Heterozygosity for \( \text{Lepr}^{\text{ob}} \) or \( \text{Lepr}^{\text{db}} \) affects body composition and leptin homeostasis in adult mice

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Chung, Wendy K., Kristen Belfi, Melvin Chua, Jennifer Wiley, Ronald Mackintosh, Margery Nicolson, Carol N. Boozer, and Rudolph L. Leibel. Heterozygosity for \( \text{Lepr}^{\text{ob}} \) or \( \text{Lepr}^{\text{db}} \) affects body composition and leptin homeostasis in adult mice. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R985–R990, 1998.—In an effort to understand the genetics of human obesity, we have studied the physiology and molecular genetics of rodent models with monogenic forms of obesity including the leptin gene-defective \( \text{Lepr}^{\text{db}} \)/\( \text{Lepr}^{\text{ob}} \) and leptin receptor gene-defective \( \text{Lepr}^{\text{db}} \)/\( \text{Lepr}^{\text{ob}} \) mouse. In the experiments reported here, we investigated the effects of heterozygosity at \( \text{Lepr}^{\text{db}} \) and \( \text{Lepr}^{\text{ob}} \) on body composition and circulating leptin concentration in +/-, \( \text{Lepr}^{\text{db}} \)/+, and \( \text{Lepr}^{\text{db}} \)/+ adult mice to identify possible gene dosage effects of these mutations that might elucidate their physiology. Adult mice heterozygous for the \( \text{Lepr}^{\text{db}} \) or \( \text{Lepr}^{\text{ob}} \) allele had equivalent fat mass and percentage body fat, which was increased 27–47% and 23–35%, respectively, relative to +/- littermates. Plasma leptin concentrations adjusted for fat mass were 6.5 ng/ml in the \( \text{Lepr}^{\text{db}} \)/+ mouse and 9.6 ng/ml in the +/-, and 11.5 ng/ml in the \( \text{Lepr}^{\text{db}} \)/+ mouse. Sex had no effect on plasma leptin after controlling for fat mass. These data, and data from a small number of mice heterozygous at both \( \text{Lepr}^{\text{db}} \) and \( \text{Lepr}^{\text{ob}} \) (compound heterozygous), suggest that leptin protein produced per mass of body fat is reduced in \( \text{Lepr}^{\text{db}} \)/+ mice and that body fat is increased in \( \text{Lepr}^{\text{db}} \)/+ mice until plasma leptin concentrations reach that of a normal +/- mouse. The elevated plasma leptin concentration in the \( \text{Lepr}^{\text{db}} \)/+ mice suggests that \( \text{LEPR} \) may mediate autocrine suppression of \( \text{Lep} \) expression. These results raise the possibility that human mutations that have even subtle effects on the leptin/leptin receptor system in either the homozygous or heterozygous state may have significant effects on adiposity.

leptin receptor; fat mass; obesity; diabetes

OBESITY IS A MAJOR HEALTH problem in the western world due to its contributions to cardiovascular disease, non-insulin-dependent diabetes mellitus, and cancer (16). The high concordance rates for obesity in identical twins (44, 45), familial clustering of increased body fat (23, 29), and single gene syndromes associated with obesity including Prader-Willi (37), Bardet-Biedel (3, 5), Alstrom (1), and Cohen syndromes (15) suggest a genetic component to obesity in humans.

