0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(12):5057–5063

doi: 10.1210/jc.2006-1442

Association between Neuromedin U Gene Variants and Overweight and Obesity

Irena Hainerová,* Signe S. Torekov,* Jakob Ek, Marie Finková, Knut Borch-Johnsen, Torben Jørgensen, Ole D. Madsen, Jan Lebl, Torben Hansen, and Oluf Pedersen

Department of Pediatrics and Centre for Research of Diabetes, Metabolism, and Nutrition (I.H., M.F., J.L.), Third Faculty of Medicine, Charles University, 100 34 Prague 10, Czech Republic; Steno Diabetes Center and Hagedorn Research Institute (S.S.T., J.E., K.B.-J., O.D.M., T.H., O.P.), DK-2820 Gentofte, Denmark; Kennedy Institute-National Eye Clinic (J.E.), DK-2600 Glostrup, Denmark; Research Center for Prevention and Health (T.J.), Glostrup University Hospital, DK-2600 Glostrup, Denmark; and Faculty of Health (K.B.-J., O.P.), University of Aarhus, DK-8000 Aarhus, Denmark

Background: Neuromedin U (NMU) is an anorexic neuropeptide expressed in the hypothalamus. Mice lacking the *NmU* gene are hyperphagic and obese, whereas mice overexpressing *Nmu* are hypophagic and lean.

Objective: Our objective was to investigate whether variants in *NMU* are associated with human obesity.

Design: The coding region of *NMU* was analyzed for variants in obese Czech children and obese Danish adults. Identified missense variants were investigated for cosegregation with obesity in families or association with obesity in the general population.

Setting: The study was performed at Steno Diabetes Center, Denmark, and Department of Pediatrics, Charles University, Czech Republic.

Subjects and Methods: A total of 289 Czech children and adolescents with early-onset obesity and 84 Danish obese adults were analyzed for variants in *NMU*. A *NMU* Ala19Glu polymorphism was genotyped in 5851 Danish subjects of the Inter99 cohort, and a rare *NMU* Arg165Trp mutation was sequenced in the proband family and in 53 lean and unrelated Czech subjects.

Results: The rare *NMU* Arg165Trp variant cosegregated with childhood obesity in a Czech family. Homozygous carriers of the Glu allele of the *NMU* Ala19Glu polymorphism were more common in the overweight and obese subjects; the Glu/Glu frequency was 0.4 (95% confidence interval, 0.2–0.6) among 2586 lean subjects (BMI < 25 kg/m^2) and 0.9 (95% confidence interval, 0.7–1.1) among 3265 overweight and obese subjects (body mass index ≥ 25 kg/m^2) [odds ratio, 2.5 (1.2–5.3); $P = 0.01$].

Conclusion: Amino acid variants in *NMU* associate with overweight and obesity, suggesting that NMU is involved in energy regulation in humans. **(*J Clin Endocrinol Metab* 91: 5057–5063, 2006)**

THE WORLD HEALTH Organization (WHO) has proclaimed obesity as a global epidemic, and the increasing prevalence of obesity has launched an interest in understanding the molecular basis of energy homeostasis (1). Recently, many peripheral and central neuropeptides influencing feeding behavior and energy expenditure have been identified (2), and among these is human neuromedin U (NMU). Pre-pro-NMU, which contains the 25-residue-long biologically active peptide NMU, is a 174-amino-acid protein encoded by 10 exons localized at chromosome 4q12 (3) (Fig. 1). NMU is widely expressed in the central nervous system (CNS) with particularly high expression in the hypothalamus, a region known for its importance in appetite regulation (4, 5). Mice lacking the gene encoding NMU ($NmU^{-/-}$ mice) are hyperphagic and show decreased energy expenditure resulting in increased adiposity and eventually obesity. These knockout (KO) mice demonstrate obesity-pathophysiological features such as hyperleptinemia, hyperinsulinemia, hyperlipidemia, and late-onset hyperglycemia (6). Correspondently, transgenic mice that ubiquitously overexpress *NmU* are hypophagic and leaner in comparison with wild-type mice. In addition, they are insulin sensitive and have increased mRNA expressions of hypothalamic neuropeptide Y, proopiomelanocortin, and melanin-concentrating hormone (7). The role of NMU in the central regulation of feeding behavior was further supported by the finding that intracerebroventricular administration of NMU to free-fed rats leads to decreased food intake, loss of body weight, increased locomotor activity, elevation of body temperature, and increased heat production (8, 9). The NMU receptor 1 (NMU1R) is abundantly expressed in various peripheral tissues, whereas expression of the NMU receptor 2 (NMU2R) is limited to the CNS with particularly high expression in the hypothalamus (8). A study of a *NMU2R* haplotype in 500 whites from the United Kingdom showed no association with obesity-related traits but identified two highly prevalent ancestral forms of this receptor (10).

