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Melanocortin-4 Receptor Mutations and Polymorphisms Do Not Affect Weight Loss after Bariatric Surgery

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Abstract

Bariatric surgery is the most effective long term weight-loss therapy for severe and morbidly obese patients. Melanocortin-4 Receptor (MC4R) mutations, the most frequent known cause of monogenic obesity, affect the regulation of energy homeostasis. The impact of such mutations on weight loss after bariatric surgery is still debated. The objective is to determine the impact of MC4R status on weight loss in obese subjects over one year after bariatric surgery. A total of 648 patients, who were referred to bariatric surgery in a single clinical nutrition department, were genotyped for their MC4R status. The following four groups were categorized: functional MC4R mutations, MC4R single nucleotide polymorphisms (SNPs): Val103Ile (V103L) and Ile251Leu (I251L), MC4R variant rs17782313 (downstream of MC4R) and MC4R SNP A-178C on the promoter. Each patient was matched with two randomly paired controls without mutation. Matching factors were age, sex, baseline weight and type of surgery procedure (Roux-en-Y gastric bypass and adjustable gastric banding). We compared weight loss between cases and controls at 3, 6 and 12 months after surgery. Among 648 patients, we identified 9 carriers of functional MC4R mutations, 10 carriers of MC4R V103L and I251L SNPs, 7 carriers of the rs17782313 variant and 22 carriers of the A-178C SNP. Weight loss at 3, 6 and 12 months did not differ between cases and controls, whatever the MC4R mutations. This is the first case-control study to show that MC4R mutations and polymorphisms do not affect weight loss and body composition over one year after bariatric surgery.

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Introduction

Obesity is a rapidly growing global public health challenge [1]. It is directly related to an increased risk for type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease and overall mortality [2]. Bariatric surgery is the most effective therapy for long-term weight lost in severe or morbidly obese subjects [3,4]. Its use has dramatically increased during the last decade [1]. However, the individual weight loss response varies widely. Clinical and genetic factors may influence its effect. Genetic background accounts for 40% to 70% of an individual predisposition to obesity [5] with strong determinants of weight loss following bariatric surgery [6].

Melanocortin-4 receptor (MC4R) mutations are the most frequent monogenic causes of severe early onset human obesity (up to 6%) [7]. MC4R expressed in the hypothalamic nuclei is involved in the leptin-melanocortin signaling pathways. The central melanocortin system influences energy intake and expenditure and the balance between lipid utilization and storage. MC4R affects leptin-melanocortin signaling system and regulates

food intake and maintain long-term energy homeostasis by integrating signals provided by its agonist, α -melanocyte stimulating hormone, and antagonist, Agouti related peptide [8]. By this way, MC4R mutations may play a role in weight loss, especially after bariatric surgery.

Several MC4R mutations affect differently this system. Partial or total loss of MC4R function, as well as the variant rs17782313 mapped 188 kb downstream of MC4R, are positively associated with obesity [9–11]. In contrast, two SNPs of the MC4R gene: I251L and V103L have been shown to be negatively correlated with obesity [12,13]. Promoter mutations could be associated with obesity [14], although the effect of the polymorphism A-178C was not specifically investigated.

We tested whether these deoxyribose nucleic acid (DNA) sequence variations in and downstream of the MC4R gene affects weight loss and body composition after the two most frequent bariatric procedures: Roux-en-Y gastric bypass (RYGBP) and adjustable gastric banding (AGB) [15].

Table 1. Clinical characteristics according to MC4R genotypes (functional *MC4R* mutations, MC4R polymorphisms and the variant rs17782313 downstream *MC4R*).