Molecular cloning of the genes defective in the \( \text{Lepr}^{\text{ob}} \)/\( \text{Lepr}^{\text{ob}} \) and \( \text{Lepr}^{\text{db}} \)/\( \text{Lepr}^{\text{ob}} \) mice, leptin (\( \text{Lep} \)) (52) and leptin receptor (\( \text{Lepr} \)) (8, 9, 30, 47), respectively, has identified a novel hormone-hormone receptor system by which quantities of stored somatic triglycerides can be signaled to the hypothalamus and thereby influence energy intake, expenditure, calorie partitioning, and various endocrine functions (31). Recent identification of human subjects with a loss-of-function mutation in \( \text{Lep} \) (34) and \( \text{Lepr} \) (K. Clement, C. Vaissel, N. Lah lou, S. Cabrol, V. Pelloux, D. Cassotul, M. Gourmelen, C. Dina, J. Chambaz, J.-M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel, and B. Guy-Grand, unpublished observations) suggests that leptin participates in the regulation of body fat in humans. Statistical linkage of the \( \text{Lep} \) genomic region with extreme obesity was independently observed in two Caucasian populations (13, 38), and polymorphisms within \( \text{Lep} \) have been associated with increased adiposity in multiple populations (48; P. Behn, W. K. Chung, C. Iannotti, K. Clement, M. Province, C. Wellig, J. McGill, S. Dagogo-Jack, P. Froguel, R. L. Leibel, and M. Permutt; Y. Chagnon, W. K. Chung, L. Perusse, M. Chagnon, S. Roy, R. L. Leibel, and C. Bouchard; and M. Karvonen, U. Pesonen, S. Leal, R. Sipilainen, A. Rissanen, H. Naukanen, W. K. Chung, R. L. Leibel, M. Laakso, M. Uusitupa, and M. Koulu, unpublished observations).

Given the high prevalence of obesity in western societies and the relative rarity (despite extensive searches) of subjects homozygous for loss-of-function mutations in genes associated with monogenic rodent obesity (6, 11, 19, 32), it is unlikely that a significant proportion of the variation in adiposity in humans will be explained by homoyzosity for gross alterations in \( \text{Lep} \) or \( \text{Lepr} \). However, more subtle and common allelic variants, or heterozygosity for loss-of-function alleles at these and other genes, may act in concert to determine genetic predisposition to increased adiposity within a permissive environment. Subtle heterozygous phenotypes, such as increased length of survival during a fast (17) in \( \text{Lepr}^{+/+} \) and \( \text{Lepr}^{+/} \) mice and increased adiposity in 7-day-old \( \text{Lepr}^{+/+} \) pups (49), have been described.

We produced +/-, \( \text{Lepr}^{+/+} \), \( \text{Lepr}^{+/} \), and \( \text{Lepr}^{+/+} \) mice to determine whether differences in circulating leptin and body composition could be detected in adult mice that were heterozygous for \( \text{Lepr}^{ob} \) and/or \( \text{Lepr}^{db} \). Such differences might provide insights into the regulation of leptin metabolism and identify a mechanism whereby heterozygosity for sequence variants of this ligand/receptor system might contribute to human obesity.
METHODS

Animals. Eighteen pairs of C57BL/6J Leprdb/+ or C57BL/6J Leprob/+ males and C57BL/6J +/+ females (Jackson Laboratories, Bar Harbor, ME) were bred. Preliminary data on compound heterozygotes (Leprdb/+, Leprob/+) were also generated by breeding Leprdb/+ C57BL/6J males and Leprob/+ C57BL/6J females. The offspring were weaned at 21 days of age and separated into same-sex cages. All animals were kept in a pathogen-free environment at 25°C. Autoclaved water and irradiated chow (Pico Lab mouse breed chow 5058, 9% fat; Purina Mills, St. Louis, MO) were provided ad libitum. The mice were on a 12:12-h light-dark cycle. After weaning, weekly body weight and nasoanal length were determined. Mice were killed by carbon dioxide asphyxiation between the ages of 39 and 120 days after a 2-h fast. At death, sex, body weight, and nasoanal length were determined. Body mass index (BMI) was calculated as body weight divided by nasoanal length squared. Approximately 1.0 ml of blood was removed by cardiac puncture and mixed with 50 μl of 82 μM EDTA anticoagulant. The spleen was removed and frozen at −80°C for DNA extraction. Gastrintestinal contents were removed from each mouse to reduce error in body composition determination. Plasma was decanted and stored at −80°C. Carcasses were stored at −80°C.

Genotyping. Genomic DNA was prepared from the frozen spleens by phenol-chloroform extraction (2). Genotypes at Leprdb and Leprob were determined as previously described (10).

