Extreme Obesity May Be Linked to Markers Flanking the Human *OB* Gene

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Mice with mutations of the *ob* gene are extremely obese, and the human homologue (*OB*) has been cloned and physically mapped. The protein product of the *ob* gene (leptin) reduces body fat in mice when given exogenously, and leptin has been proposed to provide a lipostatic signal that regulates adiposity. Variation in the *OB* gene may be one genetically determined cause of obesity in human populations. To test this hypothesis, we genotyped siblings from 78 families at markers flanking the human *OB* gene. Pairs of siblings with extreme obesity (BMI ≥40; n = 59) shared haplotypes identical-by-descent for the region containing the *OB* gene at greater than chance levels (corrected P = 0.04). Furthermore, one haplotype containing the *OB* gene was transmitted by heterozygous parents to extremely obese (BMI ≥40) offspring more frequently than expected by chance, indicting significant allelic disequilibrium (corrected P = 0.027). One explanation for these linkage findings is that some individuals with extreme obesity have an allelic variant of the *OB* gene, although other nearby genes could contribute to obesity in these families. *Diabetes* 45:691–694, 1996

Mice with mutations of the *ob* gene are extremely obese. The mouse obesity (*ob*) gene and its human homologue (*OB*) have been cloned (1), and the human gene has been physically mapped to chromosome 7q31.3 (2,3). Injections of the protein derived from the *ob* gene (leptin) reduce body fat in the mouse (4–6), and human leptin may have a similar effect. In humans, leptin appears to be produced exclusively in adipose tissue (7–9) and is secreted into the blood (10). In mice, several lines of evidence suggest that leptin and insulin may interact in metabolic processes. Leptin expression is positively related to plasma insulin levels, with mice rendered diabetic by streptozotocin injections producing less leptin than euglycemic mice. This slowing of leptin production is rapidly reversed by insulin administration (11). Likewise, adipose tissue *ob* mRNA is rapidly induced by a single injection of insulin (12). Mice with mutations of the *ob* gene are also hyperinsulinemic and hyperglycemic, with the severity of these features predicated upon the background strain (13).

The emerging interaction between leptin and insulin levels and the comorbidity of diabetes and obesity in human populations have raised the question of whether mutations or variations in the *OB* gene could contribute to human obesity and diabetes. Stirling et al. (14) found no increased sharing of alleles identical-by-state near the *OB* gene among sibling pairs with NIDDM, but extremely obese sibling pairs were not studied. Considine et al. (8) sequenced the exons of the *OB* gene in five obese subjects (BMI ~42), but did not find the same mutation in the human gene that led to the premature stop codon in the *ob/ob* C57BL/6J mouse. Likewise, Hamilton et al. (15) sequenced the exons of the *OB* gene in two obese patients, but found no mutations.

Although human variation of the *OB* gene has not yet been associated with obesity and diabetes, it is reasonable to expect that mutations of the *OB* gene could result in severe obesity. Plasma leptin levels correlate positively with BMI and body fat percentage in humans (9,10,15), and subjects with morbid obesity (BMI >40) have plasma leptin levels that vary over an order of magnitude (10). One possible cause of this variability might be the presence of low- or high-activity alleles of the *OB* gene. It is reasonable to presume further that the phenotypic expression of low- or no-activity alleles of the *OB* gene could lead to a human phenotype that is as extreme as the mouse phenotype.

To test for linkage between the *OB* gene and human obesity, we examined extreme obesity in 78 families. Haplotypes were constructed using markers closely flanking the human *OB* gene for obese and lean family members, and the average number of haplotypes shared identical-by-descent was determined for obese sibling pairs. If obese siblings shared more haplotypes identical-by-descent at the *OB* gene locus than expected by chance, this result would suggest that variation in this region can confer an obese phenotype.

RESEARCH DESIGN AND METHODS

Subjects. A total of 78 families were identified from an ongoing study of obesity in humans conducted at the University of Pennsylvania. To participate, families had to have at least one obese sibling with a BMI ≥40 kg/m^2, a second obese sibling with a BMI ≥30 kg/m^2, at least one lean parent, and one lean sibling (BMI ≤27 kg/m^2). Some families recruited early in the study lacked a lean parent or a lean sibling or had a somewhat less obese proband (BMI between 35 and 40 kg/m^2). Probands, siblings, mothers, and fathers were measured for height and weight. Body composition was assessed by bioelectric impedance, skinfold thickness (biceps, triceps, subscapular, and supra-iliac), and

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waist and hip circumference. In ~20% of cases, relatives provided only blood and self-reported height and weight when they could not be directly measured. When a family member had died, the surviving spouse or the proband provided the deceased person's height and weight. Information about age at obesity onset, dieting and exercise habits, and medical history was obtained from questionnaires. This protocol was approved by the Institutional Review Board at the University of Pennsylvania, Philadelphia, Pennsylvania.