	Functional MC4R mutations			Variant rs17782313			MC4R polymorphisms: Val130Ile, Ile251Leu			MC4R polymorphism: A-178C		
	Carriers	Non carriers		Carriers	Non carriers		Carriers	Non carriers		Carriers	Non carriers	
Number, n	9	18	7	14	10	20	22	44				
Age, years	36.2 (13.3)	34.2 (8.4)	45.4 (9.2)	46.1 (9.9)	39.2 (12.2)	42.4 (9.1)	46.1 (7.9)*	43.3 (9.1)*				
% Female	100	100	71.4	71.4	80	80	86.4	86.4				
Surgery Type	3	6	0	0	2	4	0	0				
	6	12	7	14	8	16	22	44				
Weight, kg	139.2 (14.3)	139.8 (12.1)	142.0 (23.9)	133.4 (15.5)	121.6 (18.2)	125.1 (16.0)	126.4 (16.4)	124.3 (13.9)				
BMI, kg/m ²	50.4 (4.6)	50.8 (4.9)	49.0 (7.6)	48.8 (6.3)	46.0 (6.1)	46.0 (5.6)	46.1 (6.4)	46.1 (5.3)				
Fat mass, %	47.9 (4.1)	49.7 (4.9)	45.3 (4.3)	46.3 (5.5)	45.5 (4.3)	45.1 (7.6)	45.3 (4.9)	45.6 (6.2)				
Glycemia, mmol/L	5.1 (1.0)	6.0 (2.2)	7.2 (2.7)	6.3 (1.7)	5.5 (1.3)	6.1 (2.9)	6.1 (1.7)	6.4 (2.8)				
Cholesterol, mmol/L	5.0 (1.3)	4.8 (0.8)	4.5 (0.8)	4.5 (0.8)	5.2 (1.0)	5.0 (0.9)	4.9 (0.8)	5.1 (1.3)				
Percentage of Weight, %	84.9 (6.5)	84.1 (6.8)	83.1 (7.6)	81.3 (4.3)	84.8 (4.6)	83.3 (6.8)	83.0 (3.7)	82.4 (4.3)				
	80.0 (10)	78.8 (9.2)	76.1 (10.6)	74.4 (5.6)	78.9 (7.2)	77.3 (8.3)	76.5 (6.2)	74.5 (5.9)				
	74.1 (13.4)	72.5 (10.4)	70.9 (13.3)	68.0 (7.1)	74.3 (9.5)	72.4 (10.5)	70.2 (7.9)	68.7 (6.8)				

Data are mean (SD) or % when specified.

Difference between carriers and non-carriers were tested with Wilcoxon paired rank test.

*p value<0.05.

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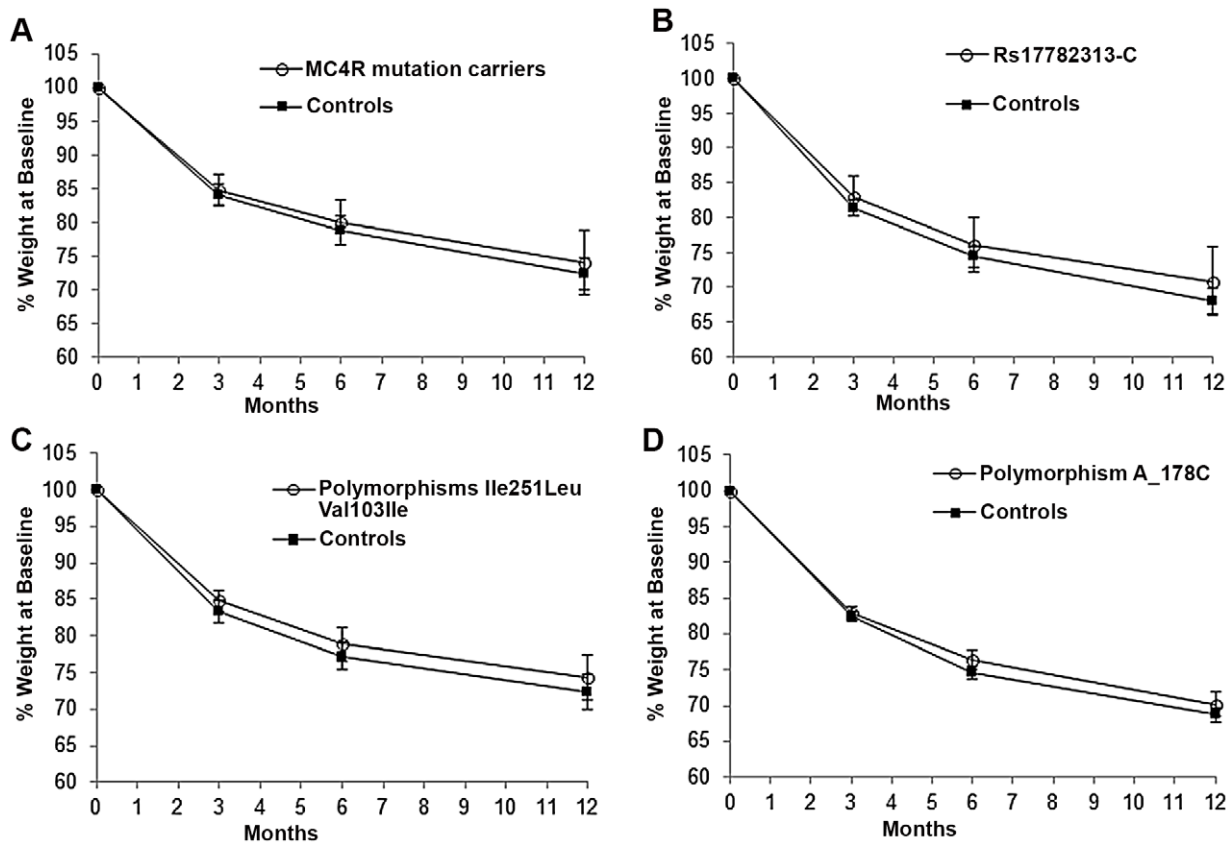