Linkage studies in white Europeans have, with a LOD score of 2.3, found suggestive genetic linkage of obesity to the chromosomal location of 4q12, which harbors numerous genes, including *NMU* (11). Based on the anorexic effect of

First Published Online September 19, 2006

* I.H. and S.S.T. contributed equally to this work.

Abbreviations: BED, Binge eating disorder; BMI, body mass index; CI, confidence interval; CNS, central nervous system; KO, knockout; NES, night eating syndrome; NMU, neuromedin U; NMU1R, neuromedin 1 receptor; NMU2R, neuromedin 2 receptor; OR, odds ratio.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

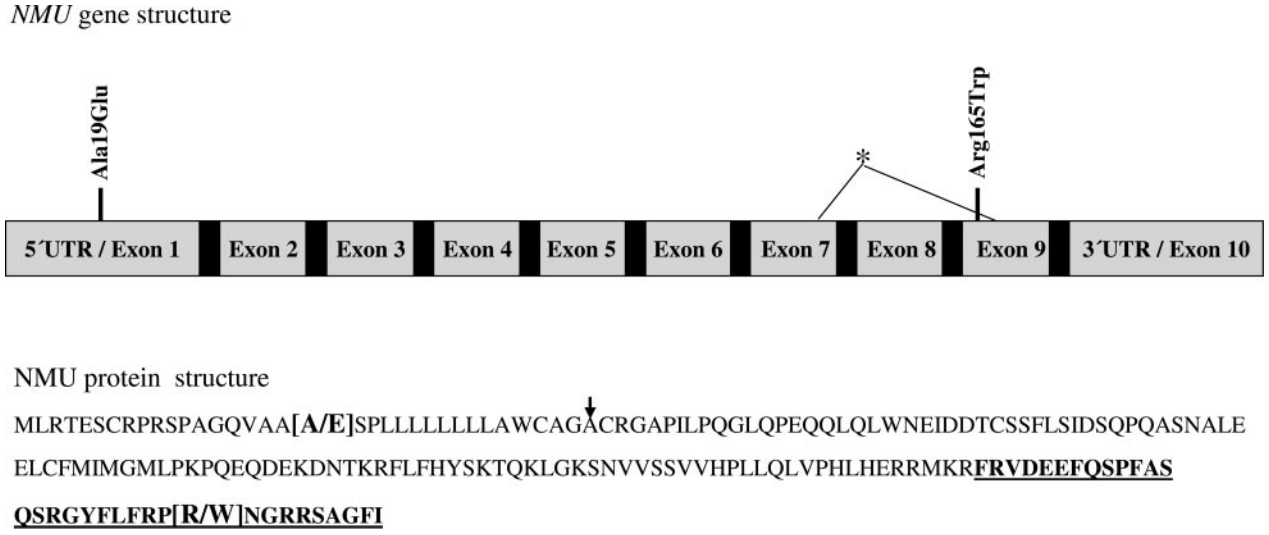

FIG. 1. The structure of the human NMU pre-propeptide. The human NMU precursor contains 174 amino acids including the 25-residue NMU peptide near the C terminus of the precursor. The NMU peptide sequence is in *bold*, and its location is demonstrated by an *asterisk*. The *vertical arrow* indicates the signal peptide cleavage site in humans. The identified variant Ala19Glu is located in the signal peptide of the pre-pro-NMU, and the Arg165Trp mutation is located in the active peptide.

Nmu in rodents and the linkage studies in humans, we consider *NMU* a positional and biological candidate gene in the pathogenesis of human obesity. Here we report the mutation analysis of the coding region and exon-intron boundaries of *NMU* in a cohort of 289 Czech obese children and 84 Danish obese adults and the association of identified novel amino acid variants to childhood obesity and overweight/obesity in the general population of middle-aged Danish whites.

Subjects and Methods

Subjects

We performed a mutation analysis of a cohort of 289 unrelated Czech children and adolescents with early-onset obesity and 84 unrelated Danish obese adults for variants in *NMU*. The Czech subjects (157 girls, 132 boys) aged 1–18 yr were recruited during the years 2003 and 2004 from pediatric endocrinologists and centers specializing in children's weight management. The probands were included in the present study if obesity was observed before the age of 11 yr and if body mass index (BMI) (kg/m^2), retrospectively evaluated, exceeded the 97th percentile for sex and age according to Czech national references (12). The mean age at obesity onset was 4.9 ± 3.1 yr (range, 0.5–11.0 yr), and the mean Z-BMI was 4.3 ± 1.7. All Czech probands were examined for *MC4R* mutations to exclude *MC4R* mutations as a cause of obesity.

The Danish obese subjects (40 men, 44 women), aged 50 ± 15 yr, derived all from obese families, were recruited at Steno Diabetes Center in Copenhagen. The mean BMI was 36 ± 5 kg/m^2.

Fifty-three normal-weight Czech subjects (28 girls/women, 25 boys/men) aged 5–44 yr were randomly recruited from the Department of Pediatrics in Prague and were analyzed for the *NMU* Arg165Trp mutation.

