar levels of receptor based on the bungarotoxin-Texas Red labeling and determined the cAMP response to the MC4R agonist melanotan II (MTII), normalized to the maximal response induced by forskolin in the same cell (Figure 4). We included the D90N mutation of MC4R as a negative control, because a previous report showed that this mutant had normal cell surface localization and agonist binding but reduced cAMP signaling [19]. The I137T and C326R mutants had a small but significant impairment of cAMP signaling compared to the wild-type receptor (p<0.01), whereas all other mutants responded.

Figure 3. Expression of MC4R mutants. A) ELISA of HEK293 cells expressing wild-type MC4R or mutations. The cell surface expression was normalized to total expression for each mutant and then to wild-type receptor for that batch (* denotes p<0.05 compared to wild-type by a one way ANOVA with Dunnet’s post-hoc test). □ MC4R mutant D90N was not found in our cohort. B) Cell surface localization of HEK-293 cells expressing mutant or wild-type (BBS-MC4R) constructs labeled with Bungarotoxin-Texas Red. C) Normalized GFP loading reveals no differences in HA-MC4R mutant lifespan. HA-MC4R expression was normalized to GFP expression and plotted as a percentage of wild-type for each blot (n=3).

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Figure 4. cAMP Assay of MC4R mutants. cAMP production of MC4R mutants after stimulation with 10 nmol/L melanotan II (MTII) and then 100 μmol/L forskolin to activate maximum receptor-independent cAMP response. MTII stimulation was normalized to baseline cAMP production and plotted as a percentage of forskolin in the same cell. Mutants similar to wild-type (Black □) are designated with the symbol (Gray □). MC4R novel mutant G34A (Red ○) and those that have different statistically altered cAMP signaling (p<0.01 compared to wild-type by a one way ANOVA with Dunnet’s post hoc test) I137T (Green □), D90N (Blue ○) and C326R (Purple ○) are highlighted with different colored symbols. The D90N variant was not found in this cohort, but included as a control.

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equally to the MTII stimulus (Figure 4).

The G34A variant has not been reported previously. We measured the MC4R activated cAMP production using an EIA on cells stably expressing wild-type MC4R and G34A and found no difference in α-MSH EC50 for cAMP production (Figure S1). The α-MSH induced cAMP production for the remaining mutants have been reported and our data is consistent with the literature for both cell surface and cAMP signaling for the previously reported mutants [13,27,28,31,33,36,37]. The cAMP EIA corroborates the FRET data and validates the imaging assay as a useful single cell measure of cAMP with the ability to determine the maximum cAMP level in the same cells and to select cells expressing similar levels of receptor.

Discussion

We and others have reported that, in general, carrying an MC4R variant is not deterministic for poor outcome after RYGB [15,38–40]. However, in all of those studies the relationship between the cellular signaling for a given mutant and the post-RYGB outcome in the subjects carrying the corresponding variants were not examined. Examining the effects of MC4R rare variants in weight-loss after surgery by matching subjects for other variables minimizes confounding factors that cannot be eliminated or addressed in population studies of obesity. We matched MC4R variant carriers to non-carriers for the most critical common predictors of weight-loss: gender, age, starting BMI, and diabetic status [41] to ask whether carrying an MC4R variant that does or does not affect expression or function can be predictive of post-RYGB phenotype. We find that only 3 patients, carrying V95L, I137T and L250Q, failed to lose comparable weight to matched patients with reference alleles. While these variants have only been reported in obese populations, we did not find a consistent and clear defect in their expression, trafficking and signaling, except for ~25% reduction in maximal cAMP signaling by I137T. Surprisingly, variants that displayed alterations in expression (R305Q) or signaling (C326R) did not have any phenotypic consequences after RYGB. We conclude that carrying a rare variant of MC4R, while associated with obesity, does not affect weight-loss after gastric bypass surgery. This is consistent with reports of weight-loss in rodents after RYGB where only complete loss of MC4R function had a significant effect on weight-loss [15,39].

These findings compel us to propose a dual role of MC4R in obesity and clinical outcomes after gastric bypass.

