

ORIGINAL ARTICLE

Binge Eating as a Major Phenotype of Melanocortin 4 Receptor Gene Mutations

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ABSTRACT

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BACKGROUND

Obesity, a multifactorial disease caused by the interaction of genetic factors with the environment, is largely polygenic. A few mutations in these genes, such as in the leptin receptor (*LEPR*) gene and melanocortin 4 receptor (*MC4R*) gene, have been identified as causes of monogenic obesity.

METHODS

We sequenced the complete *MC4R* coding region, the region of the proopiomelanocortin gene (*POMC*) encoding the α melanocyte-stimulating hormone, and the leptin-binding domain of *LEPR* in 469 severely obese white subjects (370 women and 99 men; mean [\pm SE] age, 41.0 \pm 0.5 years; body-mass index [the weight in kilograms divided by the square of the height in meters], 44.1 \pm 2.0). Fifteen women and 10 men without a history of dieting or a family history of obesity served as normal-weight controls (age, 47.7 \pm 2.0 years; body-mass index, 21.6 \pm 0.4). Detailed phenotypic data, including information on body fat, resting energy expenditure, diet-induced thermogenesis, serum concentrations of leptin, and eating behavior, were collected.

RESULTS

Twenty-four obese subjects (5.1 percent) and one control subject (4 percent) had *MC4R* mutations, including five novel variants. Twenty of the 24 obese subjects with an *MC4R* mutation were matched for age, sex, and body-mass index with 120 of the 445 obese subjects without an *MC4R* mutation. All mutation carriers reported binge eating, as compared with 14.2 percent of obese subjects without mutations ($P < 0.001$) and 0 percent of the normal-weight subjects without mutations. The prevalence of binge eating was similar among carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers. No mutations were found in the region of *POMC* encoding α melanocyte-stimulating hormone.

CONCLUSIONS

Binge eating is a major phenotypic characteristic of subjects with a mutation in *MC4R*, a candidate gene for the control of eating behavior.

THE PREVALENCE OF OBESITY, A MULTIFACTORIAL disease caused by the interaction of genetic factors with the environment,¹ is rapidly increasing worldwide.² Sedentary lifestyles, high-fat, energy-dense diets, and a genetic predisposition to obesity all contribute to the epidemic.

Several genes apparently involved with appetite have been identified. The adipocyte-derived hormone leptin^{3,4} signals nutritional status to the hypothalamus by binding to the leptin receptor⁵ and triggers the production of α melanocyte-stimulating hormone by means of proopiomelanocortin (POMC) neurons.⁶ α Melanocyte-stimulating hormone binds to the melanocortin 4 receptor (MC4R),⁷ effecting the anorectic properties of the hormone.

In contrast to occasional case reports of monogenic obesity associated with rare mutations in the genes for leptin,^{3,4,8} leptin receptor (LEPR)^{5,9} and POMC,¹⁰ the MC4R gene has been implicated in the development of obesity in up to 4 percent of persons with a body-mass index (the weight in kilograms divided by the square of the height in meters) above 35.¹¹ Mice with mutations and deficiencies of MC4R have hyperphagia, obesity, and hyperinsulinemia^{12,13} and demonstrate increased caloric efficiency.¹⁴ In 1998, mutations implicating genetic variation in MC4R as a monogenic cause of human obesity^{15,16} were first reported. To date, approximately 30 different MC4R mutations have been associated with obesity,^{11,15-25} although similar frequencies of MC4R mutations in subjects with a body-mass index below 30 and those with a body-mass index of 30 or more were reported in a recent study.²⁶ Hyperphagia, a common feature of obesity, is associated with genetic mutations in the leptin-melanocortin pathway. Leptin gene mutations have been detected in severely obese subjects who have low serum leptin levels (less than 3 ng per milliliter) and hyperphagia.^{4,8,27} A truncated LEPR gene was found in patients with elevated serum leptin levels (more than 150 ng per milliliter) and altered eating behavior,^{9,27} and the rare POMC gene mutation was linked to increased appetite.¹⁰ MC4R mutations were also associated with excessive hunger, food-seeking behavior, and hyperphagia.^{15,17,21,24}

Binge-eating disorder, recognized as an independent diagnosis,²⁸ occurs in 2 to 5 percent of non-obese subjects^{29,30} but in 30 to 90 percent of obese subjects,^{29,31-33} raising the question of whether mutations in the leptin-melanocortin pathway may be responsible for binge eating in severely obese subjects. We studied eating behavior in normal-weight

and severely obese subjects undergoing comparative sequencing of the leptin-binding domain of LEPR, the region of POMC encoding α melanocyte-stimulating hormone, and the entire coding region of MC4R.