Large-scale genetic epidemiological studies were done in 5851 treatment-naïve subjects of the Inter99 cohort, which is a population-based randomized nonpharmacological intervention study for prevention of cardiovascular disease conducted at the Research Center for Prevention and Health in Glostrup, Denmark (13). The Inter99 participants were analyzed according to their BMI: 1) BMI less than 25.0 kg/m^2 (n = 2586), mean age 45.1 ± 7.9 yr, and mean BMI 22.5 ± 1.8 kg/m^2 and 2) BMI at least 25.0 kg/m^2 (n = 3265), mean age 46.9 ± 7.8 yr, and mean BMI 29.1 ± 3.8 kg/m^2.

Informed consent was obtained from parents of all child probands as well as from all adult participants. The study protocol was approved by the Ethics Committee of the Third Faculty of Medicine, Charles University in Prague, and Ethical Committee of Copenhagen County and was conducted in accordance with the Helsinki Declaration II.

Biochemical measurements

Blood samples for analyses of biochemical variables were drawn in the morning in the fasting state after an overnight fast. Plasma glucose, serum insulin, serum triglyceride, and serum cholesterol measured in the Danish subjects were analyzed using Steno Diabetes Center's standard methods and as previously reported (13).

In the Czech family with the rare *NMU* Arg165Trp mutation, blood samples were analyzed for several biochemical and hormonal variables. Plasma glucose concentration was measured by the enzymatic hexokinase method using the automatic analyzer Konelab 60 (Thermo Clinical Labsystem Oy, Espoo, Finland). C-peptide and insulin in serum were analyzed by a chemiluminescent immunometric technique using the commercial sets Immulite 2000 C-peptide and Immulite 2000 insulin (DPC, Los Angeles, CA). Serum levels of lipids, TSH, and free T_4 were measured using standard assays (Laboratory of the University Hospital, Královské Vinohrady, Prague).

Anthropometrics and eating behavior

Height and weight were measured in light indoor clothes without shoes. BMI was calculated as body weight in kilograms divided by the square of the height in meters. Body circumferences were measured according to the WHO recommendations (14).

All carriers of Arg165Trp variant were asked to fill in the eating inventory (15), the night eating syndrome (NES) (16), and binge eating disorder (BED) (17) questionnaires. Z-scores of BMI, height, and weight were calculated with data obtained from the Czech population as a reference (12). The Z-score represents the number of SD an individual subject deviates from the mean of the age- and sex-matched general population.

Mutation analysis of the coding region of NMU

Analyses of the coding region of *NMU* (NCBI accession no. X76029) were performed by denaturing high-performance liquid chromatography and subsequent confirmation using direct sequencing on both strands (ABI Prism Dye primer cycle sequencing kit) on genomic DNA extracted from whole blood using the QIAamp DNA blood kit (QIAGEN GmbH, Hilden, Germany). To identify homozygous carriers of rare *NMU* variants, the DNA of cases was mixed with wild-type DNA. Primers for mutation detection are available on request. *NMU* Ala19Glu

TABLE 1. Location and frequency of identified variants of *NMU* among Czech and Danish study subjects

Exon	Variant name	RS number	Variant frequency in obese Czech children (%)	Variant frequency in obese Danish adults (%)
1	−151C>T		4.5	0
	Ala18Glu	3828555	4.5	1.0
	Ala19Glu		1.5	7.0
	+23C>G		0.5	0.5
	+70C>T	3792703	4.0	0
2	−100G>A		12.0	7.0
3	−40T>A		0.2	0
4	−46T>C		0.2	0
	Asn76Asn	3805383	42.0	49.0
5				
6	+106A>G	2412666	22.0	37.0
7	−35T>C	3805382	32.0	24.0
	+9T>C	1873091	29.0	36.0
	+38G>A	1873090	29.0	24.0
8	−134A>G	11727729	12.0	15.0
	−172G>A	11722645	12.0	15.0
	+31A>T		0.2	13.0
	+36C>T		21.0	0
	+102A>G		0.2	0
9	−126delT		0.2	0
	Arg165Trp		0.2	0
	+38T>C		13.0	17.0
10	T128C		0.5	0
	+40G>T	2087319	45.0	35.0

The variants were identified in 289 obese Czech children and 84 obese Danish adults. Positions are relative to the nearest exon; minus indicates nucleotide position before the start of the exon; plus indicates position after the end of the exon. A frequency of 0.2 represents one carrier of a particular variant. RS number is given if known. RS, reference single-nucleotide polymorphism.

was genotyped by TaqMan allelic discrimination (KBioscience, Hoddesdon, UK). The genotype success rate was 98%, and the discrepancy rate in the examination of 909 samples was 0%. All genotype distributions obeyed Hardy-Weinberg equilibrium.

