Identification of quantitative trait loci affecting body composition in a mouse intercross

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Gravimetric analysis and dual energy x-ray absorptiometry densitometry were used to determine lean, fat, and bone tissue traits in a F_2 mouse population from a C57BL/6J and CASA/Rk intercross (B6CASAF2). These traits were used in a linkage analysis to identify quantitative trait loci that affect body composition. Linkage mapping showed that body weight (BW) loci on proximal chromosome 2 occurred in the same region as body length, lean tissue mass, and bone mineral content and on chromosome 13 in the same region as lean tissue mass, bone mineral density, and bone mineral content. Fat-related loci occurring on mid-chromosome 2 near 60 cM, proximal chromosome 6, and mid-chromosome 10 were distinct from BW, lean tissue, and bone tissue loci. In B6CASAF2 females, heterozygotes and CASA/Rk homozygotes at the chromosome 6 locus marker had higher body fat percentages, and this locus was responsible for 11% of the variance for body fat percentage. Female heterozygotes and C57BL/6J homozygotes at the chromosome 15 locus marker had higher bone mineral densities, and this locus could explain 8% of that trait's variance. A survey of the literature did not reveal any previous reports of fat-specific loci in the chromosomal 10 region near 42 cM reported in this study. The results of this study indicate that BW and BMI have limited usefulness as phenotypes in linkage or association studies when used as obesity phenotypes.

body mass index | bone mineral density | dual energy x-ray absorptiometry | lean tissue mass | obesity

Body composition analysis can be a useful technique for risk assessment in both health and disease. Analysis of body composition has applications in the fields of nutrition, endocrinology, cardiology, and fitness. Body mass index (BMI), body fat percentage, bone density, and other parameters derived from this type of analysis are important in the assessment of conditions such as obesity, diabetes, metabolic syndrome, and osteoporosis. A greater understanding of the genetic determinants of body composition parameters may lead to important advances in the study of these and other disease states.

To investigate the genetics of complex traits like BMI, body fat, and bone density, linkage analysis has been a widely used approach. For example, this approach has been used to compile >400 human and animal quantitative trait loci (QTLs) for obesity-related phenotypes (1). For bone density, 25 QTLs on 19 chromosomes have been identified with whole-genome scans of human populations (2). Linkage maps in the mouse have identified bone mineral density (BMD) and osteoporosis QTLs on chromosomes 1–7, 9, and 11–19 (3).

Linkage analysis in mice has been used by investigators to study the genetic regulation of body composition (4–6), but these studies focused primarily on gravimetrically determined phenotypes. The purpose of this study was to analyze body composition in an intercross between two mouse strains, C57BL/6J (B6) and CASA/Rk (Rk) and to use the phenotypes in a linkage analysis to detect QTLs for body composition, with an emphasis on fat-related and bone density parameters. Gravimetric methods and dual energy x-ray absorptiometry (DEXA) were used to determine the phenotypes. DEXA measures bone and soft tissue content by determining the attenuation of two photon energies with a photon detector (7) and has been used in linkage experiments for lean body mass and body length (BDLN) (8) and BMD (9–12). Loci identified with these phenotypes were compared with previously described loci related to body composition. A search of the literature and the mouse genome revealed several candidate genes for body composition regulation located within these loci.

Results

Parental Phenotypes. At 11 weeks of age, B6 mice are docile and slow-moving, whereas Rk mice at this age are easily excitable and quick-moving. Eleven-week-old B6 mice weighed more and were longer than Rk mice. Males weighed 40% more and were 16% longer, and females weighed 29% more and were 14% longer (Table 1). Male B6 mice also had a significantly greater BMI than male Rk mice. Female B6 mice also tended to have a greater BMI compared with female Rks, but this difference was not significant. In both males and females, lean tissue mass (LBM) by DEXA was greater in B6 mice compared with Rk mice. Total body fat tissue mass (FTM) was greater in B6 mice, but this difference was significant only in males, whereas abdominal fat mass (ABFM) was significantly greater in B6 mice irrespective of gender. Despite differences in body weight (BW) and BMI, the two strains had similar total percent body fat (PBF) and percent abdominal fat (PAF). The similarity in PBF also was observed when body composition was determined by using a lipid extraction technique (data not shown). However, BMD, bone area (BA), and bone mineral content (BMC) were significantly higher in B6 compared with Rk for both genders.