METHODS

SUBJECTS

From January 1999 to December 2000, 469 consecutive, unrelated, severely obese white subjects (99 men and 370 women; mean [\pm SE] age, 41.0 \pm 0.5 years; range, 17 to 70; mean weight, 121.7 \pm 5.6 kg; mean body-mass index, 44.1 \pm 2.0 [range, 35.1 to 70.3]) were referred to our clinic for refractory obesity. Nineteen percent of the subjects took antihypertensive agents, 3.6 percent took hypoglycemic agents, and 1.7 percent took lipid-lowering medications. Exclusion criteria were a body-mass index below 35 and an age of less than 17 or more than 70 years. For comparison, 25 normal-weight volunteers were studied (10 men and 15 women; mean age, 47.7 \pm 2.0 years [range, 24 to 62]; mean weight, 63.8 \pm 2.0 kg; mean body-mass index, 21.6 \pm 0.4 [range, 18.5 to 25.0]) with no family history of obesity, no use of medications in the previous five years, no history of dieting, and normal blood-chemical values. These strict inclusion criteria were set to avoid any obesity trait. All subjects gave written informed consent. The study was approved by the local ethics committee and was compliant with the Declaration of Helsinki.

A physician specializing in obesity obtained phenotypic data, including sex, age, weight, height, body-mass index, and age at the onset of obesity. A standard family history of obesity (with respect to the subjects' parents, siblings, and children) was routinely obtained. Data were entered prospectively into our self-developed computer data base, ObesityBase 2000 (Zurich, Switzerland).

From each subject 20 ml of blood was collected into a tube containing EDTA for candidate-gene analysis. All analyses were performed in a blinded manner with respect to the phenotypic characteristics of the study groups. Gene analysis identified 24 severely obese subjects with mutations in MC4R (5 men and 19 women; mean age, 43.8 \pm 2.7 years; mean body-mass index, 44.4 \pm 1.0). The remaining 445 subjects were not carriers (94 men and 351 women; mean age, 42 \pm 1 years; mean body-mass index, 43.9 \pm 0.3). Complete phenotypic data were available for all normal-weight controls (except for

data on indirect calorimetry), and for 20 of the 24 obese subjects with MC4R mutations. These 20 subjects were matched for age (mean, 43.7 ± 2.8 years), sex, and body-mass index (mean, 43.1 ± 1.3) with 120 obese noncarriers in order to compare phenotypic data.

DETAILED PHENOTYPIC DATA

Eating Behavior

Each subject completed a questionnaire based on the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, diagnostic criteria for binge-eating disorder²⁸ using the fully validated eating disorder questionnaire of Spitzer et al.³⁰ (German translation³⁴). To validate the questionnaire data, a certified dietitian and a psychologist conducted independent, semistructured interviews with each subject. Finally, the physician specializing in obesity conducted a structured interview using this questionnaire. Team members were unaware of the subjects' behavioral diagnosis and genotype. Unanimity among all three professionals characterizing each subject's eating behavior was required for a diagnosis of binge eating. Diagnostic criteria for binge eating included at least twice-weekly binge eating over a minimum of six months. A binge was defined as rapid consumption of an unusually large amount of food in the absence of hunger, causing the subject to feel embarrassed, depressed, or guilty and out of control. There was no purging behavior. Subjects who did not fulfill all criteria for binge-eating disorder, determined unanimously by the team, were described as "non-bingers."

Serum Leptin Levels, Total Body Fat, and Resting Energy Expenditure

Serum venous blood was obtained after an overnight fast of 12 hours for genetic analysis and measurement of serum leptin levels (Prof. Krech und Partner) with the use of a radioimmunoassay (Linco Research). Body fat was determined by dual-energy x-ray absorptiometry (model QDR 4500A, Hologic), as previously described.³⁵ Since obese subjects occasionally did not fit in the scanner, body fat in all subjects was calculated on the basis of half-body scans.³⁶ Resting energy expenditure and diet-induced thermogenesis were measured by indirect calorimetry (Deltatrac II, Datex N2) before 8 a.m. after a 12-hour fast.³⁵ Thereafter, subjects consumed a standard Swiss breakfast consisting of one whole-wheat roll, one slice of whole-wheat toast, 20 g of butter, 25 g of jam, 1.5 dl of orange juice, 2 dl of

whole milk, and 2 dl of coffee (700 kcal, 28 g of fat, 18 g of protein, and 96 g of carbohydrate). Smoking was not permitted after 8 p.m. on the preceding evening. Resting energy expenditure and diet-induced thermogenesis (defined as the excess energy expended after a standard meal and expressed as a percentage of resting energy expenditure) were determined from continuous indirect calorimetry for three hours after the meal.

