

## GENETIC VARIATION IN THE $\beta_3$ -ADRENERGIC RECEPTOR AND AN INCREASED CAPACITY TO GAIN WEIGHT IN PATIENTS WITH MORBID OBESITY

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**Abstract** *Background.* The  $\beta_3$ -adrenergic receptor, located mainly in adipose tissue, is involved in the regulation of lipolysis and thermogenesis. The potential relevance of this receptor to obesity in humans led us to screen obese French patients for a recently identified mutation in the gene for the receptor.

*Methods.* We used the polymerase chain reaction to amplify a region of the gene for the  $\beta_3$ -adrenergic receptor encoding amino acid residues 27 to 110 in genomic DNA extracted from leukocytes from 185 patients with morbid obesity (body-mass index [the weight in kilograms divided by the square of the height in meters],  $>40$ ) and 94 normal subjects. A mutation resulting in the replacement of tryptophan by arginine at position 64 (Trp64Arg) was detected by an analysis of restriction-fragment-length polymorphisms with the use of the endonuclease BstNI, which discriminates between the normal and mutant sequences.

*Results.* The frequency of the Trp64Arg allele was similar in the morbidly obese patients and the normal subjects (0.08 and 0.10, respectively). However, the patients with morbid obesity who were heterozygous for the Trp64Arg mutation had an increased capacity to gain weight; the mean weight in the 14 heterozygous patients was 140 kg, as compared with 126 kg in the 171 patients without the mutation ( $P=0.03$ ). There were no homozygotes in this sample. The cumulative 25-year change in weight (from the age of 20 years) was 67 kg in the Trp64Arg heterozygotes, as compared with 51 kg in those without the mutation. The maximal weight differential (the maximal lifetime weight minus the weight at 20 years of age) in the Trp64Arg heterozygotes was 74 kg, as compared with 59 kg in the patients without the mutation ( $P=0.02$ ).

*Conclusions.* People with the Trp64Arg mutation of the gene for the  $\beta_3$ -adrenergic receptor may have an increased capacity to gain weight. (*N Engl J Med* 1995;333:352-4.)

**B**OTH environmental and genetic factors are involved in the onset and progression of weight gain.<sup>1</sup> Morbid obesity in humans (body-mass index [the weight in kilograms divided by the square of the height in meters],  $>40$ ) appears to have a particularly strong genetic component.<sup>2,3</sup> Like non-insulin-dependent diabetes mellitus, obesity appears to be polygenic in nature; no single gene is likely to make a person obese.

Obesity results from an imbalance between caloric intake and energy expenditure. Adipose tissue, which plays a crucial part in regulating the storage and mobilization of energy, has been the focus of efforts to identify candidate genes for obesity. One such gene is that for the  $\beta_3$ -adrenergic receptor,<sup>4,5</sup> which is the main receptor involved in the regulation of thermogenesis and lipolysis in brown and white adipose tissue in rodents.<sup>6</sup> In humans, the  $\beta_3$ -adrenergic receptor<sup>4</sup> is expressed predominantly in fat and adipocytes lining the gastrointestinal tract.<sup>7</sup> The receptor's primary role is thought to be the regulation of the resting metabolic rate and lipolysis.<sup>8</sup>

The postulated role of the  $\beta_3$ -adrenergic receptor in fat metabolism, its functional deficiency in genetically obese mice,<sup>9,10</sup> and the results of studies in which the gene for the receptor has been disrupted in mice<sup>11</sup>

prompted us to investigate the role of the  $\beta_3$ -adrenergic receptor in patients with morbid obesity. We determined the prevalence of a mutation of the gene for the  $\beta_3$ -adrenergic receptor that results in the replacement of tryptophan by arginine at position 64 (Trp64Arg) in normal subjects and patients with morbid obesity in France.

### METHODS

#### Patients

The prevalence of the mutation was determined in a group of 94 normal subjects and 185 unrelated patients with morbid obesity. The normal subjects (60 women and 34 men) had a mean ( $\pm$ SD) body-mass index of  $25\pm 5$  and a mean age of  $59\pm 11$  years. The morbidly obese patients were randomly recruited from the Department of Nutrition at Hôtel Dieu Hospital in Paris. The patients (152 women and 33 men) had a mean body-mass index of  $47\pm 7$  and a mean age of  $47\pm 12$  years. Their weight ranged from 90 to 221 kg. Sixty-seven patients (36 percent) had diabetes mellitus, and 28 (15 percent) had glucose intolerance, according to the criteria of the World Health Organization. All the patients underwent physical examinations, and full family histories were obtained. Blood samples were drawn for the extraction of genomic DNA from leukocytes. The study protocol was approved by the hospital ethics committee, and all the subjects gave written informed consent.