Figure 1. Weight loss over 12 months after bariatric surgery according to MC4R genotype (mean \pm SEM). Weight loss was expressed as a percentage of weight at baseline (surgery). Carriers and non-carriers were matched for age, sex, weight and surgery procedure (gastric banding or bypass). A) Weight loss in carriers and non-carriers of functional MC4R mutations. B) Weight loss data in carriers and non-carriers of the allele rs17782313-C. C) Weight loss data in carriers and non-carriers of MC4R polymorphisms Ile251Leu, Val103Ile, D) Weight loss data in carriers and non-carriers of MC4R polymorphism A_178C.
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Materials and Methods

Subjects

Subjects were patients followed-up in the Department of Nutrition of Pitié-Salpêtrière hospital (Paris, France). This study enrolled 648 severely obese adults with a body mass index (BMI) >35 kg/m² who underwent primary bariatric surgery from 1998 until 2010. Two laparoscopic procedures (AGB and RYGBP) [15], were performed by the same surgical team (J.L. Bouillot) in the Department of Surgery of Hotel-Dieu hospital. All patients underwent a preoperative assessment and were also examined at 3, 6 and 12 months after surgery. The clinical investigation was approved by the Ethics Committee of Hotel-Dieu Hospital. All subjects gave their written informed consent.

Anthropometric and biochemical measurements

Anthropometric parameters, including BMI and fat mass determined by biphotonic absorptiometry DEXA (dual-energy x-ray absorptiometry; Hologic, Waltham, MA) were assessed before and at 3, 6 and 12 months after surgery. Height was obtained at the initial visit and weight measurement obtained at each visit. BMI was calculated as the weight (kilograms) divided by the square of the height (square meters). Blood samples for DNA extraction were collected on EDTA after an overnight fast at baseline. Biochemical parameters including fasting glycemia and total cholesterol were measured using routine techniques.

Blood sampling and genomic sequencing

Genomic DNA was extracted from peripheral blood according to standard methods (ExtraGene extractor (Genomic SA) using Wizard Genomic DNA purification kit (Promega). Screening for MC4R variants and mutations was performed by direct sequencing of four PCR products (primers sequences available on demand) mapping the whole MC4R coding sequence as well as 230pb downstream of the ATG site (ABI 3730 sequencer, Applied Biosystems, Foster City, USA). Sequence data were aligned using the Seqscape 2.5 software and compared to the MC4R sequence (NM_005912.2). In *silico* prediction of the deleterious effect of the mutant was performed with the Alamut Mutation Interpretation Software (Interactive Biosoftware, Rouen, France) and the PolyPhen Web site (<http://genetics.bwh.harvard.edu/pph>). Among the various MC4R variants studied in this work, two new mutations (Cys196Tyr, Leu134Pro) had been deposited in GeneBank. Their publications are in process and their accession numbers are JX515607 and JX51608.

Study design and statistical analysis

Patients were categorized as carriers of: functional MC4R mutations, V103L and I251L SNPs, rs17782313 variant or SNP A-178C. Each case was matched with two randomly paired controls on the following covariates: age, sex, baseline weight, surgery procedure. Difference in weight change between case and their matched controls of each group was computed at 3, 6 and 12