ANALYSIS OF CANDIDATE GENES

The complete coding and flanking 5' and 3' untranslated regions of MC4R, the region of POMC encoding α melanocyte-stimulating hormone, and the leptin-binding domain of LEPR were sequenced and compared with a reference sequence. DNA was isolated from blood with the QIAamp DNA Blood Midi Kit (100) (model 51185, Qiagen). The DNA concentration was determined by fluorescence measurement with PicoGreen molecular probes (P-7589) in a 96-well fluorimeter (SpektraMax, Molecular Devices). The MC4R reference sequence (GenBank accession number S77415), the two coding exons defining the POMC structural sequence (GenBank accession number V01510), and the respective sequences of LEPR (assembled in-house with the use of GenBank accession numbers AC097063.2 and U59257.1) were dissected into suitable polymerase-chain-reaction (PCR) fragments (details of PCR are provided in Supplementary Appendix 1, available with the full text of this article at <http://www.nejm.org>). Primers were designed with a proprietary program (GenProfile) to have the same melting temperatures. Thus, all fragments could be amplified under identical conditions with use of a thermocycler (model PTC-225, MJ Research) with CombiPol (Invitex) for amplification of all three genes and deoxynucleoside triphosphate (Roti-Mix PCR3 L785.2, Roth) under standard reaction conditions (total reaction volume, 50 μ l). PCR products were cleaned with the QIAquick96 PCR purification kit (Qiagen) before sequencing. Both strands were routinely amplified and sequenced to ensure maximal accuracy in variation analysis. PCR primers were also used as sequencing primers. Additional internal primers were used for PCR products longer than 600 bp to ensure that there was double-stranded sequence information for the whole PCR fragment. Sequencing was performed on ABI377 and ABI3700 automated DNA sequencers with the BigDye Terminator V2 Cycle Sequencing Kit (Applied Biosystems). Polyphred software together with Phred,

Phrap, RepeatMasker, and Consed³⁷ were used to detect polymorphisms. Of the 469 DNA samples, 466 to 467 genotypes (99.4 to 99.6 percent) at each polymorphic site of MC4R were determined with maximal accuracy and included in the analysis, and 462 to 469 genotypes (at least 98.5 percent) were determined for LEPR.

STATISTICAL ANALYSIS

Statistical analysis was performed with the use of SPSS software (Advanced Models 11.0 for Windows). Analysis of variance with post hoc Bonferroni's correction, the Kruskal-Wallis test, and the Mann-Whitney U test were used where appropriate. Relations between leptin and body fat were assessed with Spearman's correlation analysis. Values are expressed as means ±SE. All P values are two-sided.

RESULTS

GENETIC VARIATION IN MC4R

Twenty-four severely obese subjects (5.1 percent) and one normal-weight subject (4 percent) had genetic variants in MC4R. Nine different mutations or polymorphisms were detected in the gene segment spanning base-pair positions 408 to 1419 relative to the reference sequence (Table 1). The first four MC4R variants (Table 1) are known,^{11,17,20,23} whereas the next five were not identified in a Medline search. The first three mutations changed amino acids, the fourth was synonymous, and the last affected the 3' untranslated region. All mutations or polymorphisms were present on only one allele; allele frequencies ranged from 0.001 to 0.016. One subject had a mutation and a polymorphism (Table 1).