***OB* chromosomal map location, marker selection, genotyping, and haplotype construction.** The human *OB* gene (accession number U18915) lies in human chromosome 7q31.3, with flanking markers in the following order: D7S1873–D7S1874–D7S680–D7S514–*PAX4*–D7S635–D7S504–*OB*–D7S1875 (2). The physical distance between D7S1873 and D7S1875 is slightly more than 2 Mb. Family members were genotyped for these markers and several markers lying immediately outside this region using previously described methods (16). Details about markers (primer sequence, allele size) were found by consulting the Genome Data Base (GDB) at the official source node at Johns Hopkins in Baltimore, Maryland (17). Haplotypes were constructed by tracing the parental origins of the alleles at markers flanking the *OB* gene and identifying all physical recombination events between markers.

Nonparametric linkage analysis. The mode of inheritance for obesity in human families is not known. We therefore selected nonparametric sibling methods because they do not require the specification of a mode of inheritance and they appear to be the most appropriate for complex phenotypes. Increasingly extreme BMI values were used to define obesity (30–40 kg/m^2), and these increasing thresholds were used to evaluate the prior hypothesis that *OB* gene linkage would be detected only in extremely obese sibling pairs. Small sample size limited the power for tests of higher thresholds (e.g., BMI >45 kg/m^2). An examination of affected sibling pairs (obese-obese) avoided the problems of reduced penetrance, e.g., subjects maintaining a lean phenotype through dieting or exercise. Most families (80–95%) included two or three affected siblings, depending on the obesity criteria used (BMI >30, 35, or 40 kg/m^2), and 82% of families included only two siblings (one pair) for the highest threshold (BMI >40 kg/m^2).

The average proportion of haplotypes shared identical-by-descent was determined for each pair of concordant (obese-obese) sibling pairs. Those values were tested for deviation from the proportion of haplotypes shared under the hypothesis of no linkage (0.50) using the SIBPAL computer program of the S.A.G.E. package (18). The nominal *P* values were corrected for multiple tests associated with ascending cutoff points of BMI (Bonferroni correction). Because the three BMI thresholds are correlated, a correction based on independent tests may be too conservative and thus the true *P* values may be lower.

Transmission test for linkage disequilibrium. The evidence for linkage disequilibrium between the region containing the human *OB* gene and obesity was evaluated by constructing a haplotype using the two closest flanking markers to the *OB* gene (D7S504 and D7S1875) and determining whether the transmission of a particular haplotype from a heterozygous parent to an obese offspring occurred more frequently than expected by chance (19).

RESULTS

For all families studied, extremely obese siblings (BMI ≥40; n = 59) shared more haplotypes from the chromosomal region containing the *OB* gene than expected by chance (P = 0.013; corrected for three tests: P = 0.04; Table 1). The most frequent haplotype immediately flanking the *OB* locus (D7S504 and D7S1875) in the parents was the "1-5" (n = 64; 20.8%) haplotype. No other haplotype accounted for >15% of the total number of observed haplotypes. The 1-5 haplotype was transmitted to extremely obese offspring (BMI ≥40) from heterozygous parents significantly more often than expected by chance (P = 0.009; corrected for three tests: P = 0.027; Table 2).

TABLE 1
Mean proportion of the *OB* gene haplotypes identical by descent for obese-obese sibling pairs

Obese cutoff	Pairs (n)	Proportion identical by descent	t value	Nominal P value	Corrected P value
≥30	213	0.51 ± 0.33	0.24	0.4038	NS
≥35	135	0.50 ± 0.34	0.03	0.4333	NS
≥40	59	0.60 ± 0.33	2.28	0.0132**	0.0396*

Data are means ± SD. Obesity is defined at ascending values of BMI (30–40 kg/m^2). The levels of statistical significance were corrected for three tests using a Bonferroni correction: *$P \leq 0.05$; **$P \leq 0.01$.

DISCUSSION

The combined linkage and linkage-disequilibrium results presented here, as well as the comparable findings obtained in an independent study of French families by Clement et al. (19a, in this issue), make the *OB* gene a strong candidate for obesity in humans. The numerical values of the threshold for extreme obesity that showed linkage to the *OB* region were somewhat less in the French sample (BMI >35) than in the U.S. sample (BMI ≥40). However, the BMI values reflect similar population prevalences of ~1–1.5% (20,21). Thus, these observations are consistent with the hypothesis that mutations or alleles of the *OB* gene confer an extreme phenotype and therefore must be rare in human populations.

