Neuromedin β: a strong candidate gene linking eating behaviors and susceptibility to obesity1–3

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ABSTRACT

Background: Obesity is frequently associated with eating disorders, and evidence indicates that both conditions are influenced by genetic factors. However, little is known about the genes influencing eating behaviors.

Objective: The objective was to identify genes associated with eating behaviors.

Design: Three eating behaviors were assessed in 660 adults from the Québec Family Study with the use of the Three-Factor Eating Questionnaire. A genome-wide scan was conducted with a total of 471 genetic markers spanning the 22 autosomes to identify quantitative trait loci for eating behaviors. Body composition and macronutrient energy intakes were also measured.

Results: Four quantitative trait loci were identified for disinhibition and susceptibility to hunger. Of these, the best evidence of linkage was found between a locus on chromosome 15q24-q25 and disinhibition (P < 0.0058) and susceptibility to hunger (P < 0.0001). After fine-mapping, the peak linkage was found between markers D15S206 and D15S201 surrounding the neuromedin β (NMB) gene. A missense mutation (p.P73T) located within the NMB gene showed significant associations with eating behaviors and obesity phenotypes. The P73T homozygotes were 2 times as likely to exhibit high levels of disinhibition (odds ratio: 1.8; 95% CI: 1.07, 2.89; P = 0.03) and susceptibility to hunger (odds ratio: 1.9; 95% CI: 1.15, 3.06; P = 0.01) as were the P73 allele carriers. Six-year follow-up data showed that the amount of body fat gain over time in T73T subjects was 2.7 times that in P73P homozygotes (3.6 compared with 1.5 kg; P < 0.05).

Conclusion: The results suggest that NMB is a very strong candidate gene of eating behaviors and predisposition to obesity. Am J Clin Nutr 2004;80:1478–86.

KEY WORDS Cognitive dietary restraint, disinhibition, susceptibility to hunger, behavioral genetics, Three-Factor Eating Questionnaire, quantitative trait locus

INTRODUCTION

The obesity epidemic has become the most important public health problem of this generation (1). Despite recent advancements in our understanding of the etiology and physiopathology of obesity, our capacity to prevent weight gain and to treat obesity is far from adequate. Although several genes have been shown to be associated with obesity, little is known about the genes influencing eating behaviors in humans, despite evidence that abnormal eating behaviors and disorders are frequently encountered in obese subjects (2).

The Three-Factor Eating Questionnaire (TFEQ) is the most widely used scale to quantify eating behaviors in normal-weight and obese person as well as in subjects with eating disorders such as anorexia nervosa, bulimia nervosa, and binge eating disorders. The 3 eating behavioral traits assessed by the TFEQ are cognitive dietary restraint, disinhibition, and susceptibility to hunger (3). A relation between eating behaviors and obesity was suggested in several studies. Obese subjects generally exhibit high disinhibition scores and susceptibility to hunger compared with lean subjects (4, 5). In the Québec Family Study (QFS), disinhibition and susceptibility to hunger were positively associated with BMI, body fatness, and waist circumference (6), and 6-y changes in dietary restraint were negatively correlated with body weight changes (7). Several studies showed the importance of eating behaviors in the context of weight-loss programs. In general, a high level of restraint or a decrease in disinhibition is associated with greater weight loss during dieting (8–11) and to better weight maintenance after weight loss (9, 10, 12).

There is also evidence that these behaviors are governed by genetic factors. In the Amish community, heritability estimates of 28%, 40%, and 23% for cognitive restraint, disinhibition, and susceptibility to hunger, respectively, were reported (13). In the QFS, the heritability of disinhibition and susceptibility to hunger was found to be 19% and 32%, respectively, whereas the heritability of cognitive restraint was not statistically significant (14). Persons who binge eat or who have bulimia nervosa or

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anorexia nervosa are also characterized by dysfunctional levels of cognitive dietary restraint, disinhibition, and susceptibility to hunger compared with normal subjects (4, 15), and they also have an important heritability component (16, 17). Despite the fact that eating behaviors are partly heritable traits, little is known about the genes influencing them.

