Identification of an Obesity Quantitative Trait Locus on Mouse Chromosome 2 and Evidence of Linkage to Body Fat and Insulin on the Human Homologous Region 20q

Audra V. Lembertas,* Louis Pérusse,‡ Yvon C. Chagnon,‡ Janis S. Fisler,* Craig H. Warden,* Deborah A. Purcell-Huynh,* France T. Dionne,‡ Jacques Gagnon,‡ André Nadeau,§ Aldons J. Lusis,* and Claude Bouchard‡

**Department of Medicine, Department of Microbiology and Molecular Genetics, and Molecular Biology Institute, University of California, Los Angeles, California 90095-1679; and ‡Physical Activity Sciences Laboratory and §Diabetes Research Unit, Laval University, Ste-Foy, Quebec G1K 7P4, Canada*

Abstract

Chromosomal synteny between the mouse model and humans was used to map a gene for the complex trait of obesity. Analysis of NZB/BINJ × SM/J intercross mice located a quantitative trait locus (QTL) for obesity on distal mouse chromosome 2, in a region syntenic with a large region of human chromosome 20, showing linkage to percent body fat (likelihood of the odds [LOD] score 3.6) and fat mass (LOD score 4.3). The QTL was confirmed in a congenic mouse strain. To test whether the QTL contributes to human obesity, we studied linkage between markers located within a 52-cM region extending from 20p12 to 20q13.3 and measures of obesity in 650 French Canadian subjects from 152 pedigrees participating in the Quebec Family Study. Sib-pair analysis based on a maximum of 258 sib pairs revealed suggestive linkages between the percentage of body fat ($P < 0.004$), body mass index ($P < 0.008$), and fasting insulin ($P < 0.0005$) and a locus extending approximately from *ADA* (the adenosine deaminase gene) to *MC3R* (the melanocortin 3 receptor gene). These data provide evidence that a locus on human chromosome 20q contributes to body fat and insulin in a human population, and demonstrate the utility of using interspecies syntenic relationships to find relevant disease loci in humans. (*J. Clin. Invest.* 1997. 100:1240–1247.) Key words: body mass index • *ADA* • *ASP* • *MC3R*

Introduction

Approximately one-third of the adult population in the United States is now overweight or obese (1), and obesity is associated with increased risk for many diseases, including heart disease, diabetes, hypertension, and cancer. The evidence for a genetic basis for obesity is compelling (2–7), and an understanding of the pathways regulating body mass and body composition is beginning to emerge. Single gene mutation mouse models of obesity have been particularly informative and have led to the identification of leptin and other proteins controlling body fat stores (8–13). Nevertheless, the genetic factors contributing to differences in susceptibility to obesity in human populations remain largely unknown. Human obesity is multifactorial, with important environmental influences as well as multiple genetic factors, making analysis of the genetic contributions difficult.

The dissection of complex traits into their underlying genetic elements has begun for a number of diseases, using both human and animal populations (14–17). One method for identifying genetic loci that contribute to complex traits in animals is quantitative trait locus (QTL)[1] mapping, which involves interbreeding two strains of mice which differ in the phenotype of interest for two generations to produce F2 mice. Each of these F2 mice represents a genetically unique combination of parental genes and phenotypes. The genome is then tested for the parental origin of the genetic elements, using polymorphic genetic markers in a whole-genome scan. Finally, statistical association of the inheritance of particular parental traits with unique genetic elements identifies loci which contain genes affecting these traits. These loci are broad, encompassing distances of 15–30 cM. Resolution is limited by the number of genetic recombinations represented in the population examined, and, unlike simple Mendelian traits, a multigenic phenotype may require the coinheritance of minor genetic elements for the effect of the major locus to be seen.

A genetic locus can be isolated from other genes controlling the trait by incorporating a small chromosomal segment containing the gene of interest from one mouse strain onto the genome of a second mouse strain by selective breeding to create a congenic strain (18–21). Thus, comparison of a phenotype of a congenic strain with the phenotype of its background strain allows study of the effects of single genes derived from the donor strain, isolated from the effects of other donor strain genes. The isolation of more than 40 minor histocompatibility genes (22) and the recent production of congenic mouse strains isolating epilepsy QTLs (23), nonobese diabetic mouse–derived diabetogenic genetic intervals (20), and SLE-susceptibility genes (21) demonstrate the feasibility of using congenic strains to dissect the complex pathogenic mechanisms of polygenic disease.

Because of the evolutionary relationship between mice and humans, many ancestral chromosomal segments have been

Reprint requests to A.J. Lusis, Ph.D., Division of Cardiology, 47-123 CHS, UCLA Medical Center, Los Angeles, CA 90095-1679. Phone: 310-825-1359; FAX: 310-794-7345; E-mail: jlusis@medicine.mcdsch.ucla.edu

Address correspondence to Janis S. Fisler, Ph.D., Research Cardiologist, UCLA, 2023 E. Sims Way #245, Port Townsend, WA 98368. Phone: 360-385-2089; FAX: 360-385-7546; E-mail: jfisler@ucla.edu C.H. Warden's current address is Med:Rowe Program, University of California, Davis, CA 95616.

Received for publication 30 December 1996 and accepted in revised form 2 June 1997.

J. Clin. Invest.

0021-9738/97/09/1240/08 $2.00
Volume 100, Number 5, September 1997, 1240–1247
http://www.jci.org

1. *Abbreviations used in this paper:* ADA, adenosine deaminase; ASP, agouti signaling protein; BMI, body mass index; i.b.d., identical by descent; LOD, likelihood of the odds; MC3R, melanocortin 3 receptor; PCK1, phosphoenolpyruvate carboxykinase; PLC1, phospholipase C; QTL, quantitative trait locus.

conserved, where the same genes occur in the same order within discrete regions. Chromosomal rearrangements have placed these syntenic regions on different chromosomes; however, maps of linkage and synteny homologies between mice and humans (24) make it relatively straightforward to test in humans the candidate loci found in mouse studies. Mouse/human synteny relationships have been recently summarized by DeBry and Seldin (25) (http://www.ncbi.nlm.nih.gov/Omim/Homology/). Homologues of single genes that cause obesity in rodents were examined in several human populations with both positive (26, 27) and negative (28–30) results.

We report here the use of a whole-genome search in a mouse cross to identify a gene contributing to multigenic obesity on distal mouse chromosome 2. This locus was subsequently confirmed in a congenic mouse strain. Examination of the homologous region in adult sibships from the Quebec Family Study shows that a locus on human chromosome 20q contributes to body fat and insulin in a human population, and demonstrates the utility of using interspecies syntenic relationships to find relevant disease loci in humans.

Methods

Mouse studies. NZB/BINJ and SM/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). NZB/BINJ females were mated with SM/J males to produce F1 progeny, which were brother–sister mated to produce F2 intercross mice. The NZB/BINJ × SM/J cross was initially set up to study genetic factors in lipoprotein metabolism, and a complete description of the cross and the lipoproteins in these mice was reported previously (31). 84 female 21-d-old F2 mice were weaned onto rodent chow containing 12% calories as fat (Purina 5001; Ralston Purina, St. Louis, MO), then at 3–5 mo were fed an atherogenic diet containing 30% calories as fat (Teklad 90221; Teklad Premier Laboratory Diets, Madison, WI) for 15 wk. Chromosome 2 congenic mice, B10.LP/SnJ (H3, H13), and the corresponding background strain, C57BL/10SnJ, were also purchased from The Jackson Laboratory. The congenic and background mice (n = 5 mice per group) were fed rodent chow (Purina 5001; Ralston Purina) until time of killing at 8 mo. The mice were housed and cared for under conditions meeting AAALAC accreditation standards. All mice were caged individually and given free access to food and water. A 12-h light–dark cycle was maintained throughout the experiment.