One haplotype constructed from the markers most closely flanking the *OB* region is in linkage disequilibrium with extreme obesity. The 1-5 haplotype from marker D7S504 and D7S1875 (respectively) is transmitted by heterozygous parents to obese offspring more often than expected by chance. The 1 allele from D7S504 is common in the population (P = 0.23), as is the 5 allele at D7S1875 (P = 0.34). The significantly greater transmission of this haplotype in preference to others by heterozygous parents to obese children suggests that a mutation or mutations arose on a relatively common background haplotype.

The region of chromosome 7 used to construct the haplotypes in the present analysis is quite small (<2 Mb) and contains the human *OB* gene. However, one other gene (*PAX4*) has also been mapped to this area (2). *PAX4* has a paired-box motif, which encodes a DNA-binding protein, and is a member of a larger gene family. No mutations in the *PAX4* gene have been described in either mice or humans (17). Several human genetic syndromes have been associated with mutations of other genes in the PAX family, such as Waardenburg's syndrome type 1 (*PAX3*) and aniridia (*PAX6*) but neither of these syndromes is characterized by an obese phenotype (17). Although *OB* represents a strong candidate gene for obesity in humans, mutations or alleles of *PAX4* or

TABLE 2
Transmission disequilibrium of a haplotype flanking the *OB* locus (D7S504 and D7S1875) from heterozygous parents

BMI of sibling	1–5 transmitted/ not transmitted	% transmitted	χ^2 (1 df)	Nominal P value	Corrected P value
≥30	71/50	58.7	3.64	0.056	NS
≥35	60/39	60.6	4.45	0.035*	NS
≥40	46/24	65.7	6.91	0.009**	0.027*

The size of allele 1 from D7S504 is 145 bp; the size of allele 5 from D7S1875 is 214 bp. The levels of statistical significance were corrected for three tests using a Bonferroni correction: *$P < 0.05$; **$P < 0.01$.

other undescribed genes in the region cannot be ruled out as contributors to the obesity observed in these families.

One sibling study has demonstrated linkage between the *KELL* locus and BMI in normal-weight French-Canadian families (22). The *KELL* locus is located in 7q33, telomeric to *OB*. The distance between the *KELL* locus and the *OB* gene region is not known, but appears to be about 25 cM based on physical and genetic maps (E.D.G., unpublished observations). This distance makes it difficult to determine whether variation in the *OB* gene could have accounted for the observed linkage in that study. If the linkage reported was due to the *OB* gene, it would suggest that common variation in the *OB* gene may influence the relatively mild obesity present in the French-Canadian sample.

The protein associated with the recently cloned *OB* gene is postulated to encode a lipostatic signal (1,4–6). These linkage results raise the possibility that a mutation or variability in the *OB* gene or its regulatory elements may exist, resulting in the impairment of the proposed negative-feedback loop between adipose tissue and the appetite regulation areas in the hypothalamus. Previous research has demonstrated that ventromedial hypothalamic lesions lead to a marked upregulation of *ob* gene expression (23), further supporting a role for the *ob* gene product in the regulation of body fat. Although the mechanism by which leptin may function in the metabolic control of food intake and body weight regulation is currently unknown, it is clear that a deficit of biologically active leptin (as in the case of the *ob* mouse mutation) results in extreme obesity. If null alleles of the *OB* gene exist in humans, we would expect a similarly obese phenotype.

Finally, linkage studies of complex multigenic traits such as obesity have special problems, such as the lability of the phenotype due to environment and behavior, presumed genetic heterogeneity, and the lack of uniform genetic background. Further problems arise concerning the interpretation of levels of statistical significance for linkage for complex traits. Lander and Kruglyak (24) have proposed a P value of 7×10^{-4} for suggestive evidence for linkage as a correction for multiple tests in a comprehensive genome screen. However, the present study tested an a priori hypothesis for a single candidate gene in a single-point test. As the authors note, these stringent P values may be relaxed in the case of a highly relevant candidate gene (24). Another issue concerns the correction for tests of related phenotypes. A Bonferroni correction of significance levels may be too conservative when related phenotypes are considered (such as increasing levels of BMI to define obesity), and our results suggest that even after these conservative corrections are imposed, the increased sharing of haplotypes from the *OB* region by extremely obese siblings was unlikely to arise because of chance. The present findings and those in the accompanying study by Clement et al. (19a) are consistent with linkage of the *OB* gene and human obesity, but ruling out linkage to other genes in 7q31.3 and even a false-positive linkage result will depend ultimately on the identification of specific allelic variants or mutations associated with obesity.