#### Analysis of Restriction-Fragment–Length Polymorphisms

Amplification of DNA by the polymerase chain reaction (PCR) was carried out under standard conditions.<sup>12</sup> The amplified fragments were digested with BstNI and analyzed by agarose-gel electrophoresis, as described elsewhere<sup>13</sup> (Fig. 1).

#### Statistical Analysis

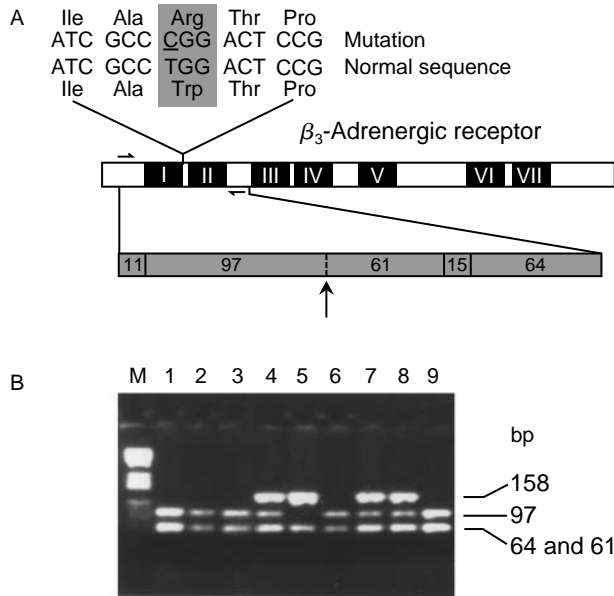
Statistical analyses were performed with the chi-square test for qualitative variables and the nonparametric Mann–Whitney U test for quantitative variables (Statview II statistical package, Abacus Concepts, Berkeley, Calif.). All data are expressed as means  $\pm$ SD.

### RESULTS

Despite the difference in the mean body-mass index between the two groups ( $25\pm 5$  in the normal subjects

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Supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the University of Paris VII, the Ministry for Research and Technology, the Bristol-Myers Squibb Company, the Ligue Nationale contre le Cancer, the Fondation pour la Recherche Médicale Française, and the Association pour la Recherche contre le Cancer. Dr. Manning is the recipient of a European Union Human Capital Mobility Grant.



**Figure 1.** PCR Amplification and Agarose-Gel Electrophoretic Analysis of the Region of the  $\beta_3$ -Adrenergic Receptor Encompassing the Mutation in Transmembrane Domain I.

In Panel A the  $\beta_3$ -adrenergic receptor is shown with the transmembrane domains indicated by the numbered black boxes. The positions of the forward and reverse PCR primers are indicated. The polymorphic region is shown above the receptor, with the mutated nucleotide underlined and the changed codon shaded. The final 248-bp PCR product is represented as a shaded box with the fragment sizes after restriction-enzyme digestion. The arrow shows where the mutation ablates the *Bst*NI site.

Panel B shows an ethidium bromide-stained 3 percent agarose gel of *Bst*NI-digested fragments of the  $\beta_3$ -adrenergic receptor after PCR amplification. Lanes 1, 2, 3, 6, and 9 contain normal receptors (*Trp*64); lanes 4, 7, and 8 contain receptors from *Trp*64Arg heterozygotes; and lane 5 contains receptors from a *Trp*64Arg homozygote. With the normal receptors, only two fragments appear: a 97-bp fragment and a doublet of 64 and 61 bp, which are nonresolvable. *Trp*64Arg homozygosity causes the disappearance of the 61-bp and 97-bp bands and the appearance of a 158-bp band. Heterozygotes have the 158-bp band, the 97-bp band, and the 64-bp and 61-bp doublet. The 15-bp and 11-bp fragments, present in all the digests, are too small to be seen on the gel. M denotes a 1-kb marker (*BRL*). PCR amplification was carried out with the following primers: forward, 5' CCAGTGGGCTGCCAGGGG3'; and reverse, 5' GCCAGTGGCGCCCAACGG3'.

and  $47 \pm 7$  in the morbidly obese patients), the allelic frequency was similar (0.08 in the morbidly obese patients and 0.10 in the normal subjects), even when adjustments were made for differences in age and sex. Thus, there was no direct correlation between the presence of the *Trp*64Arg mutation and the development of morbid obesity.