Quantitative phenotyping and genotyping of microsatellite markers were done as previously described (31, 32). Mice were fasted ~ 15 h before collection of blood and time of killing at 3 h into the light phase of the diurnal cycle. The mice were anesthetized using isoflurane and killed by cervical dislocation. Blood samples were collected from the retroorbital sinus in plasma separator tubes containing EDTA, placed on ice, and centrifuged to prepare plasma. Plasma insulin was determined in congenic and background mice by RIA using a kit incorporating a rat insulin standard (ICN Biomedicals, Inc., Costa Mesa, CA). Kidneys, liver, and spleen were collected for DNA isolation. In congenic and background mice, four fat pads consisting of three intraabdominal fat pads, the retroperitoneal, mesenteric, and gonadal fat pads, and the femoral fat pad, which is a subcutaneous fat pad on the outer thigh, were dissected, weighed, and returned to the carcass. Body composition was based on the carcass remaining after removal of the liver, kidneys, and spleen. The carcass was dried to constant weight at 90°C, homogenized, and aliquots taken for extraction of lipid in a Soxhlet apparatus. Carcass water and lipid were determined gravimetrically. 84 female mice were analyzed in the F2 intercross. Five female congenic mice and five female background mice were analyzed in the congenic test.

All genotyping proceeded by PCR amplification of microsatellite markers. PCR primers for microsatellite markers were purchased from Research Genetics (Huntsville, AL), and are listed in the figures according to their commercial names. PCR was performed using mouse DNA at a concentration of 2 ng/μl in a volume of 10 μl. The ^{32}P-labeled primers were allowed to anneal at 53°C and extend at 72°C for 25 cycles. The PCR products were visualized by running them on 5% acrylamide denaturing gels, and exposing the dried gels to x-ray film.

ANOVA and regression analysis were done using the Statview 4 statistical program for the Macintosh computer (Abacus Concepts, Inc., Berkeley, CA), while the linkage map and likelihood of the odds (LOD) scores for QTL mapping of the intercross were calculated using the MAPMAKER/QTL program (provided by S. Lincoln and E. Lander, Whitehead Institute for Biomedical Research, Cambridge, MA). An LOD score of 4.3 or greater was considered statistically significant evidence of association in intercross mice (33). The data were adjusted for the effects of age by regression analysis.

Human studies. Selection criteria and the phenotypes measured in the Quebec Family Study have been described previously (34). Quantitation of various measures pertaining to obesity was performed in 650 individuals (351 females and 299 males) from 152 pedigrees during a 1-d visit of the families to the laboratory. Measures included body mass index (BMI = weight in kilograms divided by height in squared meters), percent fat, and subcutaneous fat. Body density was measured by the underwater weighing procedure, and percentage of body fat was computed from the estimate of body density using the Siri equation (35). Fat mass and fat-free mass were computed from percent body fat and body mass. The amount of subcutaneous fat was assessed by summing skinfold thickness (in millimeters) measured at six sites (abdominal, suprailiac, subscapular, medial calf, tricep, and bicep) on the left side of the body with a Harpenden skinfold caliper (Quinton #03496-001; Quinton Instrument Co., Seattle, WA 98121-2791), following the procedures recommended by the International Biological Programme (36). Serum insulin was determined by RIA (37) in fasting subjects. These quantitative traits were adjusted by multiple regression procedures for the effects of age and sex, and standardized. Informed consent was obtained from all subjects after the nature and possible consequences of the study were explained.

All genotyping, with the exception of the melanocortin receptor 3 gene (*MC3R*) marker, was performed by PCR amplification of microsatellite markers, which are listed in the figures and tables according to their commercial names. PCR primers were obtained from Research Genetics except for the marker for the adenosine deaminase gene (*ADA*), which was constructed as described (38). PCR was performed using human DNA at a concentration of 10 ng/μl in a volume of 5 μl. The ^{32}P-labeled primers were allowed to anneal at 58°C and extend at 72°C for 25 cycles. The PCR products were visualized by running them on 5% acrylamide denaturing gels, and exposing the dried gels to x-ray film. The alleles were numbered and identified consistently across families.

RFLP typing of *MC3R* was performed using PstI digestion of 5 μg DNA (New England Biolabs Ltd., Mississiauga, Ontario, Canada). The sizes of the resultant allelic fragments were 4.4 and 3.3 kb, measured by electrophoresis using λDNA digested with EcoRI and HindIII as standards. The alleles were detected by Southern blot analysis, using a 1.1-kb *MC3R* probe labeled with ^{32}P by random priming, and exposing the blots to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) with intensifying screens for 72–120 h at −70°C.

Linkage analysis was performed using the single locus sib-pair linkage method (39). The sib-pair method has the advantage of being a nonparametric method of linkage analysis that requires no a priori knowledge of the mode of inheritance of the phenotype under investigation. The rationale underlying this method is that, in the presence of linkage between a marker locus influencing the quantitative trait, sib pairs sharing a greater proportion of genes (alleles) identical by descent (i.b.d.) at the marker locus will tend to have more similar phenotypes than sib pairs sharing fewer alleles. A negative relationship between the number of alleles shared i.b.d. and within sibship variance is therefore expected under the presence of linkage. Sib-pair analysis was performed using the program SIBPAL (version 2.7.2)

from the S.A.G.E. genetic statistical software package (S.A.G.E., 1994. *Statistical Analysis for Genetic Epidemiology, Release 2.2.*, available from the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH).

Results

Linkage analysis in mice. 84 female F2 mice from a cross between NZB/BINJ and SM/J (31) were typed for measures of obesity and 126 genetic markers on all chromosomes except Y, with an average spacing of < 15 cM. Because the number of loci contributing to the variation in obesity in this cross is unknown, we chose to construct a map in which any gene would on average lie within 10 cM of a typed marker, so the power of detection of linkage would be maximized. The F2 mice ranged from 17.2 to 38.6 g (mean 24.2, SD 4.0) in weight, from 1.12 to 14.82 g (mean 4.48, SD 2.63) in fat mass, and from 6.0 to 38.4 (mean 17.6, SD 7.1) in percentage of body fat. We used the MAPMAKER/QTL program to find genetic loci (QTLs) underlying these traits. The distribution of these traits showed a right-sided skew towards higher values, and, although the untransformed data gave LOD scores of 3.5 or greater, a log transformation normalized the data and was used for subsequent analysis. The only locus in this cross showing significant linkage with obesity-related traits was found on mouse chromosome 2, bordered by markers *D2Mit28* and *D2Mit22.* This locus showed linkage to percent body fat (LOD score 3.6), fat mass (LOD score 4.3), and body weight (LOD score 4.8) (Fig. 1). Variation at this locus explained 36% of the phenotypic variation in percent body fat in the NZB/BINJ × SM/J cross. At this locus, no QTLs > 3 were found for triglycerides or FFA. As this cross was not constructed to study obesity, plasma insulin was not measured in the NZB/BINJ × SM/J cross.