Statistical and in silico analyses

Logistic regression with adjustment for sex and age was applied to test for significant differences in genotype distribution in the case-control studies. We did not detect any signs of population stratification in the study sample of 6360 subjects from Inter99 when we applied 39 unlinked markers using the Structure program (18). Differences in quantitative phenotypes between the genotype groups were tested using a general linear model that included genotype and sex as fixed factors and age as covariate. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 13. A P value < 0.05 was considered significant.

Prediction of secondary peptide structure change was done at www.cmpharm.ucsf.edu/~nomi/nnpredict.html. Analysis of conservation of amino acid sequence among species was done at www.ncbi.nlm.nih.gov.

Results

We identified 23 variants in the coding region and exon-intron boundaries of *NMU* by examining 289 obese children and adolescents and 84 adults (Table 1). Large-scale genotyping was restricted to missense variants with an allele frequency above 5% to have enough statistical power to detect association with obesity with an odds ratio (OR) of 1.3 in 5851 subjects. One variant met these criteria, the novel *NMU* Ala19Glu missense polymorphism with an allele frequency of 7%. Furthermore, one novel missense mutation in exon 9 (Arg165Trp) was identified in only one obese child (Table 1).

The Ala19Glu polymorphism was genotyped in the Inter99 cohort and analyzed in a case-control setting for association with overweight and obesity. Homozygous carriers of the Glu allele had an increased prevalence of overweight

TABLE 2. Association study of the *NMU* Ala19Glu polymorphism with overweight/obesity in Danish whites

Group	n	Ala/Ala (%)	Ala/Glu (%)	Glu/Glu (%)	MAF (95% CI)	P, Ala/Ala + Ala/Glu + Glu/Glu	P, Ala/Ala *vs.* Ala/Glu + Glu/Glu	P, Ala/Ala + Ala/Glu *vs.* Glu/Glu
All								
BMI < 25 kg/m^2	2586	2237 (86.5)	340 (13.1)	9 (0.4)	6.9 (6.2–7.6)	0.07	0.2	0.01 [OR, 2.5 (1.2–5.3)]
BMI ≥ 25 kg/m^2	3265	2784 (85.3)	453 (13.9)	28 (0.9)	7.8 (7.1–8.4)			
Men								
BMI < 25 kg/m^2	1013	879 (86.8)	132 (13.0)	2 (0.2)	6.7 (5.6–7.8)	0.07	0.1	0.04 [OR, 4.8 (1.1−20.8)]
BMI ≥ 25 kg/m^2	1906	1613 (84.6)	275 (14.4)	18 (0.9)	8.2 (7.3–9.0)			
Women								
BMI < 25 kg/m^2	1573	1358 (86.3)	208 (13.2)	7 (0.4)	7.1 (6.2–8.0)	0.7	0.9	0.4
BMI ≥ 25 kg/m^2	1359	1171 (86.2)	178 (13.1)	10 (0.7)	7.3 (6.3–8.3)			

P values are calculated using logistic regression with sex and age as covariates and describe the significance level comparing lean and overweight/obese subjects. OR is given with 95% CI. MAF, Minor allele frequency.

TABLE 3. Association studies of the *NMU* Ala19Glu with obesity-related quantitative traits in Danish whites

Trait	Ala/Ala (%)	Ala/Glu (%)	Glu/Glu (%)	*P*, Ala/Ala + Ala/Glu + Glu/Glu	*P*, Ala/Ala *vs.* Ala/Glu + Glu/Glu	*P*, Ala/Ala + Ala/Glu *vs.* Glu/Glu
All						
n	5021	793	37			
BMI (kg/m^2)	26.2 (26.1–26.3)	26.1 (25.8–26.4)	27.4 (25.9–28.8)	0.2	0.9	0.1
Waist (cm)	86.5 (86.1–86.9)	86.2 (85.3–87.1)	91.1 (86.9–95.3)	0.08	0.9	0.03
Hip (cm)	100.8 (100.6–101.1)	100.7 (100.1–101.4)	104.0 (100.9–107.1)	0.2	1.0	0.05
Men						
n	2492	407	20			
BMI (kg/m^2)	26.7 (26.6−26.9)	26.7 (26.3–27.1)	28.6 (26.9–30.4)	0.1	0.9	0.04
Waist (cm)	92.9 (92.5–93.4)	92.8 (91.7–93.8)	98.4 (93.7–103.2)	0.07	0.9	0.02
Hip (cm)	101.3 (100.9–101.6)	101.6 (100.8–102.3)	106.4 (102.9–109.9)	0.01	0.2	0.005
Women						
n	2529	386	17			
BMI (kg/m^2)	25.7 (25.5–25.9)	25.6 (25.1–26.0)	26.0 (23.6–28.3)	0.9	0.7	0.8
Waist (cm)	80.1 (79.7–80.6)	79.4 (78.1–80.6)	82.5 (76.9–88.1)	0.4	0.3	0.4
Hip (cm)	100.4 (100.0−100.9)	99.8 (98.7−100.9)	101.1 (95.9–106.3)	0.6	0.3	0.7