GENETIC VARIATION IN THE REGION OF POMC ENCODING α MELANOCYTE-STIMULATING HORMONE AND THE LEPTIN-BINDING DOMAIN OF LEPR

No severely obese or normal-weight subjects had mutations in the region of POMC encoding α melanocyte-stimulating hormone. In contrast, 178 severely obese subjects (38.0 percent) and 8 controls (32.0 percent) had mutations or polymorphisms in the leptin-binding domain of LEPR (Table 2). Five variants not found in a Medline search were detected in severely obese subjects, and two were identified in normal-weight subjects. Three mutations or polymorphisms (one in a control subject; all three in obese subjects) affected the amino acid sequence

Table 1. Mutations in the Melanocortin 4 Receptor Gene Identified among 469 Severely Obese Subjects and 25 Normal-Weight Controls.*

Mutation	Change in Amino Acid Sequence	No. Affected		Allele Frequency	
		Obese Subjects	Controls	Obese Subjects	Controls
Known					
C728T	Thr112Met†‡	2	0	0.002	
C886T	Arg165Trp‡§	1	0	0.001	
A700G	Val103Ile†‡§¶	11	1	0.012	0.016
A1144C	Ile251Leu†‡	5	0	0.005	
Novel					
A424G	Thr11Ala¶	1	0	0.001	
T544C	Phe51Leu	1	0	0.001	
A991G	Met200Val	2	0	0.002	
C408T	Thr5Thr	1	0	0.001	
A1419G 3' UTR	—	1	0	0.001	

* Position numbers are given relative to GenBank reference sequence S77415. UTR denotes untranslated region.
 † Data are from Farooqi et al.²⁰
 ‡ Data are from Hinney et al.¹⁷
 § Data are from Vaisse et al.¹¹
 ¶ The A700G mutation and the A424G polymorphism were found in the same subject.
 || Data are from Rosmond et al.²³

Table 2. Mutations in the Leptin-Binding Domain of the Leptin Receptor Gene Identified among 469 Severely Obese Subjects and 25 Normal-Weight Controls.*

Mutation	Change in Amino Acid Sequence	No. of Homozygotes/No. of Heterozygotes		Allele Frequency	
		Obese Subjects	Controls	Obese Subjects	Controls
T88641C	Ser343Ser	26†/149	0/7	0.214	0.14
G88642A	Val344Ile	0/1	0/1	0.001	0.02
T96008C	Thr548Thr	1†/0	0/0	0.002	
G97244A	Arg612His	0/1	0/0	0.001	
T97307A	Val633Asp	0/1	0/0	0.001	

* Position numbers are given relative to GenBank reference sequence AC097063.2.
 † The T88641C polymorphism and the T96008C mutation were found in the same subject.

and were present on only one allele. Two mutations or polymorphisms were synonymous. Polymorphism T88641C occurred with a similar frequency in obese and normal-weight subjects (0.214 and 0.14, re-

spectively; $P=0.41$). Twenty-six severely obese subjects (14.9 percent) were homozygous for this polymorphism, as compared with none of the controls (Table 2). The T96008C mutation was found in one obese subject. One obese subject had a mutation and a polymorphism in *LEPR*.

PHENOTYPIC CHARACTERIZATION

The 20 severely obese carriers of *MC4R* mutations or polymorphisms (5 men and 15 women; mean age, 43.7 ± 2.8 years; mean body-mass index, 43.1 ± 1.3) and 120 matched noncarriers (30 men and 90 women; mean age, 44.0 ± 0.8 years; mean body-mass index, 42.6 ± 0.6) had similar clinical characteristics. The normal-weight carrier of an *MC4R* mutation was male, was 45 years of age, and had a body-mass index of 24.7.

Obesity occurred before the age of 18 years with a similar frequency among obese carriers of an *MC4R* mutation and noncarriers (65.0 percent and 55.0 percent, respectively; $P=0.41$). Although carriers were more likely than noncarriers to have an obese mother (75.0 percent, as compared with 31.7 percent; $P<0.001$), no statistically significant differences were found between carriers and noncarriers in the percentages of those who had an obese father ($P=0.14$), obese siblings ($P=0.25$), or obese children ($P=0.54$). In contrast, there were no significant differences in the time of onset of obesity or family history of obesity between carriers of mutations or polymorphisms in the leptin-binding domain of *LEPR* and noncarriers.

Eating Behavior

Carriers of an *MC4R* mutation and noncarriers had markedly different eating behavior. All carriers met all criteria for binge eating, as compared to 14.2 percent of obese subjects who did not have an *MC4R* mutation ($P<0.001$) and 0 percent of normal-weight subjects without an *MC4R* mutation ($P<0.001$). In contrast, there was no significant difference in the prevalence of binge eating between carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers (28.8 percent and 25.0 percent, respectively; $P=0.62$).