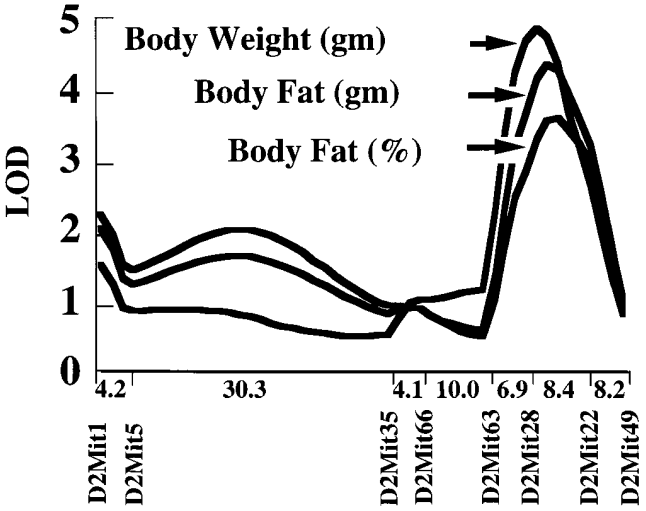

Figure 1. LOD plot for body weight, fat mass, and percent body fat adjusted for a linear effect of age, at the chromosome 2 locus in NZB/BINJ × SM/J intercross (F2) mice (31). Phenotyping and genotyping were done as previously described (31, 32). The *y* axis shows the LOD score calculated by the MAPMAKER/QTL program (67) at 2-cM intervals. The *x* axis shows genetic distance of markers, calculated by the MAPMAKER program (67) as percent recombination (cM). All markers are linked to each other with log likelihood scores of at least 5. The order of these markers is supported by odds ratios of at least 100:1.

We reasoned that the genetic variations influencing obesity in the NZB/BINJ and SM/J cross may be relatively common, since many inbred strains of laboratory mice vary in body fat content and other traits related to obesity (40). Therefore, we examined a previously constructed congenic strain to determine the effect of this chromosomal locus on obesity in mice in the absence of the contributing effects of other genes. The congenic genome consists of DNA which is ~ 98% identical to the background strain, with a small chromosomal segment donated by selective breeding from a second strain. Congenic strain B10.LP/SnJ consists of background strain C57BL/10SnJ, with a region containing the *H3* and *H13* histocompatibility loci on mouse chromosome 2 donated from strain LP/J. The borders of the donated region were mapped and lie ~ 2 cM distal to *Ada* and 2 cM proximal to *H3* (Fig. 2 *A*). When compared to the background strain, the congenic strain had a 7% decrease in body weight [congenic, 20.65±0.56 (standard error), background, 22.21±0.36, $P < 0.0478$], a 66% decrease in retroperitoneal fat pad weight (0.036±0.01, 0.106±0.01, $P < 0.0019$), a 35% lower percent body fat (9.73±1.0, 15.02±0.93, $P < 0.0066$), and 27% lower fasting plasma insulin (6.96±0.92, 9.56±1.59, $P < 0.19$) (Fig. 2 *B*). Plasma triglycerides did not differ in congenic and background mice. FFA were not measured. Together, analysis of the NZB/BINJ × SM/J cross and comparison of the background and congenic strains demonstrate that distal mouse chromosome 2 contains a genetic locus with a substantial influence on body fat.

Linkage analysis in humans. To test whether the corresponding human chromosomal region contributes to multigenic obesity, subjects from the Quebec Family Study were examined. All of human chromosome 20 is homologous to the region of distal mouse chromosome 2 beginning with the gene *Snrbp* at 72 cM and extending to the gene *Acra4* at 108 cM (25). 11 markers within this region of chromosome 20 spanning a 52-cM region extending from approximately 20p12 to 20q13.3 were typed. Since earlier data from The Quebec Family Study indicated linkage of obesity to ADA isoforms (41), typing began with a marker within *ADA* and expanded in either direction. A maximum of 258 sib pairs (Table I), of which 130 pairs (78 female, 52 male) were of the same sex and 128 pairs were of opposite sex, were studied. This population was characterized for the obesity-related phenotypes listed in Table II. The subjects varied from 18 to 94 yr in age and from 4 to 60% body fat.

Results of linkage analysis are presented in Fig. 3. Suggestive evidence of linkage was observed between the percentage of body fat and the markers *PLC1* (phospholipase C gene) ($P < 0.013$), *ADA* ($P < 0.0079$), *D20S17* ($P < 0.0078$), *D20S120* ($P < 0.004$), and *MC3R* ($P < 0.037$). These results suggest the presence of two linkage peaks for percent body fat, one between *PLC1* and *D20S17*, and another ~ 20–25 cM telomeric at *D20S120* and *MC3R* (Fig. 3). The reason for the absence of linkage at *D20S197* and *D20S176* is unclear, given the comparable heterozygosity of these markers. One possibility is that these peaks are not part of the same linkage group. We tested this possibility by using the proportion of alleles shared i.b.d. among siblings to determine linkage groups and to order loci (42). This analysis indicated that these two peaks are actually part of the same linkage group. Another explanation is that there are two closely mapping genes which underlie percentage of body fat, although a total absence of linkage at markers between genes in close proximity is unlikely. Another possibil-

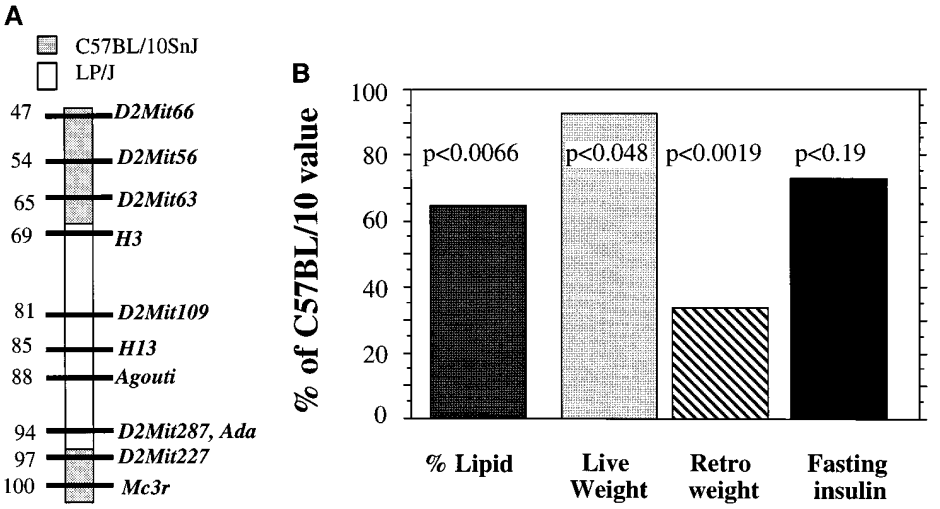

Figure 2. (*A*) The boundaries of the congenic region in B10.LP/SnJ mice: the shaded area represents DNA donated by the C57BL/10SnJ mouse and the open area represents DNA donated by the LP/J mouse. The boundaries were determined by genotyping all indicated markers except for *H3*, *H13*, *Agouti*, and *Ada.* Map distances from the top of the chromosome are indicated. (*B*) Percent differences between the congenic mice (B10.LP/SnJ) and the background strain (C57BL/10SnJ) for percent body fat (*Lipid*), live body weight, retroperitoneal fat pad weight, and fasting insulin. Congenic mice, $n = 5$, and background mice, $n = 5$, were maintained under standard vivarium conditions (chow diet and water ad libitum, 12-h light–dark cycle) before time of killing at 5 mo. The congenic strain was significantly different from the background strain in percent body fat ($P < 0.0066$), body weight ($P < 0.0478$), and retroperitoneal fat pad weight ($P < 0.0019$), but not in insulin ($P < 0.19$).