Recently, Steinle et al identified 5 chromosomal regions or quantitative trait loci (QTL) for eating behaviors assessed by the TFEQ (13), but the genes influencing cognitive dietary restraint, disinhibition, and susceptibility to hunger within these regions were not recovered. Quantitative trait linkage analyses have to be performed in other populations to confirm or provide new QTL and to allow the identification of the genes influencing the eating behaviors. To replicate previous findings, to provide new chromosomal regions, and to identify genes influencing eating behaviors, a genome-wide scan linkage analysis was undertaken in the QFS.

### SUBJECTS AND METHODS

#### The Québec Family Study

The QFS is a prospective family study designed to investigate the genetics of obesity and its comorbidities (18). Participants in the study include 274 men and 386 women (274 M and 331 F, 26 M and 331 F) were measured on 2 occasions over an average follow-up period of 6.0 ± 0.9 y. Obesity-related phenotypes and direct measures of fat mass were assessed as previously described (18). Briefly, body fatness was assessed from body density measurements obtained from underwater weighing as described elsewhere (19). Total energy intake and the percentage of energy derived from macronutrients were measured with a 3-d dietary record as previously described (20). The study was approved by the Laval University Ethics committee.

#### Eating behavior measurements

Eating behaviors were assessed with the use of the TFEQ (3) validated for the French population (21). The 3 eating behaviors assessed by the 51 questions of the TFEQ are cognitive dietary restraint (21 questions), disinhibition (16 questions), and susceptibility to hunger (14 questions). Cognitive dietary restraint is a conscious behavior aimed at limiting food intake to control body weight. Disinhibition measures how easily external factors, such as environmental events and emotional reactions, disinhibit the control of eating. Susceptibility to hunger expresses the need for food as perceived by the individual. The TFEQ is a psychometric instrument that has been validated for internal stability and construct validity (3). Although, the TFEQ does not permit a direct measurement of eating behaviors in a specific context, studies have related scores obtained with the TFEQ to more direct measurements of eating behaviors. Accordingly, it has been shown that subjects with high dietary restraint score eat less than do persons with lower scores (22, 23). It has also been shown that the disinhibition score is strongly correlated with the severity of binge eating in many populations (24, 25).

### Genomic DNA studies

A total of 471 microsatellites and restriction fragment length polymorphism markers spanning the 22 autosomes were available for the genome scan. The average interval distance was 6.8 megabases (Mb), ranging from <1 to 32 Mb. Details on DNA preparation, polymerase chain reaction conditions, and genotyping have been described elsewhere (26, 27). Markers map locations (in Mb) were taken from the Human Genome National Center for Biotechnology Information resources (Built 31).

#### Neuromedin β gene genotyping

A previously identified p.P73T mutation (28) located in exon 2 of the NMB gene was genotyped in all subjects. The polymerase chain reaction (PCR) conditions were as follow. In a final volume of 20 μL, 20 ng genomic DNA was added to a mixture containing a final concentration of deoxynucleotide triphosphate (dNTP) (Amersham Pharmacia Biotech Inc, Piscataway, NJ), 30 μmol/L each; Taq DNA polymerase (QIAGEN, Valencia, CA), 0.3 U; buffer 1X [10X: tris-HCl, 260 mmol, MgCl2/L; pH 8.7 (20 °C)]; and flanking primers (forward 5'-TGCAGTCGCTGGTCCCTC-3'; reverse 5'-AGGCGAGA-