Leptin quantitation. Fasting plasma leptin concentrations were determined with a solid-phase sandwich enzyme immunoassay using an affinity-purified polyclonal antibody immobilized in microtiter wells as previously described (40). Body composition analysis. Carcasses were autoclaved and homogenized in a Polytron for 7–10 min. Twenty-five-milliliter aliquots were stored at −10°C before chemical analysis. Total body water was determined by drying duplicate 1-g samples of homogenate overnight at 90°C to stable weight. Total carcass lipid was determined in triplicate by chloroform:methanol extraction of homogenate samples (22). Nitrogen was determined by an adaptation of the Kjeldahl method (20, 36) and was protein calculated, assuming a nitrogen-to-protein ratio of 0.16.

Statistical analysis. The progeny were grouped by genotype at Lep and/or Lepr, and these genotypic groups were compared by analysis of covariance (ANCOVA) for the dependent variables: body fat mass, percent body fat, and plasma leptin concentration. For the fat mass and percent body fat ANCOVAs, age and sex were treated as covariates. For the plasma leptin ANCOVAs, the covariates were fat mass or age and sex. The ANCOVA requirement for parallelism of regression was found not to be violated at the P < 0.05 level for any of these analyses. Scheffé’s test for unequal n values was employed in making all post hoc pairwise comparisons between the adjusted means of the respective genotypic groups. All statistical analyses were conducted using Statistics software (Statsoft, Tulsa, OK).

RESULTS

One hundred thirty-five progeny were generated (mean age = 67.5 days ± 22.3 days SD): 64 +/+, 51 Leprdb/+, 14 Leprob/+, and 6 Leprdb/+ Leprob/+. Sexual dimorphism was evident, with females within each genotypic class having slightly lower BMI (Table 1). There was no statistically significant difference in BMI between genotypic classes of both the +/+ and Leprdb/+ genotypic classes had higher percentage body fat than the corresponding males. There was no sex effect on circulating leptin concentration adjusted for fat mass (P = 0.28).

Fat mass, adjusted for age and sex, was 47.3% higher in the Leprdb/+ animals than +/+ (P = 0.0000001) and 26.7% higher in the Leprob/+ than +/+ animals (P = 0.03), with genotype accounting for a significant difference in fat mass among the three genotypes (P = 0.0000001) (Table 2). Adjusted fat mass was not statistically different between Leprdb/+ and Leprob/+ animals.

Percentage body fat adjusted for age and sex was 35.2% higher in the Leprdb/+ animals than +/+ (P = 0.0000001) and 23.5% higher in the Leprob/+ than +/+ animals (P = 0.007), with genotype accounting for a significant difference in percent body fat among the three genotypes (P = 0.0000001) (Table 2). Percentage body fat was not statistically different between Leprdb/+ and Leprob/+ animals.

Mean plasma leptin concentration was twice as high in the Leprdb/+ animals of each sex relative to the +/+ class (12.4 vs. 6.6 ng/ml for the males; 11.8 vs. 6.2 ng/ml for the females), whereas mean plasma leptin concentrations in the +/+ and Leprob/+ mice were comparable (6.6 vs. 8.8 ng/ml for males; 6.3 vs. 5.2 ng/ml for females) (Table 1). When plasma leptin concentrations were adjusted for fat mass, leptin concentration was 19.8% higher in the Leprdb/+ animals relative to the +/+ (P = 0.03), 32.8% lower in the Leprob/+ animals relative to +/+ (P = 0.03), and 43.9% lower in the Leprdb/+ relative to the Leprdb/+ (P = 0.0001) (Table 2 and Fig. 1). Genotype accounted for a significant difference in plasma leptin concentrations adjusted for fat mass (P = 0.000003).