ity is that a small chromosomal inversion has occured within this population that has placed these two markers at the outer perimeter of the linkage group. Peak linkages for BMI, fat mass, and subcutaneous fat occurred at *MC3R*, with significance of $P < 0.008$, 0.011, and 0.017, respectively. The strongest linkage observed was to fasting insulin at *MC3R* ($P < 0.0005$). Plasma triglycerides and FFA were not examined. It is noteworthy that this region of chromosome 20 does not exert any influence on lean body mass (fat-free mass) and appears to be specifically involved in determining adiposity and fasting insulin levels.

To remove the influence of outliers on the results, the distribution of standardized phenotypes was examined. Individuals with phenotype values beyond 3 SD from the mean were identified and set aside. There were eight outliers for BMI and subcutaneous fat, seven outliers for fasting insulin, six outliers for fat-free mass, five outliers for fat mass, and only one outlier for percent body fat. Linkage analyses were repeated without these individuals, and the results remained largely unchanged for percent body fat. For BMI and subcutaneous fat, the *P* values were slightly reduced but remained significant ($P < 0.05$). The linkage for fat mass shifted slightly distally (*MC3R* $P < 0.046$, *D20S120* $P < 0.016$, *D20S104* $P < 0.011$), but removal of outliers did not affect the linkage results in this region. However, the *P* value for linkage observed between fasting insulin and the *MC3R* marker went from 0.0005 to 0.03. Because the seven outliers for insulin could contribute to a real linkage with the marker for *MC3R*, we repeated the analysis for this phenotype by using a $\log_{10}$ transformation of the data to reduce skewness, and kept the outliers in the analysis. With this

Table I. Linkage Distances, Observed Allele Number, Heterozygosity Index, and Number of Sib Pairs Examined for Six Traits at 11 Markers on Chromosome 20

				Number of sib pairs					
Marker	Distance	No. of alleles	HZ	BMI	Subcut. fat	% Fat	Fat mass	Fat-free mass	Fasting insulin
	cM								
D20S27	36.1	6	0.73	224	225	188	188	188	185
D20S104	40.6	12	0.77	146	147	140	140	140	105
D20S101	50.9	9	0.67	195	196	172	172	172	151
PLC1	58.2	15	0.77	218	219	182	182	182	176
ADA	59.4	6	0.77	226	227	190	190	190	182
D20S17	65.0	7	0.75	209	210	176	176	176	172
D20S197	65.6	12	0.82	221	222	185	185	185	178
D20S176	70.6	4	0.62	151	152	139	139	139	120
D20S120	78.4	14	0.85	210	211	174	174	174	171
MC3R	85.6	2	0.40	258	257	212	212	212	213
D20S171	88.8	13	0.81	203	204	167	167	167	168

Markers are listed in their relative genetic order, and the distance in centiMorgans is from the top of the short arm of chromosome 20. Sex-averaged recombination distances were derived from an integrated genetic and physical map available from the Genome Location Data Base (66). All phenotypes were adjusted for age and sex. *HZ,* heterozygosity index. *Subcut. fat*, subcutaneous fat measured as the sum of six skinfolds.

Table II. Phenotype Measurements of Subjects

Variable	No.	Mean	SD	Min.	Max.
Age (yr)	650	43.2	17.6	18.2	93.5
BMI (kg/m^2)	645	26.4	6.2	16.8	64.9
Subcutaneous fat (mm)	614	115.5	62.2	24	448
Body fat (%)	535	28.2	10.7	4	60
Fat mass (kg)	535	21.3	12.1	2.1	109.3
Fat-free mass (kg)	535	51.3	10.7	29.3	83.4
Fasting insulin (pmol/liter)	475	68.7	56.1	1	588

Min., minimum. *Max.*, maximum.

transformation, the linkage observed remained significant ($P < 0.0008$). Therefore, we are confident that the linkages reported in Fig. 3 are not caused by extreme phenotypic values.

Discussion

This study used chromosomal synteny between the mouse model and humans to map a gene for obesity on human chromosome 20q. An initial whole-genome scan of the NZB/BINJ × SM/J intercross progeny identified a single significant locus on distal mouse chromosome 2. The presence of an obesity gene on distal mouse chromosome 2 was confirmed using an unrelated congenic strain. As this region of mouse chromosome 2 is syntenic to human chromosome 20, we examined linkage between markers located within a 52-cM region extending from 20p12 to 20q13.3 and measures of obesity in French Canadian subjects. Sib-pair analysis of participants in the Quebec Family Study revealed suggestive linkages between percentage of body fat ($P < 0.004$) and fasting insulin ($P < 0.0005$), and a locus encompassing markers from *ADA* through *MC3R*. Suggestive linkages were also found for BMI ($P < 0.008$), subcutaneous fat ($P < 0.017$), and fat mass ($P < 0.011$), at *MC3R*. Although the mouse data predicted a locus affecting body composition in this region, the difference in fasting insulin in the congenic and background mice did not anticipate the very strong linkage seen in the humans. Because no insulin measurements were obtained in the intercross mice, it is unknown whether the chromosome 2 locus influenced insulin levels in these mice.

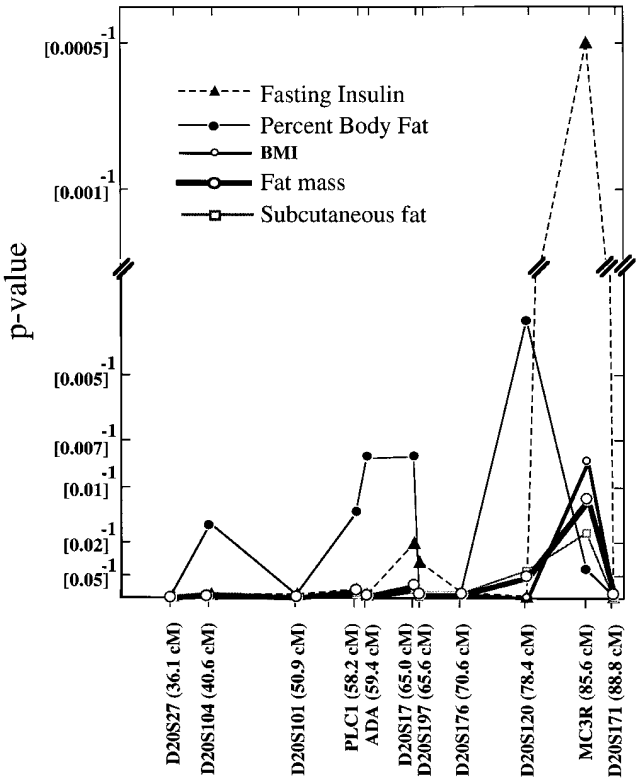

Figure 3. Significance of linkage of markers with obesity phenotypes in the Quebec Family Study subjects. The inverse of the *P* values are plotted on the *y* axis, and the positions of the markers on the chromosome-20 map are given on the *x* axis.