Linkage Analysis. Fourteen different phenotype parameters were used to characterize the F_2 progeny of C57BL/6J and CASA/Rk intercross (B6CASAF2s) [see supporting information (SI) Table 4]. This phenotype data were used to perform a linkage analysis to identify QTLs. Significant loci were identified in all parameters measured in the B6CASAF2s except PAF (Table 2). Additionally, no significant loci were identified when fat pad weights [mesenteric plus omental fat pad weight (MOF), gonadal fat pad weight (GF), and retroperitoneal fat pad weight (RF)] were analyzed by using total abdominal fat pad weight (MOF + GF + RF) as an additive covariate.

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Abbreviations: ABFM, abdominal fat mass; B6, C57BL/6J; B6CASAF2, progeny of C57BL/6J and CASA/Rk intercross; BA, bone area; BDLN, body length; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; BW, body weight; DEXA, dual energy x-ray absorptiometry; FTM, fat tissue mass; GF, gonadal fat pad weight; LBM, lean tissue mass; LOD, logarithm of odds; MOF, mesenteric plus omental fat pad weight; PBF, percent body fat; QTL, quantitative trait loci; RF, retroperitoneal fat pad weight; Rk, CASA/Rk; ROI, region of interest.

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Table 1. Body characteristics and DEXA body composition parameters in parental strains

	Males, mean ± SD		Females, mean ± SD		*t* test *P* values	
Phenotype	C57BL/6J	CASA/Rk	C57BL/6J	CASA/Rk	Male B6 vs. Rk	Female B6 vs. Rk
BW, g	27.7 ± 1.3	16.7 ± 1.1	20.4 ± 1.4	14.4 ± 0.9	<0.0001	<0.0001
BDLN, cm	9.1 ± 0.2	7.6 ± 0.4	8.7 ± 0.2	7.5 ± 0.1	<0.0001	<0.0001
BMI, g/cm^2	0.34 ± 0.015	0.30 ± 0.032	0.27 ± 0.017	0.26 ± 0.014	0.021	NS
LBM, g	18.7 ± 1.2	11.5 ± 0.88	13.8 ± 1.1	9.4 ± 0.52	<0.0001	0.0004
FTM, g	5.2 ± 0.22	3.2 ± 0.21	2.9 ± 0.21	2.4 ± 0.50	<0.0001	NS
ABFM, g	1.0 ± 0.13	0.6 ± 0.14	0.8 ± 0.04	0.5 ± 0.15	0.0020	0.0110
PBF	21.9 ± 1.6	21.8 ± 1.8	17.6 ± 1.4	20.4 ± 3.4	NS	NS
PAF	20.0 ± 2.2	19.7 ± 4.3	25.6 ± 4.3	21.0 ± 2.1	NS	NS
BMD, g/cm^2	0.057 ± 0.001	0.0446 ± 0.0006	0.047 ± 0.001	0.044 ± 0.002	<0.0001	0.0207
BA, cm^2	8.6 ± 0.39	6.5 ± 0.33	7.8 ± 0.21	6.3 ± 0.18	<0.0001	<0.0001
BMC, g	0.49 ± 0.019	0.29 ± 0.018	0.36 ± 0.013	0.27 ± 0.020	<0.0001	<0.0001

NS, not significant.

Lean Tissue Loci. Loci for BW were revealed on chromosomes 2, 5, and 13. The BW locus on chromosome 2 (34 cM) appears identical to a chromosome 2 locus for BDLN. The BW and BDLN phenotypes were determined gravimetrically; a DEXA-determined phenotype, LBM, had loci near the BW loci on chromosomes 2 (34 cM) and 13 (49 cM). The only other locus near the BW chromosome 5 (1 cM) locus was a BMI locus at 1 cM. BDLN and LBM loci appeared near BW loci, suggesting that these genomic regions are responsible for the regulation of BW as it pertains to body size and lean tissue.

Fat-Related Loci. Linkage analysis also produced a number of QTLs for phenotypes that could be grouped as fat-related. On chromosome 2, fat-related loci were identified for gravimetrically and DEXA-derived phenotypes (FTM, ABFM, MOF, and RF), but these loci were located at ≈60 or 90 cM and, therefore, do not represent the same loci as the chromosome 2 BW locus. Fat-related loci also were identified between 0 and 13 cM on chromosome 6 for FTM, PBF, MOF, GF, and RF and on chromosome 10 for PBF, MOF, GF, and RF near 40 cM. None of the fat-related regions with significant logarithm of odds (LOD) scores were located near loci identified from the BW or BMI analyses.