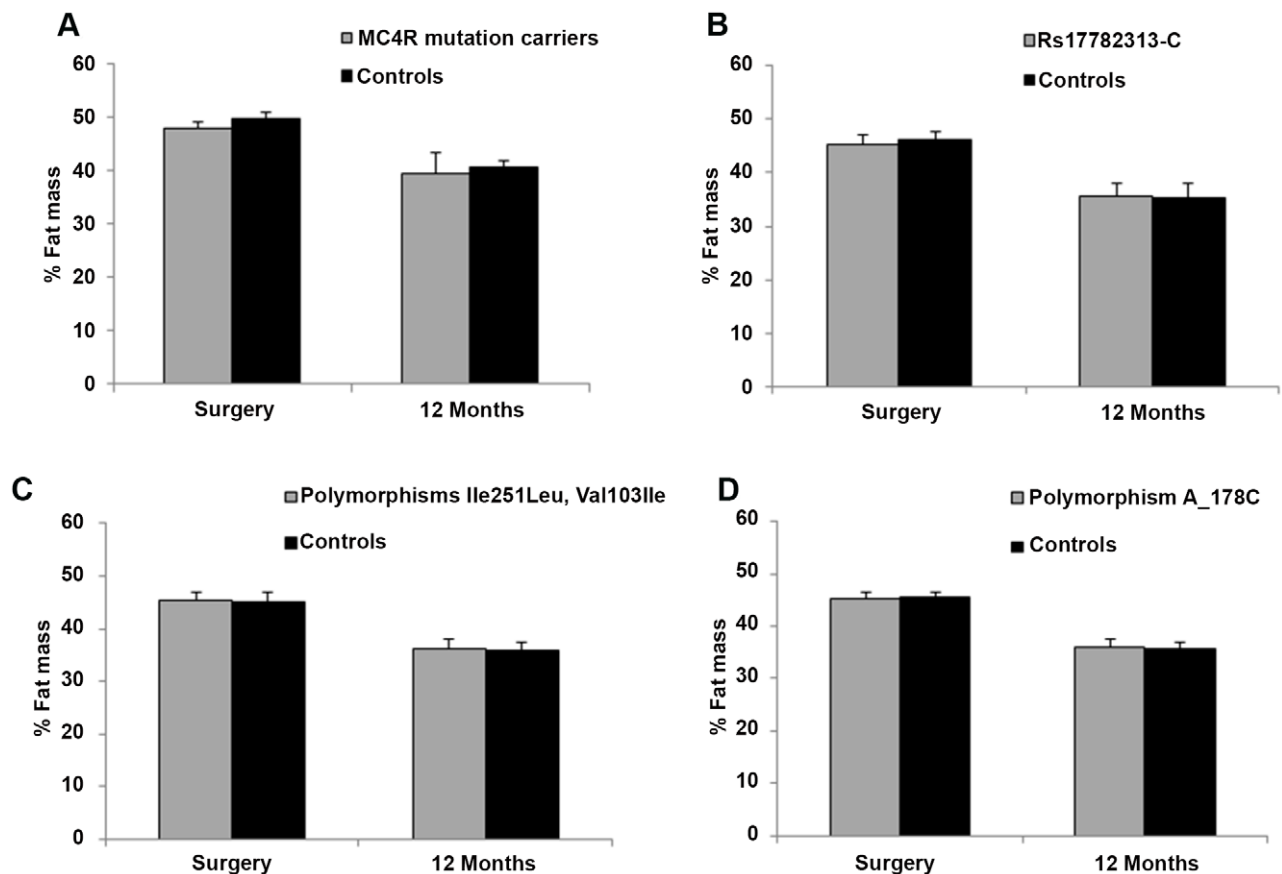


Figure 2. Fat mass percentage at 12 months after bariatric surgery according to MC4R genotype. A, plot percentage of fat mass in carriers and non-carriers of functional MC4R mutations. B, plot percentage of fat mass in carriers and non-carriers of the allele rs17782313-C. C, plot percentage of fat mass in carriers and non-carriers of MC4R polymorphisms: Ile251Leu, Val103Ile. D, plot percentage of fat mass in carriers and non-carriers of MC4R polymorphism: A_178C.
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months after surgery. Weight was expressed as a percentage of body weight in kilograms at the times of surgery. Fat mass as a percentage of weight (FM %) change was calculated at baseline and at 12 months. Wilcoxon signed rank tests were used to compare mean differences between cases and controls at each time of follow-up. All statistical analyses were performed with SAS version 9.1 (SAS institute, Cary, NC, USA).