Arguments have been made against the significance of reported linkages of the order of $P = 0.05$ arising from a high-density whole-genome scan (33). Thus, *P* value thresholds of 7.4×10^{-4} have been recommended for suggestive linkage, and 2.2×10^{-5} for significant linkage, in humans using sib-pair analysis (33). However, these guidelines may be too stringent when applied to the present data. In this study, both the QTL in the mouse cross and the difference between congenic and background strains suggested that the search be restricted to a 50-cM region of linked markers on human chromosome 20. Therefore, linkage at this site can be considered a true single-point test of significance rather than the result of a genome-wide search. However, as the number of markers tested increases, the likelihood that linkage will be found purely by chance also increases. If the critical *P* value for 11 random marker tests is $P = 0.05/n$, where *n* is the number of tests, then percentage of body fat at *D20S120* and fasting insulin at *MC3R* still meet the critical cutoff of $P = 0.0045$. Linkage with percentage of body fat at *D20S120* remains even after the removal of outliers beyond 3 SDs. To further establish the stability of these results, we calculated the 95% confidence interval of the regression coefficient for the significant linkages, and found that for *P* values < 0.017, the coefficient of regression remains negative, suggesting that most of the linkages reported in Fig. 3 are significant. However, it is important to confirm these results in additional population samples.

Measurement of multiple phenotypes likewise may require adjustment of the significance level (33). The Quebec Family Study data suggest that BMI and percent body fat likely share common genetic effects (i.e., pleiotropy), and that the bivariate heritability or common heritability between the two phenotypes is 10% (43). Thus, it is likely that one gene or a set of genes influences all of the obesity phenotypes. Although BMI and percent body fat were well correlated within individuals, the covariation between these traits is not necessarily attributable to common genetic factors and may be rather the result of environmental factors specific to each individual. However, under the pleiotropy hypothesis, we assume that the correlation between BMI and percent fat is at least partly accounted for by genetic factors. Therefore, no Bonferroni correction of the *P* values to adjust for multiple obesity phenotypes was made (33).

A complete linkage map of the mouse and human chromosomal regions examined is shown in Fig. 4, where the introgressed region of the congenic mouse model as well as the 90% confidence interval for the mouse QTL are indicated. Given that the confidence interval for the QTL is not precise, the possible genes in the syntenic human region range from the agouti signaling protein gene (*ASP*) to the phosphoenolpyruvate carboxykinase gene (*PCK1*). There are several

attractive candidate genes at this locus. Linkage for percent body fat occurred in the region of *ADA* (Fig. 3), which has been suggested to be a possible candidate gene for obesity (41). ADA is an α-adrenergic agonist with potent lipolytic and vasodilator effects which regulates both lipolysis and insulin sensitivity in human adipose tissue (44–46). Thus, variations in ADA could theoretically explain the effects of this locus on both energy balance and insulin levels. Another candidate at this locus is the *agouti* gene, mutations of which result in ectopic expression of the normal agouti protein, resulting in yellow fur, obesity, and diabetes in mice (8, 11). However, markers linked to the human homolog *ASP* showed no linkage to obesity in two prior studies (see below) (28, 29). An attractive candidate for the human locus is *MC3R*, which is located on human chromosome 20q (47), 8 cM telomeric to *D20S120*, and is linked with percent fat, fat mass, subcutaneous fat, and plasma insulin in this study. *MC3R* is expressed in the hypothalamus, which is involved in neuroendocrine and autonomic control (48). Melanocortin-3 receptors can be activated well by all members of the melanocortin family, including α–, β– and γ–melanocyte-stimulating hormone and ACTH (49). Since *MC3R* has been mapped in mice to ∼ 2 cM distal to *D2Mit52* (50), which maps distal to *D2Mit227*, *MC3R* appears to lie outside the congenic region of the B10.LP/SnJ mouse (see Fig. 2 *A*). However, *MC3R* remains a candidate for the QTL in the NZB/BINJ × SM/J cross and in the human population. Another candidate, mapping to 20q13.3, is cytosolic *PCK1* (51). When the murine homologue of *PCK1* is slightly overexpressed in transgenic rats, it leads to glucose intolerance and an increase in visceral adipose tissue in females (52). It is possible that the locus represents an unknown gene.

Evidence of linkage between various genetic markers and the amount of body fat in humans has been reviewed recently (53). Only a few loci, identified by the use of the single locus sib-pair linkage method with a large number of sib pairs, show moderate to strong linkage ($P < 0.005$) with direct measures of body fat. One of them is the Kell blood group locus (7q33), which showed a strong linkage ($P < 0.0001$) with the amount of subcutaneous fat in 402 sib pairs from the Quebec Family Study (41). More recently, another study on ∼ 250 sib pairs from the Quebec Family Study showed linkage between markers *D1S200* and *D1S476* on 1p32-p22, and fat mass ($0.009 < P < 0.02$) and subcutaneous fat ($P < 0.02$ at *D1S476)* (54). In a different population, a marker (*Tnfir24*) located near *TNF-α* (6p21.3) was shown to be linked ($P < 0.002$) to percent body fat measured by underwater weighing in Pima Indians (55). A single polymorphism was located in the promoter region of *TNF-α*, but association could not be demonstrated between alleles at the polymorphism and percent body fat by these investigators.

Several studies have examined linkage of obesity to the genes which cause monogenic obesity in mice. Positive linkage of obesity measures to the *obese* gene was observed in French obese families (BMI > 35, $P < 0.002$) (26), Mexican Americans (BMI, $P < 0.003$, trunkal skinfold thickness, $P < 0.003$, extremity skinfolds, $P < 0.00014$, waist circumference, $P < 0.00063$) (27), and an American population of extremely obese individuals (BMI > 40, $P < 0.04$) (56). However, the *obese* gene was not linked to obesity, measured as BMI > 30, in a second population of Mexican Americans (30), nor to measures of obesity and energy metabolism in Pima Indians (29). The single gene mutation, *diabetes*, and a QTL, *dietary obese 1*,