Bone-Related Loci. A number of bone-related loci also were revealed in the analysis. Significant loci for BMD were discovered on chromosomes 1, 2, 6, 13, and 15. Two of these loci were near BW loci on chromosomes 2 (34 cM) and 13 (49 cM), and two were near BMC loci on chromosomes 6 (28 cM) and 15 (43 cM). The BMD and BMC loci on chromosome 6 at 28 cM probably are distinct from the fat-related loci on chromosome 6, which clustered in a range of 0–13 cM. Three of the BMC loci were located near loci for BW on chromosomes 2 (40 cM), 9 (61 cM), and 13 (45 cM). The bone-related loci, therefore, clustered with the BW and lean tissue loci in a number of instances.

LOD Plots. To better assess the clustering of traits, the LOD plots of those traits clustering in the major loci were compared (Figs. 1 and 2). The comparison of LOD plots illustrates that BDLN, LBM, and BMC cluster along with BW on proximal chromosome 2, BDLN, LBM, BA, and BMD cluster together on distal chromosome 9, and LBM, BMD, and BMC cluster together with BW on mid-chromosome 13 (Fig. 1). The linkage analysis of phenotypes representing LBM and bone tended to reflect genomic regions that were near those for BW.

In contrast to the loci representing lean tissue and bone phenotypes, fat-related loci were distinct from BW loci (Fig. 2). FTM, ABFM, MOF, and RF clustered together on mid-chromosome 2, FTM, PBF, MOF, GF, and RF clustered near each other on proximal chromosome 6, and PBF, MOF, GF, and RF were clustered together on mid-chromosome 10.

By inspection, there appear to be bimodal peaks on LOD plots for BDLN (chromosome 9), RF (chromosome 2), and BMC (chromosome 13). To explore the possibility of multiple QTL with in the same chromosome, the fitqtl function of R/qtl was used. Analysis with the fitqtl function revealed that there may be an interaction between the two peaks (51 and 63 cM) on chromosome 13 for BMC ($P = 0.0945$), whereas there appears to be no interactions for BDLN ($P = 0.294$) or RF ($P = 0.488$).

Genotypic Means. To get a sense of the influence of genotype on the phenotypic parameters used in this cross, several phenotypes with high (>5) LOD scores were selected and the genotypic means were determined (Table 3). Heterozygotes and homozygotes for the Rk marker in the chromosome 6 locus have a higher body fat percentage than B6 homozygotes. In females, this locus appears to explain 11% of the variance for PBF. The effects of marker genotype on BMI (in the chromosome 5 locus) and LBM (in the chromosome 9 locus) exhibit similar patterns in that B6 homozygotes have the greatest BMI and LBM, whereas heterozygotes and Rk homozygotes do not differ significantly in either of these parameters. The genotypic effects on BMD (in the chromosome 15 locus) show that B6 homozygotes and heterozygotes have similar BMDs that are significantly greater than that of Rk homozygotes and that this locus in females is responsible for 8% of the variance in that phenotype.

Discussion

In this study, gravimetric and absorptiometric means were used to perform linkage analyses on various phenotypes in F_2 progeny of a cross between B6 and Rk mice. Significant loci were identified for BW on proximal 2 and mid-13 chromosomes. A BDLN locus also was found on proximal chromosome 2, and in general, phenotypes that describe lean tissue like LBM, BMC, and BMD also were found to be clustered with the BW loci. This relationship between BW, BDLN, and bone and lean tissue parameters on chromosomes 2 and 13 suggests that these chromosomal regions play a role in the genetic regulation of body size and BW contributed by lean tissue including bone, whereas fat-related tissue does not appear to be involved.