Data are means (95% CI). Means were adjusted for the effect of age. Phenotypic differences between the genotype groups were tested with a general linear model that included genotype as fixed factor and age as covariate factor.

and obesity [OR, 2.5; 95% confidence interval (CI), 1.2–5.3; $P = 0.01$). Gender-specific analysis showed that this finding was also observed in men only (OR, 4.8; 95% CI, 1.1–20.8; $P = 0.04$) but not in women only ($P = 0.4$) (Table 2). The same Inter99 cohort was examined in the setting of obesity-related quantitative phenotypes, and the variant was associated with increased waist circumference in the total Inter99 population, without known type 2 diabetic patients ($P = 0.03$). In accordance with the case-control studies, gender-specific analyses showed that the variant was associated with increased BMI ($P = 0.04$) and increased waist ($P = 0.02$) and increased hip ($P = 0.005$) circumference in men only (Table 3).

We identified one severely obese child who was heterozygous for an Arg165Trp mutation of *NMU*. On examination, the proband was a 4-yr-old girl, and her weight was 33 kg (Z-weight = 5.9), height was 113.5 cm (Z-height = 1.7), and BMI was 25.6 kg/m^2 (Z-BMI = 5.7). Her birth weight was 3.76 kg (50th percentile). A progressive and accelerated weight gain occurred from the age of 6 months when she had a weight of 9,680 g (Z-weight = 2.6), whereas her weight was 14,870 g at the age of 1 yr (Z-weight = 4.9) (Fig. 2). Since 18 months of age, her height has exceeded the 97th percentile of the national standards of height (12). All adult mutation carriers self-reported as being tall from early childhood. She has had a normal intellectual development.

Studies of the pedigree showed a cosegregation with obesity except for one family member with a history of overweight (30-yr-old man; BMI, 24 kg/m^2; maximum BMI was 26.3 kg/m^2 at the age of 26 yr) (Fig. 3). All obese mutation carriers reported obesity onset at early childhood (0.5–7.0 yr). None of the carriers reported NES or BED. Evaluation of the eating inventory questionnaires of all mutation carriers did not reveal any differences from normal range. There was no biochemical evidence of adrenal or thyroid disorders among the mutation carriers. None of the carriers had hyperinsulinemia or severe hyperglycemia. All carriers of the 165Trp variant except the proband had elevated levels of fasting serum triglycerides (Table 4). This rare mutation was absent in 53 unrelated Czech control subjects.

Discussion

With the first mutation analysis of the coding region of *NMU*, we have identified a novel Ala19Glu polymorphism that in its homozygous form associates with the combined phenotypes of overweight and obesity and with increased waist circumference. Gender-stratified analysis showed that homozygosity of this variant was associated with an increased BMI and increased waist circumference in men only. Comparable gender-gene interaction has, among others, been demonstrated for variants in the *NPY2R* gene, whose encoded protein also acts in the hypothalamus on the regulation of energy balance (19). Furthermore, a recent study shows that there exists a gender difference in the role of hypothalamic-acting hormones in the pathogenesis of obesity (20), which might be related to gender-gene interactions.

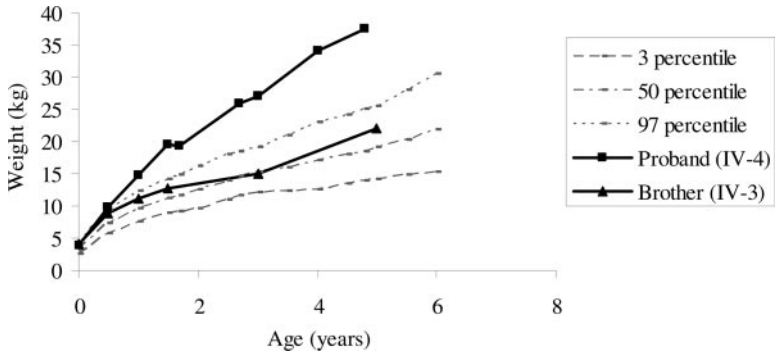

FIG. 2. Weight development of the proband carrying the *NMU* Arg165Trp mutation (IV-4) and the brother carrying the wild type (IV-3). Percentiles are according to the Czech national standard references for girls (12). The brother is included in the figure for comparison with his sister.