Further analysis revealed that the mutation may have deleterious effects on the progression of obesity. Of the 185 morbidly obese patients, 14 were heterozygous for the *Trp*64Arg mutation (Table 1); none were homozygous. The two subgroups were similar in age, ratio of women to men, and height. The mean body-mass index in the morbidly obese patients with the mutation was slightly higher than that in the patients without the mutation ( $51 \pm 9$  vs.  $47 \pm 7$ ,  $P = 0.11$ ). How-

ever, the mean weight was significantly higher in the heterozygous group ( $140 \pm 29$  kg vs.  $126 \pm 23$  kg,  $P = 0.03$ ), as was the increase in weight over a 25-year period (from the age of 20 years) ( $67 \pm 22$  kg vs.  $51 \pm 24$  kg,  $P = 0.007$ ) (Table 1). The maximal weight differential (defined as the maximal lifetime weight minus the weight at the age of 20 years) was also significantly higher in the heterozygotes ( $74 \pm 27$  kg) than in the patients without the mutation ( $59 \pm 27$  kg,  $P = 0.02$ ). There were no discernible differences between the two groups in the age at the onset of obesity, frequency of diabetes, or ratio of the circumference of the waist to that of the hips, although the last variable is difficult to measure in massively obese patients.

To determine whether hereditary obesity was associated with inheritance of the *Trp*64Arg mutation, we examined the family members of four morbidly obese, mutation-carrying probands for the presence of the mutation. Three generations were studied in one family and two generations in the other three families. The results of these analyses are shown in Figure 2. In Family A the proband (Subject 4) was a heterozygous 45-year-old woman with a body-mass index of 49. Her massively obese 65-year-old mother (Subject 1), who had doubled her weight over 40 years (from 80 to 166 kg), was homozygous. The proband's morbidly obese 37-year-old sister (Subject 3) and two daughters (Subjects 6 and 7) were heterozygous. The older daughter's weight was normal for her age (between the 50th and 75th percentiles), and the younger daughter was slightly overweight (between the 75th and 90th percentiles).<sup>14</sup> In Family B, the proband (Subject 5) was a morbidly obese woman whose two sisters were not obese. Her 64-year-old father (Subject 1) had a history of morbid obesity (maximal lifetime body-mass index, 40). In the other two families each proband was an only child. In Family C, the proband (Subject 3) was a heterozygote who had received the allele from her morbidly obese mother (Subject 2). In Family D, the proband (Subject 3) was a massively obese 47-year-old man whose homozygous father (Subject 1) had a history of obesity,

**Table 1.** Demographic and Clinical Characteristics of 185 Morbidly Obese Patients, According to the Presence or Absence of the *Trp*64Arg Mutation in the Gene for the  $\beta_3$ -Adrenergic Receptor.\*

CHARACTERISTIC	WITH MUTATION (N = 14)	WITHOUT MUTATION (N = 171)	P VALUE
Age (yr)	44 ± 11	48 ± 12	0.14
Sex (F/M)	10/4	142/29	0.46
Age at maximal weight (yr)	42 ± 6	45 ± 11	0.27
Body-mass index	51 ± 9	47 ± 7	0.11
Height (cm)	166 ± 8	164 ± 9	0.29
Weight (kg)			
Current	140 ± 29	126 ± 23	0.03
At 20 yr	73 ± 15	76 ± 19	0.70
Maximal	147 ± 33	135 ± 28	0.14
Current weight minus weight at 20 yr	67 ± 22	51 ± 24	0.007
Maximal weight minus weight at 20 yr	74 ± 27	59 ± 27	0.02

\*Plus-minus values are means ± SD.