Loci for fat-related phenotypes were identified on middle and distal chromosome 2, proximal chromosome 6, and mid-chromosome 10. In these regions, loci for FTM, ABFM, and fat pad weights clustered together on chromosome 2, and FTM, PBF, and fat pad weight traits clustered together on chromosome 6. The chromosome 10 region was shared by PBF and fat pad

Table 2. QTL for body characteristics and body composition parameters in B6CASAF2s

	Both sexes				Males				Females			
Phenotype	Symbol*	Chr	Peak, cM	LOD	Symbol*	Chr	Peak, cM	LOD	Symbol*	Chr	Peak, cM	LOD
Weight	Bw20	2	34	5.21	Bw23	9	71	4.15	Bw24	5	3	3.72
	Bw21	5	1	6.33					Bw25	8	1	3.52
	Bw22	13	49	5.09					Bw26	14	56	3.71
Length	Bdln7	2	34	4.00	Bdln9	9	72	4.42				
	Bdln8	9	59	4.80								
BMI	Bmi	5	1	6.34	Bmi	5	1	5.05				
LBM	Lbm11	2	34	6.13	Lbm14	2	36	4.19				
	Lbm12	9	63	5.88	Lbm15	9	71	5.08				
	Lbm13	13	49	3.81								
FTM	Ftm1	2	60	4.20	Ftm4	15	22	3.79	Ftm5	6	13	3.55
	Ftm2	6	0	4.36								
	Ftm3	15	15	3.92								
ABFM	Abfm1	2	62	4.13	Abfm2	2	87	3.88				
PBF	Pbf1	6	5	5.55					Pbf3	6	17	5.15
	Pbf2	10	42	5.83								
MOF	Mof1	2	66	4.32	Mof5	2	86	3.48	Mof6	10	28	3.71
	Mof2	6	1	4.40								
	Mof3	10	40	6.13								
	Mof4	18	4	3.96								
GF	Gf1	6	13	4.59	Gf4	10	42	4.43	Gf6	6	17	4.34
	Gf2	10	42	7.14	Gf5	14	48	3.49				
	Gf3	14	48	4.49								
RF	Rf1	2	96	5.14	Rf4	10	42	3.66	Rf5	5	73	3.60
	Rf2	6	1	3.89					Rf6	6	16	4.48
	Rf3	10	42	7.38					Rf7	10	4	4.55
BMD	Bmd20	1	96	4.17	Bmd25	2	37	4.34	Bmd29	1	99	3.98
	Bmd21	2	40	4.09	Bmd26	6	35	3.64	Bmd30	15	43	5.68
	Bmd22	6	28	3.79	Bmd27	9	65	4.15				
	Bmd23	13	47	5.21	Bmd28	15	61	3.99				
	Bmd24	15	45	8.03								
BA	Ba1	7	53	4.71	Ba4	7	53	4.12	Ba6	13	63	3.90
	Ba2	9	59	5.07	Ba5	9	63	5.49				
	Ba3	13	67	4.30								
BMC	Bmc1	2	40	3.87	Bmc7	2	37	3.50	Bmc10	13	61	3.78
	Bmc2	6	28	3.92	Bmc8	7	53	3.45	Bmc11	15	47	4.35
	Bmc3	7	15	4.42	Bmc9	9	65	5.64				
	Bmc4	9	61	4.96								
	Bmc5	13	45	5.31								
	Bmc6	15	43	6.12								

*Symbol names were created by using International Committee on Standardized Genetic Nomenclature for Mice Rules for Nomenclature of Genes, Genetic Markers, Alleles, and Mutations in Mouse and Rat (http://rgd.mcw.edu/nomen_rules.html).

weights. The appearance of several fat-related traits, determined by independent techniques, suggests that these regions may be responsible for the genetic regulation of fat-related tissue.

BMI was linked only to a region on chromosome 5 (1 cM), which was also linked to BW. Like BW, the BMI locus did not share any region with fat-related loci. Therefore, the BMI locus identified in this study appears to be relevant to the part of BMI that is contributed by lean tissue traits.

Furthermore, although these two murine strains differ in BW and BMI, no difference was detected in PBF in the whole mouse or when only abdominal fat was considered. The difference in BW between the two strains appears to be related to a difference in body size and tissue mass, with lean and fat tissue sharing equal proportions. Loci that were linked to BW were found to cluster with lean and bone tissue loci, but not fat tissue loci. Also in this study, the BMI locus was found only in the same vicinity as a BW locus. These findings suggest that BW and BMI may have limited use alone as obesity phenotypes in linkage or association studies, and loci that appear to regulate these traits should be verified by some independent measurement of body fat. Many clinical and linkage studies use BMI as an obesity measurement, so this limitation is an important caveat in the interpretation of such studies. It should be pointed out, however, that these relationships between obesity phenotypes may vary based on experimental conditions; perhaps BW and BMI would have been correlated with body fat phenotypes in B6CASAF2s given a high-fat diet (A. J. Lusis, personal communication).