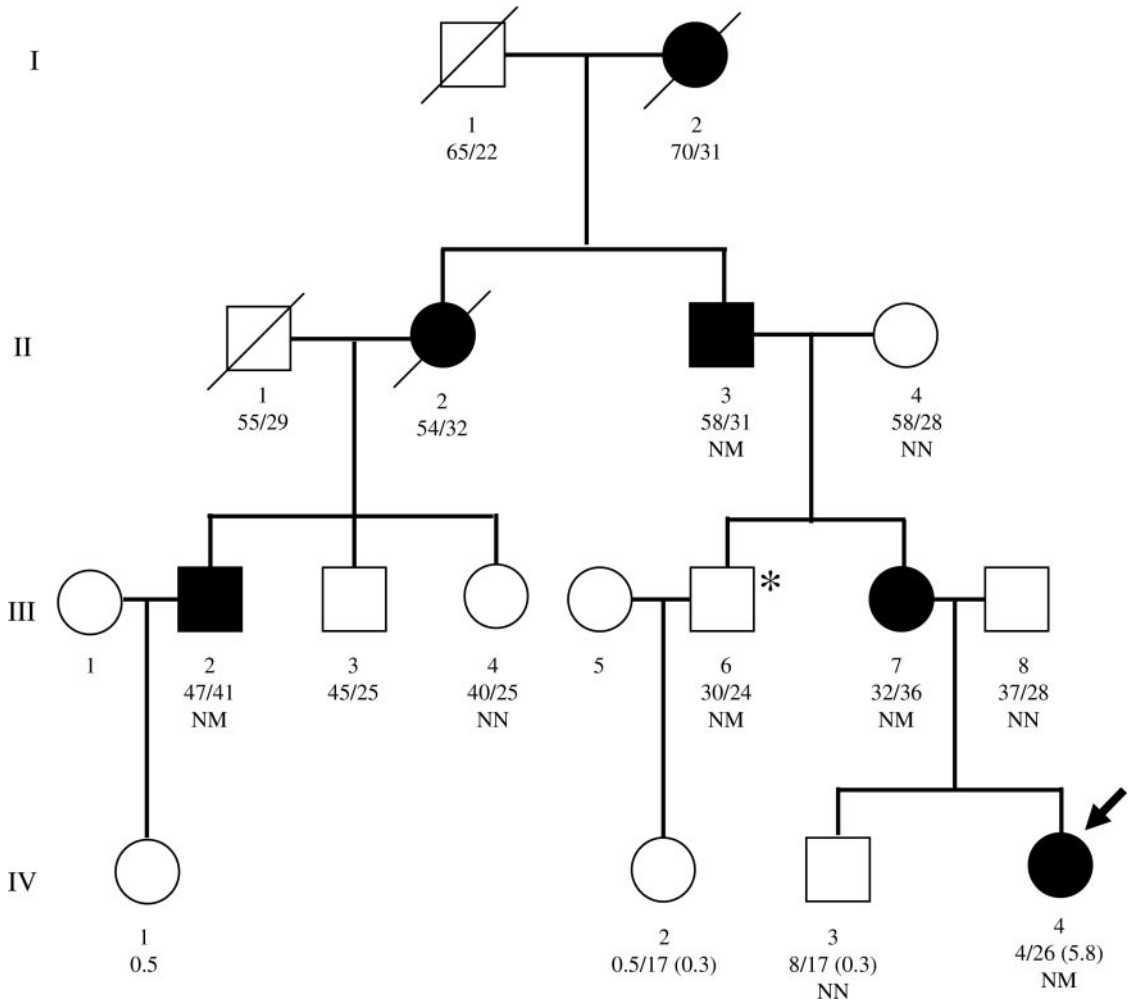

FIG. 3. Pedigree of the family with the *NMU* Arg165Trp mutation. *Black symbols* indicate obese subjects. Obesity in adults (age ≥ 18 yr) is defined as a BMI more than 30 kg/m^2. Obesity in children is defined as a BMI above the 97th percentile according to the Czech national references for a given sex and age (12). *Square symbols* represent men; *round symbols* represent women. The proband is indicated by an *arrow*. An *asterisk* indicates a nonobese mutation carrier with a history of overweight. The text below each individual represents the following: ID no., age (yr)/BMI (kg/m^2) and Z-BMI in subjects less than 18 yr old; genotype NN, no mutation; NM, heterozygous carrier of the *NMU* Arg165Trp mutation.

To date, there exists only one published study of the *NmU* KO mice. All results are shown for the male mouse, with a notice that the female mouse also developed obesity. However, because there is no real comparison reported between the genders of the *NmU* KO mouse, it is unclear whether there also is a gender effect in this specific mouse model. The *NMU* Ala19Glu polymorphism is located in the signal peptide of pre-pro-NMU (Fig. 1), which is important for the cellular translocation and export of the peptide. Ala is a hydrophobic amino acid and is important for the proper signal peptide

TABLE 4. Characteristics of Arg165Trp *NMU* mutation carriers in a Czech family

	IV-4	III-7	III-6	III-2	II-3
Age (yr)	4	32	30	47	58
Weight (kg)	34	104	82	131	94
Height (m)	1.15	1.71	1.85	1.79	1.76
BMI (kg/m^2)	26	36	24	41	31
Maximum BMI (kg/m^2)/at age (yr)	26/4	32/36	26/26	44/36	32/57
Childhood obesity	Yes	Yes	No	Yes	Yes
Obesity from age (yr)	0.5	7		7	6
Fasting serum insulin (<29 mIU/liter)	7	18	3	8	17
Fasting serum C-peptide (290–1320 pmol/liter)	515	1993	809	1257	1819
Fasting plasma glucose (3.6–6.1 mmol/liter)	3.9	6.4	5.0	4.5	5.6
Fasting serum total cholesterol (3.6–5.2 mmol/liter)	3.8	5.2	4.8	6.8	6.2
Fasting serum triglycerides (0.6–1.7 mmol/liter)	1.2	2.6	1.8	2.5	3.2