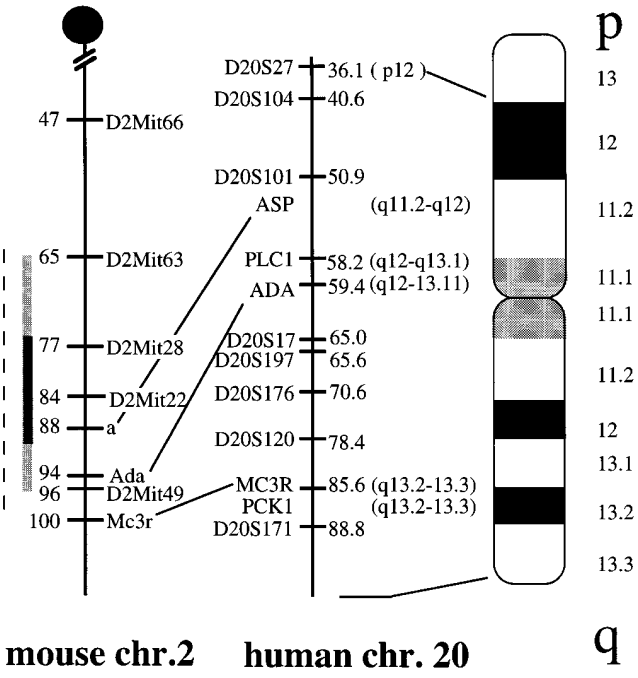

Figure 4. Integrated synteny map of mouse and human chromosomes. The dashed line to the far left of the mouse chromosomes indicates the introgressed region of the congenic mouse model. The bar to the immediate left of the mouse chromosome map indicates the region containing the QTL in the NZB/BINJ × SM/J intercross. The dark region of this bar indicates the 90% confidence interval of this QTL. Marker distances are indicated from the top of the chromosome, with the mouse distances derived from the 1994 Committee Map of the Mouse Genome, and the human from the Genome Location Data Base (66).

map to the mid-portion of mouse chromosome 4, a region syntenic to human 1p31 and 1p35–p31. In adult sibships from the Quebec Family Study, suggestive linkages between markers on human 1p32–p22, and body fat and insulin have been reported recently with the strongest association found with fat mass ($P = 0.009$) (54). Also in the Quebec Family Study, positive linkage of BMI ($P < 0.001$) and skinfold thickness ($P < 0.016$) was found with plasma isoforms of the enzyme ADA (41), the gene for which maps to distal mouse chromosome 2, an area which shows striking linkage conservation with human chromosome 20, with identical gene order but expanded length in the human genome (25). The *agouti* gene, several mutations of which cause obesity in mice (57), maps to this same region, although subsequent studies in other human populations did not find linkage of obesity and polymorphic DNA markers flanking the agouti locus (28, 29). The linkage analyses in these different populations do not necessarily need to be consistent. It is possible for one population to have alleles of a gene at a locus which promote obesity, whereas those alleles could be too rare or too prevalent in a second population for detection of significant linkage. This may be the case in one population reporting negative results (29).

Loci contributing to multigenic obesity, in addition to the one reported here, have been identified recently in humans through wide-genome searches for obesity genes. In one of these studies based on 660 markers spaced at an average of 8 cM and typed in 874 Pima Indians, two loci located on

11q21–q22 and 3p24.2–p22 showed significant linkage (P = 0.001) with percent body fat (58). In another study, 169 markers spaced at ~ 20 cM were typed in 458 Mexican Americans, and a locus located on 2p21 showed evidence of linkage (LOD score 2.7) with fat mass (59).

Results of this study based on syntenic mouse models demonstrate for the first time that a QTL on mouse chromosome 2/human chromosome 20 influences adiposity in humans. Several loci contributing to multigenic obesity, in addition to the one reported here, have been identified in various mouse models (32, 60–63). We chose to examine the human chromosome 20 locus, since the evidence in mice was particularly convincing, the region of synteny was clear, and since we previously found linkage of BMI and skinfold thickness with plasma isoforms of ADA, the gene for which maps to this region. It will be important to study in a similar manner additional loci identified in animal models. An earlier attempt to use this approach to identify the human homologue of an insulin-dependent diabetes mellitus–susceptibility gene first identified in the nonobese diabetic mouse model was not successful (64). It has been argued that the ability of this method to detect human loci depends on the frequency of the alleles conferring susceptibility to a trait in the human population examined (65). However, more recently, using mouse/human synteny relationships to find linkage to obesity with the human homologue of the mouse *diabetes* gene (54), and the mouse *obese* gene has been more successful (26, 27, 56). In the appropriate population, such coordinated studies of the genetic control of obesity in both mice and humans should provide a powerful strategy for identifying the underlying causes of this significant public health problem.

Acknowledgments

The authors thank Dr. Ping-Zi Wen, Ms. Lara Accad, and Mr. Matthew Thimgan for genotyping assistance, and Mr. Mark Cuevas for assistance with mouse phenotyping. Gratitude is also expressed to Monique Chagnon and Anne-Marie Bricault for the MC3R typing. The authors would like to acknowledge Dr. William O. Wilkison from the Glaxo Research Institute who made the MC3R probe available to us. Some of the results presented herein were obtained with the program S.A.G.E., which is supported by a U.S. Public Health Service Resource Grant (1P41 RR03655) from the National Center for Research Resources.

This work was supported by grants from the National Institutes of Health (DK-45066 and HL-42488), and from the Medical Research Council of Canada (PG-11811). A.V. Lembertas was supported by National Institutes of Health training grant DK07685, and L. Pérusse was supported by a research scholarship from Fonds de la Recherche en Sante du Québec (FRSQ)-Québec.

References

1. Kuczmarski, R.S., K.M. Flegal, S.M. Campbell, and C.L. Johnson. 1994. Increasing prevalence of overweight among U.S. adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. *JAMA (J. Am. Med. Assoc.).* 272:205–211.

2. Vogler, G.P., T.I.A. Sorensen, A.J. Stunkard, M.R. Srinivasan, and D.C. Rao. 1995. Influences of genes and shared environment on adult body mass index assessed in an adoption study by a comprehensive path model. *Int. J. Obes.* 19:40–45.

3. Stunkard, A.J., J.R. Harris, N.L. Pedersen, and G.E. McClearn. 1990. The body-mass index of twins who have been reared apart. *N. Engl. J. Med.* 322:1483–1487.

4. Tambs, K., T. Moum, L. Eaves, M. Neale, K. Midthjell, P.G. Lund-Larsen, S. Naess, and J. Holmen. 1991. Genetic and environmental contributions to the variance on the body mass index in a Norwegian sample of first- and second-degree relatives. *Am. J. Hum. Biol.* 3:257–267.

5. Price, R.A., R. Ness, and P. Laskarzewski. 1990. Common major gene inheritance of extreme overweight. *Hum. Biol.* 62:747–765.

6. Rice, T., I.B. Borecki, C. Bouchard, and D.C. Rao. 1993. Segregation analysis of fat mass and other body composition measures derived from underwater weighing. *Am. J. Hum. Genet.* 52:967–973.

7. Bouchard, C., A. Tremblay, J.-P. Després, A. Nadeau, P.J. Lupien, G. Thériault, J. Dussault, S. Moorjani, S. Pineault, and G. Fournier. 1990. The response to long-term overfeeding in identical twins. *N. Engl. J. Med.* 322:1477–1482.

8. Bultman, S.J., E.J. Michaud, and R.P. Woychik. 1992. Molecular characterization of the mouse agouti locus. *Cell.* 71:1195–1204.

9. Zhang, Y., R. Proenca, M. Maffel, M. Barone, L. Leopold, and J.M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature (Lond.).* 372:425–432.

10. Naggert, J.K., L.D. Fricker, O. Varlamov, P.M. Nishina, Y. Rouille, D.F. Steiner, R.J. Carroll, B.J. Paigen, and E.H. Leiter. 1995. Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. *Nat. Genet.* 10:135–141.