A survey of the literature reveals common regions that have been implicated in the genetic regulation of similar phenotypes. For example, Masinde *et al.* (8) used DEXA in a linkage analysis in a mouse cross and identified a LBM QTL on chromosome 2 (26.3 cM), which is near the chromosomal region associated with LBM, BW, and BDLN in this study.

Of the >600 genes, markers, and chromosomal regions previously identified as associated with human obesity phenotypes, a number could be found in the loci described in this study (see SI Table 5). Multiple obesity QTLs, described by independent laboratories, lie on chromosome 2. Mehrabian *et al.* (13) also

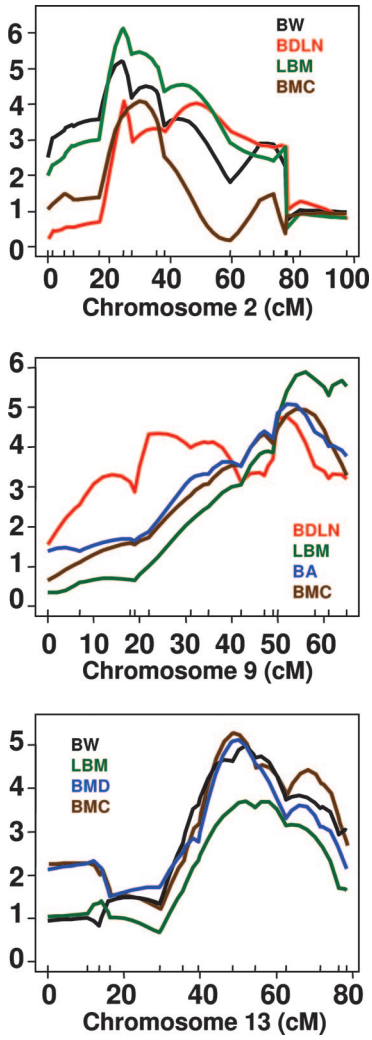

Fig. 1. Linkage maps displaying QTLs for various phenotypes in B6CASAF2s on chromosomes 2, 9, and 13. The *x* axes depict the chromosomal map positions in cM, and the *y* axes show the LOD scores. The upright tick marks on the *x* axes represent the marker positions described in ref. 30.

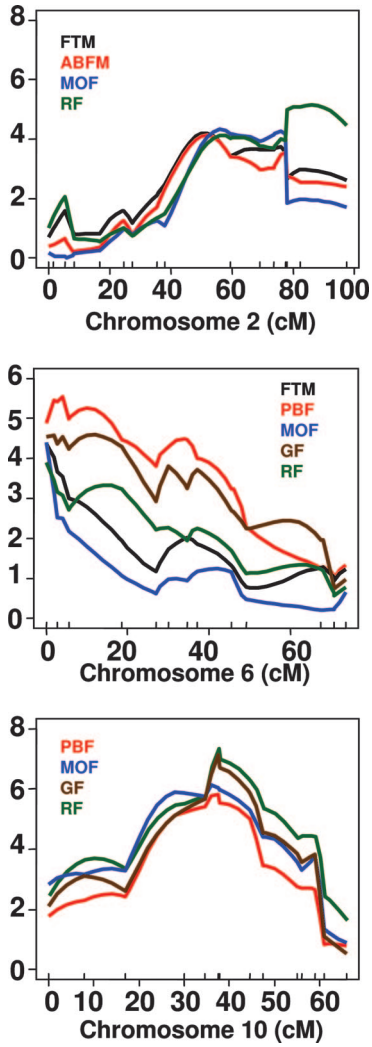

Fig. 2. Linkage map displaying QTLs for various phenotypes in B6CASAF2s on chromosomes 2, 6, and 10. The *x* axes depict the chromosomal map positions in cM, and the *y* axes show the LOD scores. The upright tick marks on the *x* axes represent the marker positions described in ref. 30.

identified central and distal chromosome 2 loci for fat-related phenotypes. In that study, mapping of a C57BL/6J and CAST/Ei intercross revealed mob6, a central locus on chromosome 2 for s.c. and retroperitoneal fat pad weights, and mob5, a distal chromosome 2 locus for percent lipid. Yi *et al.* (14) reported a chromosome 2 QTLs for various obesity phenotypes, and this work has been followed by the production of a chromosome 2 congenic mouse with Spret/Ei on a B6Lipc^{null} background containing the interval from 142.1–168.8 Mb (15). A locus Obq10 for gonadal and mesenteric fat pad weights (as a percentage of BW) was described by Taylor *et al.* (16) at central chromosome 2 in an intercross between New Zealand obese and small mice.