DISCUSSION

Adult mice heterozygous for the Leprdb or Leprob allele had equivalent fat mass and percentage body fat. These phenotypes were increased 27–47% and 23–35%, respectively, relative to +/+ littermates. Several other phenotypes have been associated with heterozygosity at Leprdb or Leprob when genotype assignment was determined by test crosses. These phenotypes include increased epididymal fat cell size in Leprdb/+ mice (26), prolonged survival of a fast in 7- to 9-mo-old Leprdb/+ and Leprdb/+ mice (17), more effective conversion of acetone to lactate in Leprob/+ and Leprob/+ mice (18), slower glucose oxidation in fat pads of Leprob/+ mice relative to +/+ (50), 40% less cholesterol in the brains of Leprdb/+ relative to +/+ mice (41), hyperinsulinemia in Leprdb/+ mice relative to +/+ controls, and increased (44%) glucose and increased (88%) insulin during glucose tolerance tests in Leprdb/+ mice relative to +/+ controls (21). The existence of a phenotype attributable to heterozygosity for these mutations has potentially important implications regarding the genetics of human obesity. Although there have been reports of human subjects with profound, early onset obesity caused by mutations in Lepr (34), Lepr (K. Clement, C. Vaissel, N. Lahlou, S. Cabrol, V. Pelleux, D. Cassutul,
Table 1. Phenotypic data by sex and genotypic class

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>n</th>
<th>BMI, g/cm²</th>
<th>Fat Mass, g</th>
<th>Percentage Fat</th>
<th>Plasma Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>Male</td>
<td>30</td>
<td>0.326 ± 0.0449</td>
<td>2.70 ± 0.76</td>
<td>9.73 ± 2.09</td>
<td>6.6 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>34</td>
<td>0.318 ± 0.039</td>
<td>2.34 ± 0.69</td>
<td>11.75 ± 2.42</td>
<td>6.3 ± 2.8</td>
</tr>
<tr>
<td>Lepob/+</td>
<td>Male</td>
<td>27</td>
<td>0.346 ± 0.035</td>
<td>3.50 ± 0.66</td>
<td>14.23 ± 1.97</td>
<td>12.4 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24</td>
<td>0.325 ± 0.036</td>
<td>3.32 ± 1.00</td>
<td>15.71 ± 3.41</td>
<td>11.8 ± 5.8</td>
</tr>
<tr>
<td>Lepob/1</td>
<td>Male</td>
<td>7</td>
<td>0.386 ± 0.030</td>
<td>4.80 ± 0.82</td>
<td>15.48 ± 2.35</td>
<td>8.8 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>0.309 ± 0.034</td>
<td>2.74 ± 0.50</td>
<td>14.3 ± 2.48</td>
<td>5.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of animals. Minimum and maximum values, respectively, are given in parentheses. Lepr, leptin receptor gene defective; Lepob, leptin gene defective; BMI, body mass index.

M. Gourmelen, C. Dina, J. Chambaz, J.-M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel, and B. Guy-Grandl, unpublished observations), and proconvertase 1 (PC-1), an enzyme active in the same proneuropeptide processing pathway as carboxypeptidase E that is mutated in the Cpeα/Cpeβ mouse (25), the number of subjects with either homozygous or compound heterozygous loss-of-function mutations in any of these genes has to date been limited to five individuals (three families) despite intensive searches (11, 19, 32, 48; K. Froguel, and B. Guy-Grandl, unpublished observations), and proconvertase 1 (PC-1), an enzyme active in the same proneuropeptide processing pathway as carboxypeptidase E that is mutated in the Cpeα/Cpeβ mouse (25), the number of subjects with either homozygous or compound heterozygous loss-of-function mutations in any of these genes has to date been limited to five individuals (three families) despite intensive searches (11, 19, 32, 48; K. Clement, C. Vaisse, N. Lahlu, S. Cabrol, V. Pelloux, D. Cassutol, M. Gourmelen, C. Dina, J. Chambaz, J.-M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel, and B. Guy-Grandl, unpublished observations). However, heterozygosity for these and yet-to-be-described mutations relating to obesity will, of course, be much more common.