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of the subjects</th>
<th>Men (n = 274)</th>
<th>Women (n = 386)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>43.8 ± 15.1</td>
<td>41.9 ± 14.7</td>
</tr>
<tr>
<td>Cognitive dietary restraint</td>
<td>5.9 ± 3.6</td>
<td>8.4 ± 4.8</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>4.5 ± 3.0</td>
<td>5.9 ± 3.4</td>
</tr>
<tr>
<td>Susceptibility to hunger</td>
<td>4.1 ± 3.5</td>
<td>3.9 ± 3.2</td>
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<tr>
<td>Weight (kg)</td>
<td>85.5 ± 21.6</td>
<td>74.4 ± 22.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.6 ± 6.9</td>
<td>29.1 ± 8.8</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>96.8 ± 17.4</td>
<td>87.2 ± 19.0</td>
</tr>
<tr>
<td>Percentage body fat</td>
<td>24.4 ± 9.4</td>
<td>33.2 ± 10.3</td>
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<tr>
<td>Fat mass (kg)</td>
<td>22.3 ± 14.2</td>
<td>25.9 ± 15.5</td>
</tr>
<tr>
<td>Total energy intake (kJ)</td>
<td>11274 ± 3105</td>
<td>8532 ± 2131</td>
</tr>
<tr>
<td>Carbohydrate intake (% of energy)</td>
<td>47.2 ± 6.6</td>
<td>48.0 ± 6.3</td>
</tr>
<tr>
<td>Protein intake (% of energy)</td>
<td>16.3 ± 3.0</td>
<td>16.4 ± 3.2</td>
</tr>
<tr>
<td>Lipid intake (% of energy)</td>
<td>34.2 ± 6.1</td>
<td>34.1 ± 5.8</td>
</tr>
</tbody>
</table>

1. All values are x ± SD.
2, 3. Significantly different from men (ANOVA): 2 P ≤ 0.0001, 3 P ≤ 0.01.
4. n = 236 M and 331 F.
5. n = 175 M and 223 F.
55 °C, 30 s; and extension at 72 °C, 5 s. Detection was done by using IRDye tagged primer, and the products were analyzed on an automated DNA sequencer (automated sequencer model 4200; LI-COR, Lincoln, NE). The p.P73T genotypes were in Hardy-Weinberg equilibrium. To validate our genotyping, 38 control subjects were sequenced, and no discrepancy was found between the sequence and the genotype.

Statistical analysis

Eating behaviors were adjusted for age and sex effects as well as for age, sex, and BMI with the use of stepwise regression procedures; only the significant \( P < 0.05 \) terms were retained. The residuals were standardized to a mean of 0 and an SD of 1 and were then used as the phenotypes for the analyses.

Two approaches were used to test for linkage between eating behaviors and the genetic markers. First, linkage was tested with the new Haseman-Elston regression-based sibpair linkage procedure (29) implemented in the SIBPAL2 software from the SAGE 4.2 statistical package (30). The maximal number of sibpairs was 315. Second, linkage was tested with the variance components–based approach implemented in the quantitative transmission disequilibrium test computer software (31). To identify region of promising linkage or QTL, we used \( P \leq 0.0023 \) or log of odds (LOD) \( \geq 1.75 \) for both linkage methods. This level represents, on average, one false positive linkage signal per genome scan of 400 markers (32). Other interesting regions (suggestive) are reported if \( P \leq 0.01 \) or LOD \( \geq 1.17 \). A chi-squared test was applied to evaluate whether genotype and allele frequencies were in Hardy-Weinberg equilibrium and to compare genotypic frequencies between groups of individuals with low, intermediate, and high scores on the eating behavior scales. Because the men expressed less disinhibition than did the women, assignment to eating behavior groups was performed in each sex separately. In men, cutoff values were 3 and 8 (0–3, 4–7, and 8–16), whereas in women the corresponding cutoffs were 4 and 10 (0–4, 5–9, and 10–16). For susceptibility to hunger, there were no sex differences and group assignments were the same in men and women with cutoff values of 2 and 7 (0–2, 3–6, and 7–14). Differences in genotypic frequencies between groups were only tested for disinhibition and susceptibility to hunger.

