

Weight Loss after Roux-en-Y Gastric Bypass in Obese Patients Heterozygous for MC4R Mutations

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Abstract

Background Heterozygous mutations in melanocortin-4 receptor (MC4R) are the most frequent genetic cause of obesity. Bariatric surgery is a successful treatment for severe obesity. The mechanisms of weight loss after bariatric surgery are not well understood.

Methods Ninety-two patients who had Roux-en-Y gastric bypass (RYGB) surgery were screened for MC4R mutations. We compared percent excess weight loss (%EWL) in the four MC4R mutation carriers with that of two control groups: 8 matched controls and with the remaining 80 patients who underwent RYGB.

Results Four patients were heterozygous for functionally significant MC4R mutations. In patients with MC4R mutations, the %EWL after RYGB (66% EWL) was not significantly different compared to matched controls (70% EWL) and non-matched controls (60% EWL) after 1 year of follow-up.

Conclusions This study suggests that patients with heterozygous MC4R mutations also benefit from RYGB and that weight loss may be independent of the presence of such mutations.

Keywords Melanocortin-4 receptor · Roux-en-Y gastric bypass · Obesity

Introduction

Lifestyle modification, such as diet and exercise, is not effective in maintaining long-term weight loss in patients with class III (BMI > 40 kg/m²) obesity. Bariatric surgery is currently the most effective therapy for these patients. Outcomes after different types of bariatric surgery are variable and the mechanisms of resultant weight loss are poorly understood [1]. It has been suggested that the outcome of these procedures could be influenced by genetic variations implicated in the predisposition to obesity [2].

Genetic factors account for 40% to 70% of an individual's predisposition to obesity [3]. Several known obesity-associated mutations involve genes of the hypothalamic leptin–melanocortin system. These include mutations in leptin, the leptin receptor, proopiomelanocortin, prohormone convertase 1/3, and melanocortin-4 receptor (MC4R) [3–5]. Specifically, heterozygous MC4R mutations are the most common genetic form of obesity, and mutations in this gene account for approximately 2.5% of severely obese individuals [6, 7].

MC4R is expressed at low levels and is found in hypothalamic nuclei involved in the regulation of food intake and in particular neurons of the paraventricular nucleus [8]. The physiological role of MC4R in food intake and body weight regulation is best demonstrated by the

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phenotype of the MC4R knockout mice [9]. Mice lacking both alleles of MC4R (MC4R^{-/-} mice) develop a maturity-onset hyperphagic obesity syndrome by 10 weeks of age. Mice heterozygous for an MC4R deletion (MC4R^{+/-} mice) show an average weight that is intermediate between that of wild-type and MC4R^{-/-} mice. This suggests that haploinsufficiency at the MC4R locus is sufficient to cause an alteration in the regulation of body weight in rodents. MC4R regulates food intake and maintains long-term energy homeostasis by integrating signals provided by its agonist α -melanocyte stimulating hormone and antagonist agouti-related peptide from leptin-sensitive neurons in the arcuate nucleus [10]. An important argument for showing that non-synonymous mutations are responsible for a disease is the demonstration of the functional relevance of these mutations (i.e., the demonstration that the mutated product behaves differently in a functional assay). Functional studies of MC4R mutations associated with obesity indicate that multiple functional alterations contribute to their pathogenicity and that more severely impaired receptor function in vitro correlates with earlier age of obesity onset and higher BMI [6].

Lifestyle interventions based on exercise, behavior, and nutrition therapy in children with MC4R mutations are not effective in providing long-term weight loss in contrast to children without MC4R mutations [11]. In addition, a controversial report suggested that carriers of non-obesity-associated genetic variations at the MC4R locus might have poorer weight loss outcomes after gastric banding [2, 12].

Here, we compare the weight loss outcome after Roux-Y gastric bypass (RYGB) in patients who are heterozygous carriers of rare functionally relevant obesity-associated MC4R mutations with that of control patients who do not carry such mutations.

Patients and Methods

Patients

Ninety-two patients who were considered appropriate candidates for RYGB were screened for MC4R mutations. Criteria used at the University of California San Francisco Bariatric Surgery Program for the selection of patients for a RYGB has been described in detail previously [13]. Briefly, all patients met the 1991 NIH Consensus Development Conference guidelines, including: BMI (weight in kilograms divided by height in meters squared) >40 or BMI between 35 and 40 with obesity-related comorbidities for at least 5 years and a documented attempt of medically supervised therapy for weight loss for at least 6 months

[14]. All patients also underwent preoperative psychological, nutritional, and medical evaluations. Active binge eating disorder is considered a contraindication for bariatric surgery at the UCSF Bariatric Program. Procedure selection was determined by patient preference and occurred after participation in a 1-h educational and screening session with a dietician and a bariatric surgeon and after medical and psychological clearances.