Identification numbers correspond to those in the pedigree (Fig. 2). Values in parentheses are normal ranges.

docking and following translocation out of the ribosome, whereas the nonhydrophobic Glu might inhibit this docking and following export of the peptide. Furthermore, the Ala allele is conserved among species (*i.e.* monkeys, mice, and rats), and replacement of the Ala-allele with the Glu allele predicts a secondary protein structure change. This information may indicate that Glu19/Glu19 pre-pro-NMU has lower cell exportation ability with following lower levels of circulating NMU, which may explain that homozygous carriers of the Glu allele are more common among overweight and obese people.

The rare *NMU* Arg165Trp mutation cosegregated with childhood-onset obesity in a Czech family with an autosomal dominant inheritance of obesity, suggesting that the *NMU* mutation may cause either haploinsufficiency or a dominant negative protein. The inheritance pattern cannot, however, be clarified because no experiments on KO *NmU* heterozygous mice have been published. One of the mutation carriers was not obese, yet he has had a history of overweight. This nonobese phenotype may be due to a limited penetrance and expression of the mutation as observed in several nonobese carriers of pathogenic *MC4R* mutations (21) and/or interaction with other BMI-regulating genetic and environmental modulators. Interestingly, the mutation carriers showed, like the phenotype of the *NmU* KO mice, hypertriglyceridemia. Although hypertriglyceridemia is frequently associated with obesity, we found hypertriglyceridemia also in the nonobese mutation carrier, suggesting that the *NMU* mutation may have impact independently on both energy and lipid balance as observed in the *NmU* KO mice (6). According to the analyses of questionnaires, the *NMU* Arg165Trp mutation was not related to NES or BED. However, because *ad libitum* food intake was not investigated, it is difficult to precisely quantify and qualify the eating habits of the mutation carriers. The replacement of Arg with Trp at codon 165 of NMU represents a major change from a basic amino acid to a hydrophobic aromatic amino acid, predicting a secondary change of the peptide structure. Moreover, the mutation is located in exon 9, which encodes the biologically active 25-residue-long NMU (NMU-25) (Fig. 1). Among species (*i.e.* rat, mouse, rabbit, pig, chicken, and frog), this is a highly conserved region of *NMU*. These findings suggest that the presence of the Trp165 allele partially disrupts the anorexic function of NMU, maybe through dominant negative antagonism of the receptor, leading to childhood obesity.

Obviously, functional studies would be appropriate to elucidate the impact of the two *NMU* mutations as well as measurements of the circulating levels of NMU. However, to date there are no assays available for measurements of the circulating human NMU protein level. Also, in future studies, it will be important to perform mutation analyses of the regulatory regions of *NMU* to elucidate whether such potential variants have impact on obesity or obesity-related phenotypes. Similarly, pertinent association studies applying tagged single-nucleotide polymorphisms that capture most of the diversity of the *NMU* locus will be of relevance. Furthermore, it is unresolved whether the identified variants in *NMU* that are associated with obesity are in linkage disequilibrium with unidentified variants, in the noncoding regions of *NMU* or in other genes in this genomic region, that are the obesity-causative variants.

In conclusion, an Ala19Glu variant of NMU was associated with an increased prevalence of the combined phenotypes of overweight and obesity in middle-aged white people, and a rare mutation, Arg165Trp, cosegregated with childhood obesity. Together these findings suggest that NMU may play a role in the regulation of the energy balance also in humans.

Acknowledgments

We are grateful to patients, families, and colleagues who referred the patients. We thank A. Forman, I. L. Wantzin, and M. Stendal for dedicated technical assistance and G. Lademann and E. Mattušová for secretarial support.

Received July 5, 2006. Accepted September 11, 2006.

Address all correspondence and requests for reprints to: Irena Hainerová, M.D., Department of Pediatrics, Šrobárova 50, 100 34 Prague 10, Czech Republic. E-mail: ihainer@hotmail.com.

The study was supported by the Czech research project of MSM No. 0021620814, a European Society for Paediatric Endocrinology (ESPE) research fellowship sponsored by Novo Nordisk, The Danish Medical Research Council, The Danish Heart Foundation, The Danish Diabetes Association, The Velux Foundation, and the European Union (EU-GENE2, LSHM-CT-2004-512013).