11. Miller, M.W., D.M.J. Duhl, H. Vrieling, S.P. Cordes, M.M. Ollmann, B.M. Winkes, and G.S. Barsh. 1993. Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. *Genes Dev.* 7:454–467.

12. Tartaglia, L.A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G.J. Richards, L.A. Campfield, F.T. Clark, J. Deeds, et al. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell.* 83:1263–1271.

13. Chen, H., O. Charlat, L.A. Tartaglia, E.A. Woolf, X. Weng, S.J. Ellis, N.D. Lakey, J. Culpepper, K.J. Moore, R.E. Breitbart, et al. 1996. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell.* 84:491–495.

14. Lander, E.S., and N.J. Schork. 1994. Genetic dissection of complex traits. *Science (Wash. DC).* 265:2037–2048.

15. Jacob, H.J., K. Lindpaintner, S.E. Lincoln, K. Kusumi, R.K. Bunker, Y.P. Mao, D. Ganten, V.J. Dzau, and E.S. Lander. 1991. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell.* 67:213–224.

16. Todd, J.A., T.J. Aitman, R.J. Cornall, S. Ghosh, J. Hall, C.M. Hearne, A. Knight, J. Love, M.A. McAleer, J.B. Prins, et al. 1991. Genetic analysis of a complex, multifactorial disease, autoimmune type 1 (insulin-dependent) diabetes. *Res. Immunol.* 142:483.

17. Ghosh, S., S.M. Palmer, N.R. Rodrigues, H.J. Cordell, C.M. Hearne, R.J. Cornall, J.-B. Prins, P. McShane, G.M. Lathrop, L.B. Peterson, et al. 1993. Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nat. Genet.* 4:404–409.

18. Snell, G.D. 1948. Methods for the study of histocompatibility genes. *J. Genet.* 49:87–108.

19. Snell, G.D. 1958. Histocompatibility genes of the mouse. I. Demonstration of weak histocompatibility differences by immunization and controlled tumor dose. *J. Natl. Cancer Inst.* 20:787–824.

20. Yui, M.A., K. Muralidharan, B. Moreno-Altamirano, G. Perrin, K. Chestnut, and E.K. Wakeland. 1996. Production of congenic mouse strains carrying NOD-derived diabetogenic genetic intervals: an approach for the genetic dissection of complex traits. *Mamm. Genome.* 7:331–334.

21. Morel, L., Y. Yu, K.R. Blenman, R.A. Caldwell, and E.K. Wakeland. 1996. Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mamm. Genome.* 7:335–339.

22. Graff, R.J., and G.D. Snell. 1968. Histocompatibility genes of mice. 8. The alleles of the H-1 locus. *Transplantation (Baltimore).* 6:598–617.

23. Frankel, W.N., E.W. Johnson, and C.M. Lutz. 1995. Congenic strains reveal effects of the epilepsy quantitative trait locus, El2, separate from other El loci. *Mamm. Genome.* 6:839–843.

24. Nadeau, J.H. 1989. Maps of linkage and synteny homologies between mouse and man. *Trends Genet.* 5:82–86.

25. DeBry, R.W., and M.F. Seldin. 1996. Human/mouse homology relationships. *Genomics.* 33:337–351.

26. Clement, K., C. Garner, J. Hager, A. Philippi, C. LeDuc, A. Carey, T.J.R. Harris, C. Jury, L.R. Cardon, A. Basdevant, et al. 1996. Indication for linkage of the human *OB* gene region with extreme obesity. *Diabetes.* 45:687–690.

27. Duggirala, R., M. Stern, B. Mitchell, L. Reinhart, P. Shipman, O. Uresandi, W. Chung, R. Leibel, C. Hales, P. O'Connell, and J. Blangero. 1996. Quantitative variation in obesity-related traits and insulin precursors linked to the OB gene region on human chromosome 7. *Am. J. Hum. Genet.* 59:694–703.

28. Xu, W., D.R. Reed, Y. Ding, and R.A. Price. 1995. Absence of linkage between human obesity and the mouse agouti homologous region (20q11.2) or other markers spanning chromosome 20q. *Obes. Res.* 3:559–562.

29. Norman, R.A., R.L. Leibel, W.K. Chung, L. Power-Kehoe, S.C. Chua, Jr., W.C. Knowler, D.B. Thompson, C. Bogardus, and E. Ravussin. 1996. Absence of linkage of obesity and energy metabolism to markers flanking homologues of rodent obesity genes in Pima Indians. *Diabetes.* 45:1229–1232.

30. Bray, M., E. Boerwinkle, and C. Hanis. 1996. OB gene not linked to human obesity in Mexican American affected sib pairs from Starr County, Texas. *Hum. Genet.* 98:590–595.

31. Purcell-Huynh, D.A., A. Weinreb, L.W. Castellani, M. Mehrabian, M.K. Doolittle, and A.J. Lusis. 1995. Genetic factors in lipoprotein metabolism: analysis of a genetic cross between inbred mouse strains NZB/B1NJ and SM/J using a complete linkage map approach. *J. Clin. Invest.* 96:1845–1858.

32. Warden, C.H., J.S. Fisler, S.M. Shoemaker, P.-Z. Wen, K.L. Svenson, M.J. Pace, and A.J. Lusis. 1995. Identification of four chromosomal loci determining obesity in a multifactorial mouse model. *J. Clin. Invest.* 95:1545–1552.

33. Lander, E.S., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* 11: 241–247.

34. Bouchard, C. 1996. Genetic epidemiology, association, and sib-pair linkage: results from the Québec Family Study. *In* Molecular and Genetic Aspects of Obesity. Vol. 5, Pennington Center Nutrition Series. G.A. Bray and D.H. Ryan, editors. Louisiana State University Press, Baton Rouge. 470–481.

35. Siri, W.E. 1956. The gross composition of the body. *Adv. Biol. Med. Phys.* IV:239–280.

36. Weiner, J.S., and J.A. Lourie. 1969. Human Biology: A Guide to Field Methods. Blackwell Publishers, Oxford.

37. Desbuquois, B., and G.D. Aurbach. 1971. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J. Clin. Endocrinol. Metab.* 37:732–738.

38. Patel, P., Y.-M.D. Lo, A. Hattersley, G.I. Bell, A. Tybjaerg-Hansen, J. Nerup, R.C. Turner, and J.S. Wainscoat. 1992. Linkage analysis of maturity-onset diabetes of the young with microsatellite polymorphisms: no linkage to ADA or GLUT2 genes in two families. *Diabetes.* 41:962–967.

39. Haseman, J.K., and R.C. Elston. 1972. The investigation of linkage between a quantitative trait and a marker locus. *Behav. Genet.* 2:3–19.

40. West, D.B., C.N. Boozer, D.L. Moody, and R.L. Atkinson. 1992. Dietary obesity in nine inbred mouse strains. *Am. J. Physiol.* 262:R1025–R1032.

41. Borecki, I.B., T. Rice, L. Pérusse, C. Bouchard, and D.C. Rao. 1994. An exploratory investigation of genetic linkage with body composition and fatness phenotypes: the Québec family study. *Obes. Res.* 2:213–219.