Results

MC4R genotyping

Among the 648 patients screened for *MC4R* mutations, 9 were heterozygous for functional *MC4R* mutations, 10 heterozygous for *MC4R* SNPs V103L and I251L, 22 were heterozygous for the SNP A-178C located on the *MC4R* promoter and 7 carriers (4 heterozygous and 3 homozygous) of the variant rs17782313 located downstream of *MC4R*. Baseline characteristics are shown in Table 1 and indicate no difference between groups in fasting blood glucose, cholesterolemia and others characteristics, except for a modest difference for age in A-178C SNP carriers compared to their controls. Moreover, fasting blood glucose and cholesterolemia did not differ 12 months after surgery between case and controls (data not presented).

Baseline and post-surgery weight loss and body composition

The A-178C carrier group showed a difference in weight at 6 months which was not maintained. At 6 months of follow-up, weight was significantly higher for polymorphism A-178C carriers (96.6 ± 14.4 kg) compared to non-carrier (92.4 ± 10.8 kg), but this difference disappeared 12 months after surgery. However, this comparison did not show any difference when weight expressed as a percentage of body weight were used instead of weight.

In the other groups, percentage and absolute amount of weight loss (Figure 1) were not related to the presence of *MC4R* mutations. Furthermore, body fat percentages at baseline and after 12 months follow-up were not different between case and controls in each group (Figure 2).

Discussion

In this case control study we suggest that functional *MC4R* mutations as well as genetic variants (SNPs V103L and I251L, variant rs17782313 and SNP A-178C) did not influence weight loss and body composition after bariatric surgery.

Previous studies in animal models and humans have pointed out the critical importance of the central melanocortineric pathway in the control of energy homeostasis in particular in the pivotal role of the melanocortin-4 receptor [16]. Based on these findings, we could have expected a different impact on weight following

bariatric surgery in human carriers of functional *MC4R* mutations, *MC4R* polymorphisms and variant rs17782313. Our findings extend results of recent observations [17–19]. A recent study in 92 morbidly obese adults found similar percentage of weight loss after RYGB between four heterozygous functional *MC4R* mutation carriers and their 8 matched controls [17]. Another study showed similar effects in 15 heterozygous carriers of functional *MC4R* mutations compared to 869 non carriers [18]. In contrary, a case report of a patient who underwent AGB and truncal vagotomy at 18 years of age, suggested that complete MC4R deficiency impairs results in poor weight-loss [20]. This result based on only one patient carrier of two mutations with different effects (a functional MC4R mutation and the SNP I251L) was inconclusive.

In 1443 patients with a minor allele frequency (MAF) of 0.27, the variant rs17782313 located downstream the MC4R gene, presumably associated with increased obesity risk [10], was not shown to be related to maximal weight loss nor weight regain after bariatric surgery [19]. Recent analysis of SNPs V103L and I251L, in a cohort of 1443 obese patients, indicated that twenty-six heterozygous carriers of I251L lost significantly more weight (~7% initial weight) than the thirty-six V103L carriers and non-carriers. However, in this report, cases and controls were not matched [21], which makes the comparison difficult between groups. Another study in 18 morbidly obese heterozygous for I251L and twenty subjects heterozygous for V103L, did not show any difference after RYGB [18]. Moreover, in a study of 175 patients including 9 carriers who underwent a gastric bypass surgery, MC4R SNPs did not affect weight excess loss, after 18 month of follow up [22]. Concerning polymorphism A-178C in the *MC4R* promoter, its effect on RYGBP outcomes was not yet studied. Our study did not find a consistent effect of this SNP in bariatric surgery outcomes.

Among the four previous published reports only one [17] was designed as a case-control study. However, it was based on a small

sample size (four cases and eight controls) which limits its statistical power. Other studies were case series without appropriate matching between cases and controls [18,19,21].

Limitations of our study include the rarity of *MC4R* mutation carriers and the short period of follow-up. The strengths are a large group of *MC4R* mutation carriers, separated analyses done according to their functionality and location and the investigation of the impact of bariatric surgery on both weight and body composition. At last, selection bias was limited since controls were randomly selected before matching to cases.

Conclusions

In conclusion, our study provides good evidence that heterozygous mutations near and in the MC4R gene, either leading to a reduced receptor function or not, did not affect weight loss and body fat mass after bariatric surgery. To have recourse to bariatric surgery as a treatment of severe obesity should not be influenced by *MC4R* mutations status.

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Author Contributions

Conceived and designed the experiments: CP KC SC. Performed the experiments: MV JLB. Analyzed the data: MV SC. Contributed reagents/materials/analysis tools: JB. Wrote the paper: MV CP JLB JB KC SC.

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