Relation of Serum Leptin Levels and Body Fat

Serum leptin levels were similar in obese carriers of an *MC4R* mutation and noncarriers (37.0 ± 4.9 and 34.7 ± 1.6 ng per milliliter, respectively; $P=0.62$). Likewise, carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers had similar se-

rum leptin levels (38.2 ± 2.7 and 33.2 ± 1.9 ng per milliliter, respectively; $P=0.14$). However, serum leptin levels in normal-weight controls (6.4 ± 0.8 ng per milliliter) were significantly lower than those in obese subjects ($P<0.001$).

Obese carriers of an *MC4R* mutation and noncarriers had similar amounts of body fat (49.9 ± 2.1 kg and 50.5 ± 1.3 kg, respectively; $P=0.85$, with no sex-based difference), as did carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers (50.1 ± 1.7 kg and 50.6 ± 1.5 kg, respectively; $P=0.84$), whereas the amount of body fat was significantly lower in normal-weight controls (14.5 ± 0.8 kg, $P<0.001$). Body fat correlated with serum leptin levels in subjects without an *MC4R* mutation ($r=0.67$, $y=0.711x-1.598$, $P<0.001$) (Fig. 1A), in 60 subjects with a mutation in the leptin-binding domain of *LEPR* ($r=0.59$, $y=0.749x-0.220$, $P<0.001$) (Fig. 1D), and in 105 subjects without a mutation in the leptin-binding domain of *LEPR* ($r=0.64$, $y=0.660x-0.576$, $P<0.001$) (Fig. 1C). In contrast, no correlation was found between body fat and serum leptin levels in 21 subjects with an *MC4R* mutation ($r=0.09$, $P=0.70$) (Fig. 1B).

Energy Expenditure

No statistically significant differences were observed between obese subjects with an *MC4R* mutation and noncarriers with respect to resting energy expenditure (2084 ± 73 kcal per 24 hours and 2074 ± 31 kcal per 24 hours, respectively; $P=0.90$) or diet-induced thermogenesis (14 \pm 2 percent and 13 \pm 1 percent, respectively; $P=0.20$). Likewise, obese carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers had similar values for resting energy expenditure (2038 ± 46 kcal per 24 hours and 2097 ± 37 kcal per 24 hours, respectively; $P=0.32$) and diet-induced thermogenesis (13 \pm 1 percent for both, $P=0.80$).

DISCUSSION

Mutations in *MC4R* occurred with a prevalence of 5.1 percent in our obese subjects, a value that is similar to the value of 4 percent described previously.¹¹ Prior studies revealed approximately 30 different *MC4R* mutations that might cause monogenic obesity.^{11,15-25} We identified five additional mutations that may also have a role in the development of morbid obesity: Thr11Ala, Phe51Leu, Met200Val, Thr5Thr, and an A1419G substitution in the 3' untranslated region. The first three mutations change

the amino acid sequence, the fourth is synonymous, and the fifth affects the 3' untranslated region of the gene. Although the last two mutations do not change the amino acid sequence in the protein, they may be involved in the development of morbid obesity by influencing the level, location, or timing of gene expression or by existing in linkage disequilibrium with an as-yet-unidentified causative mutation.³⁸

Our rigorously selected normal-weight subjects had a frequency of *MC4R* genetic variants similar to that of the obese population studied by Jacobsen et al., although obesity traits were not excluded in their control subjects.²⁶ However, the prevalence of *MC4R* variations in controls was exclusively due to the coding-region polymorphism Val103Ile, which occurred with a similar prevalence in normal-weight and obese subjects. Since our 25 stringently selected control subjects were part of a larger sample of 96 normal-weight subjects, none of whom had any of the other sequence variants found in our severely obese patients, the coding-region polymorphism Val103Ile might not predispose subjects to obesity. This possibility must be corroborated by further studies in normal-weight subjects.

Serum leptin levels correlate well with body fat,^{39,40} as we found in subjects without a mutation in *MC4R* and in both carriers of mutations in the leptin-binding domain of *LEPR* and noncarriers. However, no such correlation was seen among carriers of an *MC4R* mutation throughout a wide range of body fat (20 to 78 kg), suggesting that the negative-feedback loop between serum leptin and adipose tissue may be disrupted in these subjects.

Hyperphagia with excessive food-seeking behavior has been linked to *MC4R* mutations in humans.^{15,17,21,24} Binge-eating disorder, a well-defined trait among both obese and nonobese subjects, is especially prevalent among the severely obese, in whom it correlates with body-mass index.^{32,41} All carriers of an *MC4R* mutation were given a diagnosis of binge-eating disorder, as compared with only 14.2 percent of noncarriers. Thus, the prevalence of binge-eating disorder in our population was 26.4 percent, lower than that reported in other studies of even heavier obese cohorts,^{33,42} conducted mainly in the United States.