The chromosome 6 locus that was linked to FTM, PBF, MOF, GF, and RF in our study contains the leptin gene. Femoral fat pad weight has been shown to be linked to proximal chromosome 6 in BSB mice, used as a model for polygenic obesity (17).

A chromosomal 10 region linked to GF with a LOD of 7.14 (42 cM) and also linked to PBF (42 cM), MOF (40 cM), and RF (42 cM) was revealed in the current study. Although no fat-specific QTLs near our chromosome 10 locus have been described in the literature, a locus representing BW-based growth rate has been described in central chromosome 10 from a SM/J and LG/J intercross by Vaughn *et al.* (18). A subsequent analysis of the same cross by using gonadal fat pad weights did not reveal any fat-related QTLs on chromosome 10 (19).

Several genes reported in the literature that exist within this chromosome 10 locus have been implicated in regulation of body fat. Adipsin, a serine protease secreted by adipocytes and identical to complement factor D, is located on mouse chromosome 10 at 80.0 Mb. Although evidence for a primary role for adipsin in the etiology of obesity is lacking (20, 21), a human twin study has shown that adipsin polymorphisms are correlated with response to overfeeding (22). Promelanin-concentrating hormone (Pmch), a hypothalamic peptide involved in feeding behavior, exists near the chromosome 10 locus peak at 47.0 cm. Pmch knockout mice eat less, have reduced body fat, and increased metabolic rate (23), and when FVB mice homozygously overexpressing Pmch are fed a high-fat diet, they have a higher PBF compared with wild type (24). Guanidinoacetate methyltransferase is located at 43 cM on chromosome 10, and its deficiency causes reduced body fat mass in a knockout model (25). A search of the mouse genome (www.ensembl.org/index.html) revealed an additional candidate in lipid phosphate phosphohydrolase 2 (Ppap2c), whose product catalyzes the

Table 3. Genotypic effect on body composition parameters in B6CASAF2 females and males

Phenotype	Gender	Chromosome	Marker (cM)	Mean ± SD			*P* value*			% Variance
				BB†	BC‡	CC§	BB vs. BC	BB vs. CC	BC vs. CC	
PBF	Female	6	D6Mit272 (19)	18.9 ± 1.7	20.9 ± 3.1	22.1 ± 4.1	<0.01	<0.001	NS	11
BMD, g/cm^2	Female	15	D15Mit2 (47)	0.046 ± 0.003	0.045 ± 0.002	0.043 ± 0.002	NS	<0.001	<0.001	8
BMI	Male	5	D5Mit249 (1)	0.27 ± 0.03	0.25 ± 0.02	0.25 ± 0.02	<0.001	<0.001	NS	13
LBM, g	Male	9	D9Mit151 (72)	16.8 ± 2.1	15.3 ± 2.1	14.6 ± 2.0	<0.001	<0.001	NS	10

n values are 176 for PBF, 168 for BMD, 180 for BMI, and 181 for LBM. NS, not significant.
*By one-way ANOVA.
†C57BL/6J homozygotes.
‡Heterozygotes.
§CASA/Rk homozygote.

conversion of phosphatidic acid to diacylglycerol. Ppap2c is located within the chromosome 10 locus at 79.4 Mb.

Loci linked to bone-related parameters have been described on most of the 19 mouse autosomal chromosomes, except for chromosomes 8 and 10. In this study, bone-related loci with significant LOD scores appeared on chromosomes 1, 2, 6, 7, 9, 13, and 15, with the most significant LOD scores for BMD and BMC on chromosome 15 (LOD 8.03 and 6.12, respectively) and BMC on chromosome 13 (LOD 5.31). These chromosome 15 and 13 loci contain 8 previously described markers, genes, or QTL associated with osteoporosis-related phenotypes (see SI Table 6). The chromosome 15 locus contains the gene for tumor necrosis factor receptor superfamily member 11B precursor (Tnfrsf11b or osteoprotegerin) at 54.3 Mb. Osteoprotegerin is a regulator of osteoclastogenesis (26). Interleukin 4-induced I (chromosome 7, 23.1 cM) and procollagen, type II, α1 (chromosome 15, 54.5 cM) also have been implicated as osteoporosis candidate genes (27).