Genetic associations were assessed by analysis of covariance comparing mean phenotypic values across NMB genotypes. If significant differences were detected, Tukey’s test was used to determine differences among genotypes. Phenotypes were adjusted for age and sex with and without further adjustment for BMI. Changes over time were computed by subtracting time 2 from time 1 measurements, and the resulting 6-y delta scores were adjusted for sex, BMI at time 1, and duration of the follow-up. All family members were used in the association analyses. Relatedness among family members was adjusted for by using the sandwich estimator as implemented in the SAS mixed procedure (33, 34). Transformations were applied to nonnormally distributed variables (square root and logarithm). Reported least-squares ± SE are for untransformed variables, but \( P \) values are for transformed scores when applicable. Adjustment of the phenotypes and other statistical procedures (excluding linkage analyses) were performed with SAS software (version 8.02).

RESULTS

Genome-wide scan

The complete eating behavior multipoint linkage analyses results are shown in Figure 1, and a summary of loci showing suggestive \( (P < 0.01, \text{ an LOD} > 1.17, \text{ or both}) \) and promising \( (P < 0.0023, \text{ an LOD} > 1.75, \text{ or both}) \) evidence of linkage based on at least one linkage method is shown in Table 2. Briefly, 5 suggestive and promising evidences of linkage were found for disinhibition \((p1p3, q9q22, 15q24-q25, 17q23-q24, \text{ and } 19p13)\), and 6 were found for susceptibility to hunger \((5q31, 13q32, 15q21, 15q24-q25, 17q23-q24, \text{ and } 21q11)\). No significant linkage was found for cognitive restraint. For disinhibition, promising evidence of linkage was found on chromosome 19p13 with marker D19S215 \((P = 0.002; \text{ LOD} = 1.8)\) and LOD \( = 0.61 \). Three promising linkages were identified for susceptibility to hunger. These linkages were on chromosomes 15q21 with marker LH2NAIII \((P = 0.002; \text{ LOD} = 1.76)\), 15q24-q25 with marker D15S206 \((P = 0.0001; \text{ LOD} = 3.0)\), and 17q23-q24 with markers D17S1306 \((P = 0.0001; \text{ LOD} = 1.36)\), LOD = 2.06), D17S1290 \((P = 0.007; \text{ LOD} = 1.30)\), and 17q23-q24 with markers D17S1351 \((P = 0.002; \text{ LOD} = 1.74)\), LOD = 0.95). Interestingly, the QTLs for susceptibility to hunger on chromosomes 15 and 17 were the same as those of those found for disinhibition and were not affected by BMI adjustments (data not shown).

Fine-mapping

Our results indicate that 2 loci, 15q24-25 and 17q23-24, were linked with both disinhibition and susceptibility to hunger. To increase the density of markers around these QTL to \( \approx 1 \text{ Mb} \), 10 additional markers were genotyped on chromosome 15q and 18 markers on chromosome 17q. After fine-mapping, the QTL on chromosome 15q24-q25 remained significantly \((\text{ LOD} > 1.73)\) linked to susceptibility to hunger (Figure 2), whereas the QTL on chromosome 17 did not (data not shown). For susceptibility to hunger, the strongest evidence of linkage was found between markers and D15S201 (Figure 2; panels A and B). For disinhibition, fine-mapping did not change the results.

Association studies

The most apparent candidate gene for the linkage observed on 15q24-q25 is the NMB gene located between markers D15S206 and D15S201, just 0.4 Mb apart from marker D15S201. A previously identified (28) missense polymorphism located within exon 2 and changing proline 73 residue to threonine \((c.217C>A \text{ or } p.P73T)\) was genotyped in all subjects. As shown in Table 3, significant associations were found between this mutation and disinhibition and susceptibility to hunger with \((P = 0.006, P = 0.035)\) or without \((P = 0.027, P = 0.034)\) adjustment for BMI. The T73T subjects exhibited higher levels of disinhibition and susceptibility to hunger compared with the P73 carriers. No significant association was found between \( p.P73T \) and cognitive dietary restraint. Differences in genotypic frequencies between subjects characterized by low, intermediate, and high levels of disinhibition, and susceptibility to hunger were also tested. Results presented in Table 4 indicate that the frequency of the \( T73T \) genotype in the group of subjects with high levels of disinhibition \((17\%) \) and susceptibility to hunger \((15\%) \) is \( \approx 2 \) times that in those with low levels of these behaviors \((8\% \text{ and } 7\% \text{, respectively})\). Indeed, subjects homozygous for the mutation \((T73T)\).
were ≈2 times as likely to exhibit high levels of disinhibition (OR: 1.8; 95% CI: 1.07, 2.89; \( P = 0.03 \)) and susceptibility to hunger (OR: 1.9; 95% CI: 1.15, 3.06; \( P = 0.01 \)) than were the subjects with the 2 other genotypes (Table 4). The variant was also associated with body fatness (\( P < 0.05 \) for percentage body fat). No associations were found with macronutrient and total energy intakes (Table 3).