Post-RYGB outcomes

Post-RYGB weight-loss provides a short-term measurement in a more controlled setting, due to the physical influence of the procedure and the clinical follow-up conducted post-surgery. Our data show that modest deleterious effects of MC4R variants may not play a significant role in weight-loss following RYGB, similar to the effect seen in heterozygous Mc4r mice [15]. In contrast, Mc4r null mice fail to lose weight after RYGB [15]. Together the mouse and human data show that one functional copy of MC4R is necessary and sufficient to lose weight after RYGB. Nevertheless, a significant proportion of our patients do not respond to RYGB. Our findings suggest that the response of those patients to weight-loss is independent of their MC4R activity and may arise from other genetic and non-genetic factors. However, carriers of the I251L allele, which in vitro has increased basal activity [36], more effectively lose weight and resolve their T2D [8,15]. Additionally, selective receptor re-expression in neurons of Mc4r knock-out mice normalized weight-loss and insulin resistance in mice after gastric bypass surgery [15].

Obesity

A wealth of data exist that show association between carrying rare, presumably deleterious, MC4R variants and obesity. Our data corroborate these findings since we only found rare variant carriers in the obese population [8]. We speculate that even modestly altered MC4R activity can influence obesity due to a long-term effect that can be exacerbated by environmental factors such as food choice and/or variants in other obesity related genes. These effects are observed in rodents where heterozygous Mc4r mice develop obesity on high fat diet or even regular chow; however, on reduced fat diet these mice do not gain significant weight compared to their wild-type littermates [42]. Reduced food intake in MC4R rare variant carriers can result in significant weight-loss, but an increased effort is needed to stay on a weight-loss regimen [43]. While we do not have feeding behavior data for our patients, all patients are placed on a calorie restricted diet for 6–12 months in order to achieve some weight loss prior to RYGB.

In our cohort during the 6 month pre-surgery calorie restriction period, carriers of any rare MC4R allele lost 6.6±0.9% of their initial weight, while non-carriers lost 6.1±0.2% of their initial weight. Therefore, allele carriers can lose similar weight during calorie restriction suggesting again that in the absence of environmental factors MC4R variants do not affect the ability to lose weight. While MC4R rare variants are highly associated with obesity, external factors contribute significantly to the obese phenotype. At the cellular level no consistent change in MC4R signaling can account for the association of rare variants with obesity. For example, only two of the rare mutations, I137T and C326R, had defects in cAMP signaling, while the other mutations signaled as well as wild-type MC4R. The defects of the I137T and C326R would only account for ~25% reduction in function of one copy of MC4R. This small defect may contribute to the obesity of those carrying these variants; however, only the I137T carrier failed to lose similar weight compared to a matched subject post-RYGB. While there may be a common unifying mechanism for all the obesity associated MC4R variants, none has been reported to date.

Together these data highlight the complex nature of MC4R signaling and how it affects not only obesity and metabolism, but also weight-loss. Altered MC4R function does not rule out the effect of other factors that may impact both obesity and RYGB outcomes. We suggest that increasing MC4R function will have a positive outcome on weight-loss after caloric restriction or RYGB, as well as improved T2D remission after RYGB. While several MC4R agonists have failed in clinical trials, mainly due to unrelated side effects, they do hold significant promise for weight-loss, which may depend on concomitant modification in feeding behavior.

Supporting Information

Figure S1 cAMP dose-response of MC4R and variant G34A. cAMP dose-response in HEK cells stably expressing HA-MC4R (● solid black lines) and the mutant G34A (□ dashed line) from three independent immunoassays. The α-MSH EC50 value for WT-MC4R is 41.1 nM and for G34A is 43.0 nM which were not statistically different (ANOVA with Dunnet’s post-hoc test). (TIF)

Methods S1 Supplemental Methods. (DOCX)
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Author Contributions
Conceived and designed the experiments: TM. Performed the experiments: BSM ULMAE ANS MDB AMK KKJ TM. Analyzed the data: BSM ULMAE TM. Contributed reagents/materials/analysis tools: CDS GEB GSG DJC. Wrote the manuscript: BSM TM.

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