Surgical Technique for RYGB

The technique for RYGB has been described previously [15]. In brief, it was performed laparoscopically using staplers (US Surgical Corporation, Norwalk, CT) to create a 15- to 30-mL gastric pouch, the antecolic gastrojejunostomy, the 100-cm Roux limb, and the side-to-side jejunojunctionostomy.

Postoperative Care

Patients were given clear liquids on the first postoperative day and then advanced to a full liquid diet on the second postoperative day. Patients were seen in clinic 2 weeks postoperatively, then every 3 months for the first year. After the operation, patients received prenatal vitamins, calcium supplements, vitamin D, and acid suppression medication. Nutritional supplements were prescribed as needed for documented deficiencies. All weight measurements were obtained at the UCSF Bariatric Surgery Center. Calculation of excess weight loss was based on Metropolitan Life Insurance height and weight tables to determine ideal body weight (IBW) [16]. We defined excess weight as the weight above the patient's IBW. We calculated the total weight loss (kg) on each follow-up time and the percent excess weight loss (%EWL) after surgery, which was weight loss divided by excess weight multiplied by 100 on each follow-up point.

Control Group Matching

Controls were matched for gender and the presence of diabetes. In addition, controls were matched within one standard deviation for baseline BMI before surgery and for age. The UCSF Committee on Human Research approved the protocols, and informed written consent was obtained from all patients.

Gene Analysis

Genomic DNA was extracted from white blood cells using standard methods. Our group previously described DNA sequencing and in vitro analysis of blood samples from patients with MC4R mutations [7].

Statistical Analyses and Power Calculation

The comparison of %EWL at each follow-up time point (1, 3, 6, 9, and 12 months) between cases ($n=4$) and matched controls ($n=8$) was performed using a paired, two-tailed t test. Additionally, the comparison between the four cases and the 88 controls (8 matched controls and 80 non-matched controls) was performed using linear regression while adjusting for age, gender, self-reported race, and baseline BMI.

Results

Identification of MC4R Mutations

Ninety-two patients were screened for MC4R mutations. The mean \pm SD of BMI and age prior to surgery was 55 ± 8.5 kg/m² and 45 ± 10.4 years, respectively.

Four patients carried heterozygous MC4R mutations. One patient carried the Cys271Phe (812G>T) mutation, one patient carried the nonsense mutation Gln307stop (919C>T), and two patients carried the Arg236Cys (706C>T) mutation. All these mutations cause functional alteration in vitro [7].

Weight Loss After RYGB

Subjects who were heterozygous for known MC4R non-synonymous mutations, defined as cases, were matched to

subjects who lacked any non-synonymous MC4R mutations, defined as controls, based on age, sex, BMI, and type 2 diabetes mellitus (see “Patients and Methods”). Four cases were matched to eight controls (Table 1). We compared %EWL between MC4R mutation carriers and the entire cohort of 88 patients without MC4R mutations (4 matched controls and 80 non-matched controls) with a starting BMI > 40 kg/m² at all time points (1, 3, 6, 9, and 12 months). There was no statistically significant difference in %EWL between cases and controls at any time point (Fig. 1).

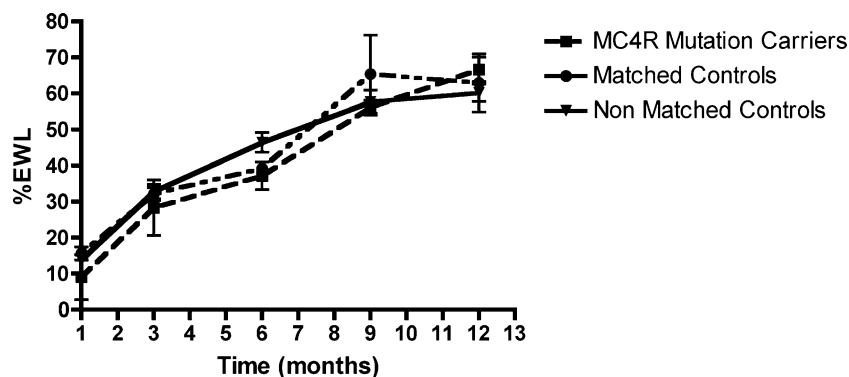
Linear regression controlling for age, sex, self-reported race, and baseline BMI was used to compare %EWL between cases and all 88 controls patients (8 matched controls and 80 non-matched controls) without MC4R mutations in the cohort at the 9- and 12-month follow-up time points. After controlling for all the above variables, MC4R mutation carriers did not have a significantly different %EWL at 9 or 12 months. At 9 months of follow-up, the mutation carriers had less %EWL compared to controls; however, at 12 months, the mutation carriers had more %EWL compared to controls. Thus, neither the rate nor the absolute amount of weight loss was related to the presence of MC4R variants. In fact, the only predictor that was significant was baseline BMI. For every 10 points greater in BMI, patients lost 10% less EWL at a follow-up time of 9 months (effect size \pm SE, 1 ± 0.38 , $p=0.006$) and 7% less EWL at a follow-up time of 12 months (effect size \pm SE, -0.7 ± 0.3 , $p=0.026$). The combination of analysis of matched pairs and linear