Significant associations were also found between the \( p.P73T \) polymorphism and 6-y changes in adiposity-related phenotypes (Figure 3). Increases in body weight (\( P = 0.03 \)), BMI (\( P = 0.04 \)), waist girth (\( P = 0.02 \)), body fat (\( P = 0.02 \)), and fat mass (\( P = 0.04 \)) with age in the \( T73T \) homozygotes were ≈2 times higher than those in the \( P73 \) allele carriers (Figure 3). The \( NMB \) variant was not associated with changes in total energy intake, but trends were observed for an increase in the percentage of total energy intake as lipids (\( P = 0.06 \)) and a reduction in protein as a percentage of total energy intake (\( P = 0.08 \)) in \( T73T \) homozygotes compared with the \( P73 \) allele carriers (Figure 3).

**FIGURE 1.** Results of variance component multipoint linkage analyses of eating behaviors in the Québec Family Study. Mb, megabases; Chr, chromosome; LOD, logarithm of odds.

**FIGURE 3.** Increases in body weight (\( P = 0.03 \)), BMI (\( P = 0.04 \)), waist girth (\( P = 0.02 \)), body fat (\( P = 0.02 \)), and fat mass (\( P = 0.04 \)) with age in the \( T73T \) homozygotes were ≈2 times higher than those in the \( P73 \) allele carriers (Figure 3). The \( NMB \) variant was not associated with changes in total energy intake, but trends were observed for an increase in the percentage of total energy intake as lipids (\( P = 0.06 \)) and a reduction in protein as a percentage of total energy intake (\( P = 0.08 \)) in \( T73T \) homozygotes compared with the \( P73 \) allele carriers (Figure 3).
Identification of 4 loci linked to eating behaviors

Genome-wide linkage analyses provide an opportunity to identify chromosomal regions (loci) harboring genes influencing complex traits, such as eating behaviors. Using this approach, we identified 4 QTL for disinhibition and susceptibility to hunger: 19p13 for disinhibition and 15q21, 15q24-q25, and 17q23-q24 for susceptibility to hunger. No evidence of linkage was found for cognitive dietary restraint, which agrees with the observation of a nonsignificant heritability estimate for this phenotype in the QFS (14). Some of the linkages uncovered in the present study were not affected by BMI adjustment, which suggests that the genes influencing disinhibition and susceptibility to hunger exert their effects independently of body weight status. This is the case for the QTL on chromosomes 15q24-q25 and 17q23-q24, which are associated with both disinhibition and susceptibility to hunger. These 2 QTL harbor candidate genes of interest for eating behaviors and obesity. On chromosome 15q24-25, 2 candidate genes were identified: aryl-hydrocarbon receptor nuclear translocator 2, which is a partner of its single-minded, drosophila, homologue 1 (SIM1) gene, which has been shown to be responsible for one case of a monogenic form of human obesity (35–37) and NMB, which modulates behaviors and food intake in many species (28, 38–41). Two genes of interest were also found on chromosome 17q23-q24: the thyroid hormone–associated protein (TRAP240) activates the transcription of the thyroid hormone (triiodothyronine), which is known to modulate energy expenditure (42, 43) and growth hormones 1 and 2, which have been associated with the metabolic syndrome including abdominal obesity (44, 45).