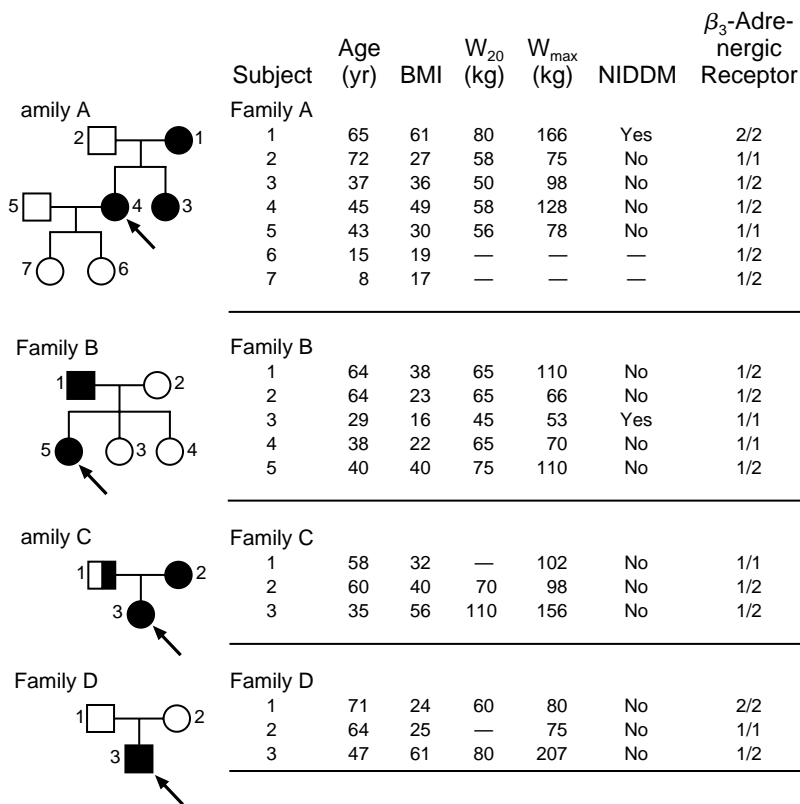


Figure 2. Pedigrees and Clinical Features of the Families of Four Morbidly Obese Patients.

The solid circles and squares represent female and male family members, respectively, with morbid obesity, the half-solid square a male family member with mild obesity, and the arrows probands. BMI denotes body-mass index,  $W_{20}$  the weight at 20 years of age,  $W_{max}$  the maximal weight during adulthood, and NIDDM non-insulin-dependent diabetes mellitus. The genotype for the  $\beta_3$ -adrenergic receptor is indicated as 2/2 (Trp64Arg homozygote), 1/2 (Trp64Arg heterozygote), or 1/1 (wild type).

myocardial infarction, and stroke. In certain family members the progression of the obesity in association with the presence of the Trp64Arg mutation was strongly influenced by environmental and health factors — for example, the restriction of food intake helped the daughters of the proband in Family A and the father of the proband in Family D to control their weight.

## DISCUSSION

This study confirms the presence of a mutation in the gene for the  $\beta_3$ -adrenergic receptor in morbidly obese patients and normal subjects in France. The similarity in the allelic frequency of the mutation in the two groups suggests that the gene is not a major determinant of obesity. However, the mutation may contribute to the capacity to gain weight in persons at high risk for obesity due to other, possibly additive, genetic, environmental, and behavioral factors. For example, in Family D, the proband's father, who was homozygous for the mutation, maintained a normal weight by means

of dietary restriction, but in Family A, the proband's homozygous mother was morbidly obese; both had heterozygous offspring with severe obesity.

The role of such a mutation in the pathogenesis of obesity is conjectural but may be related to a lowering of the resting metabolic rate, which is genetically determined.<sup>15</sup> A decrease in the metabolic rate may be caused by functional differences between the Trp64Arg mutation in the gene for the  $\beta_3$ -adrenergic receptor and the normal gene for the receptor. In obese patients with the mutation, defects in  $\beta_3$ -adrenergic-receptor binding, signal transduction, or regulatory mechanisms may result in a diminished lipolytic response in adipose tissue, thereby exacerbating the obesity.

We are indebted to Ms. Veronique Pelloux and Ms. Nathalie Deschamps for technical assistance and to Assistance Publique—Hôpitaux de Paris for help in developing the clinical protocols used in this study.

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