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REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372:425–432, 1994
2. Green ED, Maffei M, Braden VV, Proenca R, DeSilva U, Zhang Y, Chua SC, Leibel RL, Weissenbach J, Friedman JM: The human obese (*OB*) gene: RNA expression pattern and mapping on the physical, cytogenetic, and genetic maps of chromosome 7. *Genome Res* 5:5–12, 1995
3. Geffroy S, De Vos P, Staels B, Duban B, Auwerx J, de Martinville B: Localization of the human OB gene (OBS) to chromosome 7q32 by fluorescence *in situ* hybridization. *Genomics* 28: 603–604, 1995
4. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. *Science* 269:540–543, 1995
5. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* 269:543–546, 1995
6. Campfield LA, Smith F, Guisez Y, Devos R, Burn P: Recombinant mouse *OB* protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546–549, 1995
7. Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, Mori K, Tamura N, Hosoda K, Yoshimasa Y, Jingami H, Kawada T, Nakao K: Human obese gene expression: adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* 44:855–858, 1995
8. Considine RV, Considine EL, Williams CJ, Nyee MR, Magosin SA, Bauer TL, Rosate EL, Colberg J, Caro JF: Evidence against either a premature stop codon or the absence of *obese* gene mRNA in human obesity. *J Clin Invest* 95:2720–2728, 1995
9. Lönnqvist F, Arner P, Nordfors L, Schalling M: Overexpression of the obese (*ob*) gene in adipose tissue of human obese subjects. *Nature Med* 1:950–953, 1995
10. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Med* 1:1155–1161, 1995
11. MacDougald OA, Hwang C-S, Fan H, Lane MD: Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 92:9034–9037, 1995
12. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girand J, Staels B, Auwerx J: Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377:527–529, 1995
13. Coleman DL, Hummel KP: The influence of genetic background on the expression of the obese (*ob*) gene in the mouse. *Diabetologia* 9:287–293, 1973
14. Stirling B, Cox NJ, Bell GI, Hanis CL, Spielman RS, Concannon P: Identification of microsatellite markers near the human *ob* gene and linkage studies in NIDDM-affected sibling pairs. *Diabetes* 44:999–1001, 1995
15. Hamilton BS, Paglia D, Kwan AYM, Deitel M: Increased *obese* mRNA expression in omental fat cells from masssively obese humans. *Nature Med* 1:953–956, 1995
16. Reed DR, Ding Y, Xu W, Cather C, Price RA: Human obesity does not segregate with the chromosomal regions of Prader-Willi, Bardet-Biedl, Cohen, Borjeson or Wilson-Turner syndromes. *Int J Obesity* 19:599–603, 1995
17. Fasman KH, Cuticchia AJ, Kingsbury DT: The GDB(TM) Human Genome Data Base Anno. *Nucleic Acids Res* 22:3462–3469, 1994
18. Blackwelder WC, Elston RC: Statistical Analysis for Genetic Epidemiology, Release 2.1: computer program package available from the Department of Biometry and Genetics, LSU Medical Center, New Orleans. *Genet Epidemiol* 2:85–97, 1985
19. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
19a. Clement K, Garner C, Hager J, Philippi A, LeDuc C, Carey A, Harris TJR, Jury C, Cardon LR, Basdevant A, Demenais F, Guy-Grand B, North M, Froguel P: Possible evidence for linkage of the human *OB* gene region with extreme obesity. *Diabetes* 45:687–690, 1996
20. Kuczmarski RJ: Prevalence of overweight and weight gain in the United States. *Am J Clin Nutr* 55 (Suppl. 2):495S–502S, 1992

21. Rolland-Cachera MF, Cole TJ, Sempe M, Tichet J, Rossignol C, Charraud A: Body mass index variations: centiles from birth to 87 years. *Eur J Clin Nutr* 45:13–21, 1991
22. Borecki IB, Rice T, Perusse L, Bouchard C, Rao DC: An exploratory investigation of genetic linkage with body composition and fatness phenotypes: the Quebec family study. *Obesity Res* 2:213–219, 1994
23. Funahashi T, Shimomura I, Hiraoka H, Arai T, Takahashi M, Nakamura T, Nozaki S, Yamashita S, Takemura K, Tokunaga K, Matsuzawa Y: Enhanced expression of rat obese (ob) gene in adipose tissues of ventromedial hypothalamus (VMH)-lesioned rats. *Biochem Biophys Res Commun* 211:469–475, 1995
24. Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage findings. *Nature Genet* 11:241–247, 1995