Neuromedin β is associated with eating behaviors

The chromosome 15q as well as 17q regions were retained for fine-mapping for 2 reasons: 1) these regions provided evidence of linkage for both disinhibition and susceptibility to hunger, and 2) the linkage signal was not affected by adjustment for BMI. After fine-mapping, only the former still showed promising evidence of linkage and was selected for deeper analyses. As discussed above, 2 positional candidate genes at this locus, ARNT2 (arylhydrocarbon receptor nuclear translocator 2) and NMB, were of relevance for eating behaviors and obesity. Because of its proximity to the peak linkage signal (78.2 Mb), NMB (78.2 Mb) was chosen as the most promising positional candidate gene for further investigation in association studies. NMB is a member of the bombesin-like peptides widely expressed in brain, pancreas, adrenals, and gastrointestinal tract (38). This protein family is known to inhibit food intake in rats (39) and to modulate behaviors (grooming) when administered centrally (40, 41). A missense polymorphism located within exon 2 of the NMB gene (c.217C>T or p.P73T) was genotyped and tested for association with eating behavior phenotypes in all subjects of our cohort. The results showed that P73T homozygotes were ∼25% more disinhibited and susceptible to hunger or ∼2 times as likely to be in the

**DISCUSSION**

**Identification of 4 loci linked to eating behaviors**

Summary of the loci showing suggestive ($P < 0.01$ or LOD $> 1.17$) or promising ($P < 0.0023$ or LOD $> 1.75$) evidence of linkage with eating behaviors

<table>
<thead>
<tr>
<th>Phenotype and chromosome</th>
<th>Markers</th>
<th>Position</th>
<th>$P$</th>
<th>LOD score</th>
<th>QTDT, LOD score</th>
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<tr>
<td><strong>Disinhibition</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1p31</td>
<td>LEPRCA</td>
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<td>0.0111</td>
<td>1.14</td>
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</table>

$^1n=660$ subjects from 202 families. $P$ values and logarithm of odds (LOD) scores are for age- and sex-adjusted phenotypes. No significant evidence of linkage was observed for cognitive restraint. Mb, megabases; SAGE, statistical analysis for genetic epidemiology (Statistical Solutions Ltd, Cork, Ireland).

$^2$Shows promising evidence of linkage.

**FIGURE 2.** Results of fine-mapping multipoint linkage analyses of eating behavior for chromosome 15. The positions of the aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2) and neuromedin β (NMB) genes are shown. Mb, megabases. A: Age- and sex-adjusted phenotypes. B: Age-, sex-, and BMI-adjusted phenotypes. The dotted lines represent the level of suggestive [logarithm of odds (LOD) $\geq 1.17$] and promising (LOD $\geq 1.75$) evidence of linkage.
TABLE 3
Association of the p.P73T (c.217C→A) neuromedin β polymorphism with eating behaviors and adiposity-related phenotypes in subjects from the Québec Family Study