42. Elston, R.C., and B.J. Keats. 1985. Sib pair analyses to determine linkage groups and to order loci. *Genet. Epidemiol.* 2:211–213.

43. Rice, T., A. Tremblay, O. Dériaz, L. Pérusse, D.C. Rao, and C. Bouchard. 1996. Genetic pleiotropy for resting metabolic rate with fat-free mass and fat mass: the Québec Family Study. *Obes. Res.* 4:125–131.

44. Schwabe, U., R. Ebert, and H.C. Erbler. 1973. Adenosine release from isolated fat cells and its significance for the effects of hormones on cyclic 3′,5′-AMP levels and lipolysis. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 278:247–259.

45. Ohisalo, J.J., S. Ranta, and I.T. Huhtaniemi. 1984. Inhibition of adenosine 3′,5′-monophosphate accumulation and lipolysis by adenosine analogs in human subcutaneous adipocytes. *J. Clin. Endocrinol. Metab.* 58:32–35.

46. Kern, P.A., J.M. Ong, J.W.F. Goers, and M.E. Pedersen. 1988. Regulation of lipoprotein lipase immunoreactive mass in isolated human adipocytes. *J. Clin. Invest.* 81:398–406.

47. Gantz, I., T. Tashiro, C. Barcroft, Y. Konda, Y. Shimoto, H. Miwa, T. Glover, G. Munzert, and T. Yamada. 1993. Localization of the genes encoding the melanocortin-2 (adrenocorticotropic hormone) and melanocortin-3 receptors to chromosomes 10p11.2 and 20q13.2-q13.3 by fluorescent in situ hybridization. *Genomics.* 18:166–167.

48. Roselli-Rehfuss, L., K.G. Mountjoy, L.S. Robbins, M.T. Mortrud, M.J. Low, J.B. Tatro, M.L. Entwistle, R.B. Simerly, and R.D. Cone. 1993. Identification of a receptor for γ-melanotropin and other proopionmelanocortin peptides in the hypothalamus and limbic system. *Proc. Natl. Acad. Sci. USA.* 90:8856–8860.

49. Miwa, H., I. Gantz, Y. Konda, Y. Simoto, and T. Yamada. 1995. Structural determinants of the melanocortin peptides required for activation of melanocortin-3 and melanocortin-4 receptors. *J. Pharmacol. Exp. Ther.* 273:367–372.

50. Malas, S., J. Peters, and C. Abbott. 1994. The genes for endothelin 3, vitamin D 24-hydroxylase, and melanocortin 3 receptor map to distal mouse chromosome 2, in the region of conserved synteny with human chromosome 20. *Mamm. Genome.* 5:557–579.

51. Stoffel, M., K. Xiang, R. Espinosa, N.J. Cox, M.M. Le Beau, and B.I. Bell. 1993. cDNA sequence and localization of polymorphic human cytosolic phosphoenolpyruvate carboxykinase gene (PCK1) to chromosome 20, band q13.31: PCK1 is not tightly linked to maturity-onset diabetes of the young. *Hum. Mol. Genet.* 1:1–4.

52. Valera, A., A. Pujol, M. Pelegrin, and F. Bosch. 1994. Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin dependent diabetes mellitus. *Proc. Natl. Acad. Sci. USA.* 91:9151–9154.

53. Pérusse, L., Y.C. Chagnon, F.T. Dionne, and C. Bouchard. 1997. The human obesity gene map: the 1996 update. *Obes. Res.* 5:49–61.

54. Chagnon, Y.C., L. Pérusse, M. Lamothe, M. Chagnon, A. Nadeau, F.T. Dionne, J. Gagnon, W.K. Chung, R.L. Leibel, and C. Bouchard. 1997. Suggestive linkages between markers on human 1p32-p22 and body fat and insulin levels in the Quebec Family Study. *Obes. Res.* 5:115–121.

55. Norman, R.A., C. Bogardus, and E. Ravussin. 1995. Linkage between obesity and a marker near the tumor necrosis-alpha locus in Pima Indians. *J. Clin. Invest.* 96:158–162.

56. Reed, D.R., Y. Ding, W. Xu, C. Cather, E.D. Green, and R.A. Price. 1996. Extreme obesity may be linked to markers flanking the human *OB* gene. *Diabetes.* 45:691–694.

57. Wilson, B.D., M.M. Ollmann, L. Kang, M. Stoffel, G.I. Bell, and G.S. Barsh. 1995. Structure and function of ASP, the human homolog of the mouse agouti gene. *Hum. Mol. Genet.* 4:223–230.

58. Norman, R., D. Thompson, T. Foroud, W. Garvey, P. Bennett, C. Bogardus, and E. Ravussin. 1997. Genomewide search for genes influencing percent body fat in Pima Indians: suggestive linkage at chromosome 11q21-q22. Pima Diabetes Gene Group. *Am. J. Hum. Genet.* 60:166–173.

59. Comuzzie, A., J. Hixson, L. Almasy, B. Mitchell, M. Mahaney, T. Dyer, M. Stern, J. MacCluer, and J. Blangero. 1997. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat. Genet.* 15:273–276.

60. Warden, C.H., J.S. Fisler, M.J. Pace, K.L. Svenson, and A.J. Lusis. 1993. Coincidence of genetic loci for plasma cholesterol levels and obesity in a multifactorial mouse model. *J. Clin. Invest.* 92:773–779.

61. West, D.B., J. Waguespack, B. York, J. Goudey-Lefevre, and R.A. Price. 1994. Genetics of dietary obesity in AKR/J × SWR/J mice: segregation of the trait and identification of a linked locus on chromosome 4. *Mamm. Genome.* 5:546–552.

62. Pomp, D., M.A. Cushman, S.C. Foster, D.K. Drudik, M. Fortman, and E.J. Eisen. 1994. Identification of quantitative trait loci for body weight and body fat in mice. *In* 5th World Congress on Genetics Applied to Livestock Production. C. Smith, J.S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J.P. Gibson, B.W. Kennedy, and E.B. Burnside, editors. University of Guelph, Guelph, Ontario, Canada. 21:209–212.

63. West, D.B., J. Goudey-Lefevre, B. York, and G.E. Truett. 1994. Dietary obesity linked to genetic loci on chromosomes 9 and 15 in a polygenic mouse model. *J. Clin. Invest.* 94:1410–1416.

64. Hyer, R.N., C. Julier, J.D. Buckley, M. Trucco, J. Rotter, R. Spielman, A. Barnett, S. Bain, C. Boitard, I. Deschamps, et al. 1991. High-resolution linkage mapping for susceptibility genes in human polygenic disease: insulin-dependent diabetes mellitus and chromosome 11q. *Am. J. Hum. Genet.* 48:243–257.

65. Risch, N., S. Ghosh, and J.A. Todd. 1993. Statistical evaluation of multiple-locus linkage data in experimental species and its relevance to human studies: application to nonobese diabetic (NOD) mouse and human insulin-dependent diabetes mellitus (IDDM). *Am. J. Hum. Genet.* 53:702–714.

66. Morton, N.E., A. Collins, S. Lawrence, and D.C. Shields. 1992. Algorithms for a location database. *Ann. Hum. Genet.* 56:223–232.

67. Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics.* 1:174–181.