Preliminary data on six individual mice heterozygous for both Leprα and Leprβ demonstrate further increases in sex- and age-adjusted fat mass (3.84 g Lepob/1, Leprβ/1; 3.58 g Leprβ1/+; 3.08 g Leprβ1/+) and age- and sex-adjusted percentage body fat (16.7% Lepob/1, Leprβ/1; 15.5% Leprβ1/+; 14.2% Leprβ1/) over either single heterozygote. The increased adiposity associated with single allele loss of two obesity-related genes also has implications for the genetics of human obesity. Human obesity is a complex trait that in most instances is probably related to the interaction of allelic variation in several obesity-related genes and the environment. Because obesity is a convergent phenotype “resolving” multiple effects on energy intake and expenditure, there is likely to be genetic heterogeneity for obesity susceptibility (39). The responsible genes will likely vary not only among populations but among individuals within a population. Heterozygosity at several loci for alleles with relatively subtle effects on gene expression and function is likely to be a common mechanism for increased adiposity.

Plasma leptin concentration adjusted for fat mass is lowest in the Lepob/+, intermediate in +/-, and highest in Leprβ1/+ mice. Preliminary data indicate that mice doubly heterozygous for Leprα and Leprβ have leptin concentrations adjusted for fat mass that are indistinguishable from those of Leprβ1/+ mice (6.2 vs. 6.5 ng/ml, respectively). These data suggest several things about the regulation of circulating leptin concentration. The decreased plasma leptin concentrations (adjusted for fat mass) in the Leprβ1/+ mice relative to the +/- suggest that induction of the single normal allele in Leprβ1/+ mice is not sufficient to compensate completely for the defective allele, which produces none of the full-length hormone. In contrast, Leprβ1/+ mice have higher plasma leptin concentrations (adjusted for fat mass) than +/- mice, suggesting that they have a defect in feedback regulation of leptin production. Such dysregulation could be due to attenuation of autocrine effects of leptin that are conveyed by the leptin receptor, differences in fat cell size, number or anatomic distribution, or effects mediated by an unidentified feedback mechanism acting through, e.g., the hypothalamus.

Table 2. Age- and sex-adjusted means by genotypic class

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Fat Mass (Adjusted for Age and Sex), g</th>
<th>Percentage Fat (Adjusted for Age and Sex), %</th>
<th>Plasma Leptin (Adjusted for Age and Sex), ng/ml</th>
<th>Plasma Leptin (Adjusted for Fat Mass), ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>64</td>
<td>2.43</td>
<td>11.48</td>
<td>7.2</td>
<td>9.6</td>
</tr>
<tr>
<td>Lepob/1+</td>
<td>51</td>
<td>3.58</td>
<td>15.52</td>
<td>12.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Lepob/1</td>
<td>14</td>
<td>3.08</td>
<td>14.18</td>
<td>5.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Significance (P value)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANCOVA</td>
<td></td>
<td>0.00000001</td>
<td>0.00000001</td>
<td>0.00000001</td>
<td>0.00000001</td>
</tr>
<tr>
<td>+/- vs. Lepob/1+</td>
<td></td>
<td>0.00000001</td>
<td>0.00000001</td>
<td>0.00000001</td>
<td>0.03</td>
</tr>
<tr>
<td>+/- vs. Lepob/1</td>
<td></td>
<td>0.03</td>
<td>0.007</td>
<td>NS</td>
<td>0.03</td>
</tr>
<tr>
<td>Lepob/1+ vs. Lepob/1</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>0.0000006</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Overall genotype effect assessed by analysis of covariance (ANCOVA) with Scheffe’s test for subsequent pairwise differences by genotype.
Similar data demonstrating equivalently elevated circulating leptin concentrations in suckling Lepr<sup>ob</sup>/+ (51) and Lepr<sup>flak</sup>/+ rats (M. Boschmann, unpublished data) suggest that the "long" form of the receptor that includes the complete intracellular signaling domain, the only splice form defective in the Lep<sup>db</sup>/+ mouse, is the splice form responsible for dysregulated leptin production. The apparent equivalence of fat mass-adjusted leptin concentrations in the Lep<sup>ob</sup>/+ and Lep<sup>db</sup>/+, Lepr<sup>db</sup>/+ (doubly heterozygous) mice suggests that leptin is being maximally produced by the single normal Lep allele in the Lep<sup>db</sup>/+ mouse because leptin concentrations are not increased in the Lep<sup>ob</sup>/+, Lep<sup>db</sup>/+, mice despite the tendency to increase leptin production/release demonstrated by the Lep<sup>db</sup>/+ mice. The increase in circulating leptin concentrations in the Lepr<sup>db</sup>/+ mice is unlikely to be attributable to decreased clearance; otherwise Lepr<sup>ob</sup>/+, Lepr<sup>db</sup>/+ mice would have circulating leptin concentrations higher than Lep<sup>db</sup>/+ mice. Lep expression data from Zucker rat pups with a defect in Lep demonstrate increased Lep mRNA in adipose tissue of Lep<sup>ob</sup>/+ relative to +/+ pups (51) and support the hypothesis that dysregulation of leptin metabolism in rodents with heterozygous defects in Lepr is the result of increased leptin production.