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>T/T (n = 67)</th>
<th>P/T (n = 254)</th>
<th>P/P (n = 335)</th>
<th>P1</th>
<th>P2</th>
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<tbody>
<tr>
<td>Cognitive dietary restraint</td>
<td>6.8 ± 0.53</td>
<td>7.2 ± 0.31</td>
<td>6.9 ± 0.41</td>
<td>0.6102</td>
<td>0.6581</td>
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<tr>
<td>Disinhibition</td>
<td>5.2 ± 0.41</td>
<td>3.9 ± 0.21</td>
<td>4.3 ± 0.30</td>
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<td>0.0057</td>
</tr>
<tr>
<td>Susceptibility to hunger</td>
<td>4.8 ± 0.51</td>
<td>3.5 ± 0.31</td>
<td>3.9 ± 0.38</td>
<td>0.0343</td>
<td>0.0345</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.0 ± 2.7</td>
<td>64.9 ± 1.6</td>
<td>68.5 ± 2.2</td>
<td>0.1067</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 ± 0.97</td>
<td>23.1 ± 0.59</td>
<td>24.6 ± 0.86</td>
<td>0.0888</td>
<td>—</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>80.8 ± 2.3</td>
<td>79.9 ± 1.5</td>
<td>81.9 ± 1.9</td>
<td>0.3977</td>
<td>0.2930</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>28.7 ± 1.27</td>
<td>26.8 ± 0.87</td>
<td>28.6 ± 0.91</td>
<td>0.0357</td>
<td>—</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>21.0 ± 18</td>
<td>19.6 ± 1.2</td>
<td>22.2 ± 1.3</td>
<td>0.0737</td>
<td>—</td>
</tr>
<tr>
<td>Carbohydrate intake (% of energy)</td>
<td>48.1 ± 1.3</td>
<td>49.2 ± 1.1</td>
<td>49.6 ± 0.9</td>
<td>0.3680</td>
<td>0.2556</td>
</tr>
<tr>
<td>Protein intake (% of energy)</td>
<td>16.3 ± 0.7</td>
<td>15.6 ± 0.4</td>
<td>15.8 ± 0.3</td>
<td>0.6366</td>
<td>0.6593</td>
</tr>
<tr>
<td>Lipid intake (% of energy)</td>
<td>34 ± 1.2</td>
<td>33.6 ± 1.1</td>
<td>33.6 ± 1.0</td>
<td>0.5613</td>
<td>0.5468</td>
</tr>
</tbody>
</table>

1 Age- and sex-adjusted phenotypes.
2 Age-, sex-, and BMI-adjusted phenotypes. After adjustment, the T/T homozygotes were significantly different from the 2 other genotypes for disinhibition and susceptibility to hunger.
3 Least-squares ± SE (all such values).
4 Significantly different from P/T, P < 0.05 (analysis of covariance followed by Tukey’s t test).
5 Significantly different from P/P, P < 0.05 (analysis of covariance followed by Tukey’s t test).

These results suggest that age analysis of chromosome 15 conditional on the linkage found on chromosome 15q, we repeated the linkage to be fully accounted for by a single polymorphism. For comparison with those investigated in the present study, it is unlikely to expect a signal in a notable way. Thus, for complex phenotypes such as modest effects, which, in the aggregate, could affect the linkage with certainty that it is not. Indeed, one should keep in mind that loci that have alleles with major effects on a complex phenotype may also have alleles, at other sites within the gene, with modest effects, which, in the aggregate, could affect the linkage signal in a notable way. Thus, for complex phenotypes such as those investigated in the present study, it is unlikely to expect a linkage to be fully accounted for by a single polymorphism. Moreover, we cannot exclude the possibility that the association with eating behaviors is due to another unidentified functional mutation within NMB or a different gene in the vicinity.

Neuromedin β is associated with obesity

Disinhibition and susceptibility to hunger are behaviors that are known to be correlated with obesity and to influence weight gain over time (12, 46) as well as weight regain after a weight-loss program (47). In the current study, the p.P73T NMB polymorphism was shown to be associated with eating behaviors. The possibility that the T73T subjects gain more body weight and adiposity over time was thus tested. As shown in Figure 3, the results showed that increases in body fatness after an average follow-up of 6 y were ≈2 times those in homozygotes for the mutation (3.6 kg) compared with the P73 allele carriers (1.5 kg). After adjustment of adiposity-related phenotypes for eating behavior scores (data not shown), only the 6-y changes in waist girth remained significantly associated with the p.P73T NMB polymorphism, which suggested that the effect of the NMB gene sequence variation on body fat accumulation is modulated by its effect on eating behaviors.