Table 1 Clinical data from patients heterozygous for MC4R mutations and eight matched non-carrier for MC4R mutations controls

Pair	MC4R mutation	Gender	Age (years)	BMI (kg/m ²)	Dyslipidemia	DM	HTN	Perioperative complications
Pair 1								
Carrier	Cys271Phe	Female	55	53.8	–	+	+	None
Control		Female	65	55.7	+	+	+	None
Control		Female	55	51.2	+	+	–	None
Control		Female	55	68.8	–	+	+	None
Pair 2								
Carrier	Glu307Stop	Male	43	54.3	+	–	–	None
Control		Male	42	57.9	–	–	–	None
Control		Male	49	47.5	–	–	+	None
Pair 3								
Carrier	Arg236Cys	Female	47	63.9	–	–	–	None
Control		Female	55	57.3	–	–	–	None
Control		Female	49	65.7	–	–	+	None
Pair 4								
Carrier	Arg236Cys	Male	37	43.5	–	–	–	Pneumonia
Control		Male	36	40.5	–	–	+	None

DM diabetes mellitus, HTN hypertension, + presence of comorbidity, – absence of comorbidity

Fig. 1 %EWL curves of MC4R mutation carriers ($n=4$), matched controls for gender, age, race, BMI, and presence of diabetes ($n=8$) and non-matched controls ($n=80$) from 1 to 12 months post-RYGB



regression analysis provides better statistical analyses with the available cohort.

Perioperative (30 Days) Complications

Among the MC4R mutation carriers, one patient had aspiration pneumonia and was admitted to the ICU. He was discharged home on postoperative day 8. Out of eight matched control patients, none had perioperative complications.

Discussion

Bariatric surgery is an effective weight loss tool for selected severely obese patients for whom conventional medical and behavioral therapy for obesity has failed. With rising obesity rates, bariatric surgery has become a more common tool to achieve weight control and resolution of comorbidities [17]. Heterozygous MC4R mutations are the most common genetic form of obesity, and mutations in this gene account for approximately 2.5% of severely obese individuals [6, 7]. Our study is the first one to document weight loss after RYGB in patients with heterozygous MC4R mutations, and the results, although preliminary, suggest that patients with these mutations were able to lose as much weight as patients without MC4R mutations after RYGB. It is well established that obese patients can achieve a %EWL of approximately 60% after RYGB [18], and previous studies suggests that the %EWL after RYGB is superior than that obtained with gastric banding [19].

A previous report suggested that carriers of genetic variations at the MC4R locus have poorer outcomes after gastric banding [2]. This study, however, grouped many disparate mutations and polymorphisms together. A large majority of patients in the study carried MC4R variants that were not associated with obesity (i.e., found also in non-obese controls) and with no demonstrated functional effect on the MC4R protein. In addition, patients with binge

eating disorders were included, which has been implicated in poorer outcomes after bariatric surgery, making the independent effect of MC4R gene variants in the previous study unclear [12].

Other studies have demonstrated resolution of or marked improvement in type 2 diabetes mellitus (DM) after bariatric surgery [20]. Only one out of the four patients with MC4R mutations had type 2 DM prior to RYGB, which did not resolve after surgery. Due to the small sample size of MC4R mutation carriers, we could not draw conclusions about the benefits of bariatric surgery for the resolution of diabetes in patients with heterozygous MC4R mutations. In a previous cohort of 769 obese adult patients, there was no significant difference in the prevalence of type 2 DM between carriers and non-carriers of MC4R patients. Nineteen patients were found to be MC4R carriers, and the prevalence of type 2 DM was 20% among these patients, similar to our patients with MC4R mutations [6].

The limitations of our study are mostly due to the rarity of patients with MC4R mutations and the lack of power to draw definitive conclusions. Further research to understand the pathogenic mechanisms underlying obesity caused by MC4R mutations is required for the development of rational and effective treatments. This research is challenging because in addition to the relative small number of patients with MC4R mutations, there is great genetic heterogeneity of the disorder. However, our study is the first one to document weight loss after RYGB in patients with heterozygous MC4R mutations and provide preliminary information of outcomes after RYGB.

In conclusion, and in view of the limitations described above, our study shows that patients with heterozygous MC4R mutations had a similar rate of weight loss and % EWL after RYGB as patients without MC4R mutations. This suggests that heterozygous MC4R mutation status should not influence the decision to perform RYGB as a treatment for severe obesity.

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