Animals with only a single normal leptin allele (Lep<sup>ob</sup>/+) have sex- and age-adjusted plasma leptin concentrations comparable to +/+ animals. However, these "normal" plasma leptin concentrations in the Lep<sup>ob</sup>/+ mice occur in the context of a 26.7% increase in fat mass. These data support the hypothesis that a minimal threshold concentration of leptin in plasma is necessary to sustain eumetabolism i.e., normal thermoregulation, energy expenditure, and fertility (39, 43). The Lep<sup>ob</sup>/+ animals are not capable of producing as much leptin per gram of fat as the wild-type animals and therefore, in a teleological sense, increase their total body fat stores to achieve a "normal" plasma leptin concentration. Thus a single defective Lep allele is associated in this mouse model with what might be regarded operationally as an increased "set point" of somatic fat mass. Similarly, animals with a single defective Lepr allele display increased adiposity despite increased fat-adjusted leptin concentrations, suggesting that Lep<sup>db</sup>/+ mice also increase total body fat stores to achieve a "normal" intensity of postreceptor leptin signaling in relevant target organs (e.g., hypothalamus) (8, 30). The leptin signal is apparently attenuated in the Lep<sup>db</sup>/+ animals as a result of a reduced number of molecules of the intact long receptor isoform (30).

These data, obtained in single and compound heterozygotes for mutations in Lep and Lepr, indicate that these mutations are not fully recessive with regard to either somatic fat mass or the production of leptin by adipose tissue. The former has implications for the complex genetics of human obesity, the latter for the molecular physiological mechanisms underlying the regulation of the "adipostat" (24).

Finally, in none of the genotype groups in the mice was there a significant effect of sex on plasma leptin concentration adjusted for fat mass. This finding is in contrast to the two- to threefold higher plasma leptin per unit fat mass in adult female humans compared with adult males (40). Menopausal status in human females has some influence on leptin per unit fat mass, but postmenopausal women still have higher circulating leptin concentration per unit fat mass than men (40). In humans, there are fat depot-specific differences in leptin expression, with subcutaneous being higher than intra-abdominal fat (35). Exogenous gonadal steroids influence circulating leptin concentrations in rats and humans. Estrogen administration increases leptin production in rats and humans (42). Leptin concentrations decline in human males as puberty (and testosterone production) progresses (12, 33). The lack of sex-related differences in leptin and fat mass in adult mice suggests that the higher concentrations in human
females may be due primarily to their relatively greater subcutaneous adipose mass (28, 46) rather than to direct effects of gonadal steroids on leptin expression in adipose tissue.

**Perspectives**

On the basis of the linkage studies and mutation analyses of candidate genes (e.g., the β3-adrenergic receptor, leptin, the leptin receptor) performed to date, it appears unlikely that a significant fraction of human obesity will be explicable by major functional disruption of any single gene. More likely, the confutation of subtle variations in coding and promoter sequences of several genes will be the usual mechanism. The present study shows that, in this context, classical notions of genetic recessivity may be contravened. That is, that recessivity is not absolute with regard to some phenotypes. Mice heterozygous for a "recessive" mutation in either Lepr or Lep mutations are more obese than the homozygous wild type. Thus, in searching for the genetic bases of complex phenotypes such as obesity, we should not exclude heterozygotes for sequence variations in recessive candidate genes from consideration.

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