Subjects with mutations in the leptin-binding domain of *LEPR* had serum leptin levels in the normal range and had a frequency of binge-eating disorder that was similar to that of those without such mutations. Moreover, we found no mutations in the

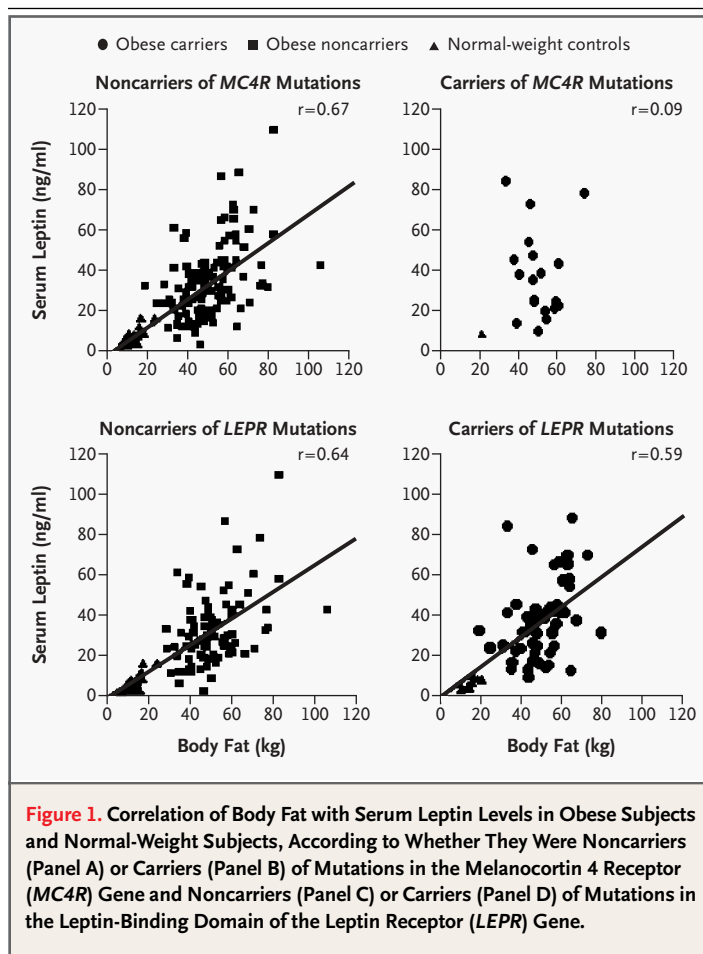


Figure 1. Correlation of Body Fat with Serum Leptin Levels in Obese Subjects and Normal-Weight Subjects, According to Whether They Were Noncarriers (Panel A) or Carriers (Panel B) of Mutations in the Melanocortin 4 Receptor (*MC4R*) Gene and Noncarriers (Panel C) or Carriers (Panel D) of Mutations in the Leptin-Binding Domain of the Leptin Receptor (*LEPR*) Gene.

region of *POMC* encoding α melanocyte-stimulating hormone in any of our subjects, suggesting that *MC4R* dysfunction is the likely cause of binge-eating disorder in subjects with an *MC4R* mutation.

MC4R deficiency has been associated not only with hyperphagia, but also with enhanced caloric efficiency in mice.¹⁴ No significant differences in resting energy expenditure were found between obese carriers of *MC4R* and *LEPR* mutations or polymorphisms and noncarriers, suggesting that neither *MC4R* nor the leptin-binding domain of *LEPR* controls resting energy expenditure. Moreover, since diet-induced thermogenesis was similar between carriers of an *MC4R* mutation and noncarriers and between carriers of a mutation in the leptin-binding domain of *LEPR* and noncarriers, the recent proposal that *MC4R* deficiency enhances caloric efficiency in mice¹⁴ does not appear to be valid in humans.

Our findings suggest that binge-eating disorder is a major phenotypic characteristic in persons with

a mutation in *MC4R* and suggest that *MC4R* is a candidate gene in the control of eating behavior. Since binge-eating disorder is a predictor of poor outcome in treatments for obesity, including gastric surgery,⁴² it remains to be determined whether mutations in *MC4R* could be one cause of long-term treatment failure. It is interesting to speculate that

melanocortin agonists currently under development⁴³ might have a specific role in the treatment of binge-eating disorder.

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