References

1. **Cummings DE, Schwartz MW** 2003 Genetics and pathophysiology of human obesity. Annu Rev Med 54:453–471
2. **Angelopoulos N, Goula A, Tolis G** 2005 Current knowledge in the neurophysiologic modulation of obesity. Metabolism 54:1202–1217
3. **Austin C, Lo G, Nandha KA, Meleagros L, Bloom SR** 1995 Cloning and characterization of the cDNA encoding the human neuromedin U (NmU) precursor: NmU expression in the human gastrointestinal tract. J Mol Endocrinol 14:157–169
4. **Ballesta J, Carlei F, Bishop AE, Steel JH, Gibson SJ, Fahey M, Hennessey R, Domin J, Bloom SR, Polak JM** 1988 Occurrence and developmental pattern of neuromedin U-immunoreactive nerves in the gastrointestinal tract and brain of the rat. Neuroscience 25:797–816
5. **Domin J, Ghatei MA, Chohan P, Bloom SR** 1987 Neuromedin U: a study of its distribution in the rat. Peptides 8:779–784
6. **Hanada R, Teranishi H, Pearson JT, Kurokawa M, Hosoda H, Fukushima N, Fukue Y, Serino R, Fujihara H, Ueta Y, Ikawa M, Okabe M, Murakami N, Shirai M, Yoshimatsu H, Kangawa K, Kojima M** 2004 Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. Nat Med 10:1067–1073
7. **Kowalski TJ, Spar BD, Markowitz L, Maguire M, Golovko A, Yang S, Farley C, Cook JA, Tetzloff G, Hoos L, Del Vecchio RA, Kazdoba TM, McCool MF, Hwa JJ, Hyde LA, Davis H, Vassileva G, Hedrick JA, Gustafson EL** 2005 Transgenic overexpression of neuromedin U promotes leanness and hypophagia in mice. J Endocrinol 185:151–164
8. **Howard AD, Wang R, Pong SS, Mellin TN, Strack A, Guan XM, Zeng Z, Williams DL, Feighner SD, Nunes CN, Murphy B, Stair JN, Yu H, Jiang Q, Clements MK, Tan CP, McKee KK, Hreniuk DL, McDonals TP, Lynch KR, Evans JF, Austin CP, Caskey CT, Van der Ploeg LHT, Liu Q** 2000 Identification of receptors for neuromedin U and its role in feeding. Nature 406:70–74
9. **Nakazato M, Hanada R, Murakami N, Date Y, Mondal MS, Kojima M, Yoshimatsu H, Kangawa K, Matsukura S** 2000 Central effects of neuromedin U in the regulation of energy homeostasis. Biochem Biophys Res Commun 277:191–194
10. **Bhattacharyya S, Luan J, Farooqi IS, Keogh J, Montague C, Brennand J, Jorde L, Wareham NJ, O'Rahilly S** 2004 Studies of the neuromedin U-2 receptor gene in human obesity: evidence for the existence of two ancestral forms of the receptor. J Endocrinol 183:115–120
11. **Deng HW, Deng H, Liu YJ, Liu YZ, Xu FH, Shen H, Conway T, Li JL, Huang QY, Davies KM, Recker RR** 2002 A genomewide linkage scan for quantitative-trait loci for obesity phenotypes. Am J Hum Genet 70:1138–1151
12. **Kobzová J, Vignerová J, Bláha P, Krejčovský L, Riedlová J** 2004 The 6th nationwide anthropological survey of children and adolescents in the Czech Republic in 2001. Cent Eur J Public Health 2001 12:126–130
13. **Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C**

2003 A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Cardiovasc Prev Rehabil 10:377–386
14. **World Health Organization** 1995 Report of a WHO Expert Committee. Physical status: the use and interpretation of anthropometry. World Health Organ Tech Rep Ser 854. Geneva: World Health Organization; 429–431
15. **Stunkard A, Messick S** 1985 The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 29:71–83
16. **Allison KC, Stunkard AJ, Their SL** 2004 Overcoming night eating syndrome. A step-by-step to breaking the cycle. Oakland, CA: New Harbinger Publications
17. **Devlin MJ, Goldfein JA, Dobrow I** 2003 What is this thing called BED? Current status on binge eating nosology. Int J Eat Disord 34(Suppl 1):S2–S18
18. **Pritchard JK, Stephens M, Donnelly PJ** 2000 Inference of population structure using mulitlocus genotype data. Genetics 155:945–959
19. **Ma L, Tataranni PA, Hanson RL, Infante AM, Kobes S, Bogardus C, Baier LJ** 2005 Variations in peptide YY and Y2 receptor genes are associated with severe obesity in Pima Indian men. Diabetes 54:1598–1602
20. **Vicennati V, Geroni L, Genghini S, Patton L, Pagotto U, Pasquali R** 2006 Sex difference in the relationship between the hypothalamic-pituitary-adrenal axis and sex hormones in obesity. Obesity (Silver Spring) 14:235–243
21. **Dubern B, Clément K, Pelloux V, Froguel P, Giradet JP, Guy-Grand B, Tounian P** 2001 Mutational analysis of melanocortin-4 receptor, agouti-related protein, and α-melanocyte-stimulating hormone genes in severely obese children. J Pediatr 139:204–209

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.