TABLE 4
Genotypic frequencies of the p.P73T neuromedin β polymorphism in subjects with low and high levels of disinhibition and susceptibility to hunger

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Low (n = 258)</th>
<th>Middle (n = 229)</th>
<th>High (n = 145)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
<th>Low (n = 258)</th>
<th>Middle (n = 229)</th>
<th>High (n = 145)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/P</td>
<td>0.53</td>
<td>0.49</td>
<td>0.53</td>
<td>—</td>
<td>—</td>
<td>0.53</td>
<td>0.49</td>
<td>0.53</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P/T</td>
<td>0.41</td>
<td>0.42</td>
<td>0.28</td>
<td>0.9 (0.64, 1.19)</td>
<td>0.396</td>
<td>0.41</td>
<td>0.39</td>
<td>0.32</td>
<td>0.9 (0.66, 1.21)</td>
<td>0.446</td>
</tr>
<tr>
<td>T/T</td>
<td>0.08</td>
<td>0.11</td>
<td>0.17</td>
<td>1.8 (1.07, 2.89)</td>
<td>0.026</td>
<td>0.07</td>
<td>0.11</td>
<td>0.15</td>
<td>1.9 (1.15, 3.06)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

1 χ² = 10.6, P = 0.032. Cutoffs were 3 and 8 (0–3, 4–7, and 8–16) in men and 4 and 10 (0–4, 5–9, and 10–16) in women.
2 χ² = 9.5, P = 0.050. Cutoffs were 2 and 7 for men and women (0–2, 3–6, and 7–14), respectively.
3 For comparison with P/P homozygotes.
4 For comparison with P carriers.
To the best of our knowledge, this is the first study to provide evidence that a gene affecting eating behavior also influences body fat gains over time. Interestingly, a recent study showed that the NMB receptor is expressed in visceral adipocytes (48), which suggests that the visceral fat depot may play a role in the regulation of food intake. Thus, NMB appears to be an excellent candidate gene for a link between eating behaviors and obesity.

The bombesin-like peptides family has many biological effects that may be related to eating behaviors and obesity, including the modulation of the serotonergic (5-HT) system (49), the regulation of thyrotropin secretion in the pituitary (50), and the stimulation of pancreatic hormones such as PYY (51). One could expect that these pathways may all be important for the NMB biological activity related to the control of eating behaviors. First, antidepressants acting on selective serotonin reuptake inhibitors are frequently used in the treatment of eating disorders (bulimia nervosa) because serotonin inhibits food intake. Second, thyroid hormones are potent physiologic stimulator of thermogenesis, which is known to stimulate food intake. Finally, a recent study showed that obese subjects, who are resistant to the effects of leptin, are not resistant to the anorectic effects of the gut hormone PYY (52). Thus, by stimulating PYY, the NMB gene could increase the satiety signal or decrease the hunger signal.

The effects of the NMB p.P73T mutation on eating behaviors seem to be of relevance for the development of obesity. Indeed, the increased levels of disinhibition and susceptibility to hunger observed in T73T homozygotes were associated with an additional increase of 2 kg of fat mass over a 6-y period compared with P73P homozygotes. By comparison, a body weight increase of 0.8 kg over 3 y was associated, at the population level, with an increase of 2.3% in the prevalence of overweight and obesity (53). Considering the increased risk of cardiovascular diseases and diabetes associated with obesity, the adiposity changes associated with increases in disinhibition and susceptibility to hunger may have substantial public health implications. However, no study has addressed the functional effect of the NMB p.P73T polymorphism on NMB expression or protein activity. On the basis of the present results and the anorectic effect of NMB, the NMB p.T73 allele should be associated with a lower NMB messenger RNA or protein levels compared with the p.P73 allele. This hypothesis has to be verified.
Summary

A genome-wide linkage analysis led to the identification of 4 chromosomal regions affecting eating behaviors. The best positional candidate gene, NMB, was located 0.4 Mb from the linkage peak on chromosome 15q24-q25. A missense mutation located in exon 2 of the NMB gene was genotyped and found to be associated with disinhibition and susceptibility to hunger as well as changes in body fatness over time. NMB is an endocrine factor that has received only limited attention in the field of eating behaviors and obesity research. Although further studies are needed to characterize the functional effects of the NMB exon 2 mutation, our findings suggest that the NMB is a strong candidate gene for eating behaviors and obesity.

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