As Corva *et al.* (28) pointed out, one should exercise caution in comparing linkage data from independent experiments. Comparisons of this nature are especially problematic if the data suggest regulation of a trait by multiple QTLs and the comparisons involve populations that are distantly related. Therefore, a stronger correlation would be expected between our data and that of Mehrabian *et al.* (13), who mapped traits in a C57BL/6J and CAST/Ei intercross.

In this study, DEXA imaging was a convenient way to perform body composition analysis on a large number of animals. However, the use of DEXA also limited this study in several ways. DEXA will overestimate fat mass, because of instrument calibration techniques or the process whereby the instrument has to extrapolate axial fat mass by applying estimates derived from the abdomen (29). The freeze/thaw process of the animal carcasses potentially could alter the structure of adipose tissue and perhaps introduce a source of error that could alter the accuracy of the imaging results.

Another potential source of error is introduced by the placement of the DEXA region of interest (ROI) (29). The ROI is the area, positioned by the DEXA operator with a computer mouse, that is subject to densitometry. Areas outside the ROI are excluded from the analysis. In small animal densitometry, the ROI is commonly used to exclude the head region. This study also used the DEXA ROI to define an abdominal area to study tissue characteristics specific to that area. Only one operator performed these placements, but because placement of the ROI is done manually, it is subject to variability.

To counteract these potential effects on the accuracy of the phenotypes, dissection and weighing of visceral fat depots also were performed and used as phenotypes in the linkage analysis, and chromosome 10 linkage maps for MOF, GF, and RF were similar to that of the DEXA-derived PBF phenotype. Similarly, chromosome 6 linkage maps for MOF, GF, and RF had peak linkages in the same chromosomal region as PBF and FTM. ABFM, FTM (determined by DEXA), MOF, and RF had linkage maps with similar shapes, especially near 60 and 90 cM. The fact that gravimetrically derived adipose tissue phenotypes produced similar linkage results to those resulting from DEXA phenotypes strengthens the validity for the findings.

In summary, linkage analyses were performed with phenotypic characteristics related to body size, fat and lean tissue, and bone density on F_2 mice from an intercross between B6 and Rk strains. Several fat-related and bone density QTLs were identified, including four fat-related loci (named Pbf2, Mof3, Gf2, and Rpf3) within a chromosomal 10 region near 42 cM. This chromosomal region is previously undescribed as to whether it contains fat-specific loci. Future studies to further define these and the other loci identified in the this study with techniques like fine mapping and/or functional studies involving candidate genes described above could prove to be useful in understanding genetic contributions in diseases such as obesity or osteoporosis.

Materials and Methods

Animals. Details on the handling of the mice used in this study are described in ref. 30. Briefly, Rk males were mated with B6 females, and the F_1 males were intercrossed with F_1 females to produce 369 B6CASAF2s. All animals were bred and housed in a single humidity- and temperature-controlled room with a 12 h dark-light cycle (6 a.m. to 6 p.m. light cycle) at the Laboratory Animal Research Center at The Rockefeller University and were provided with a single lot of Picolab Rodent Chow 20 containing 0.02% wt/wt cholesterol and water ad libitum. At 11 weeks of age, the mice were killed by exsanguination after a 5 h fast during which only water was provided. A ketamine/xylazine mix was used for anesthesia. Immediately after the mice were killed, the bile was aspirated, portions of the liver and duodenum were removed for other experiments, and the carcass was placed in −80°C for storage head down in 50-ml conical tubes (Sarstedt Newton, NC). All experiments were approved by the Institutional Animal Care and Research Advisory Committee.

Phenotype Analysis. Animals were weighed on a common laboratory scale and measured for length from the nose tip to the base of the tail before killing. Before DEXA analysis, the frozen carcass was thawed in the conical tube for 30 min in a 37°C waterbath. Time of thawing did not significantly affect DEXA results (data not shown). The left kidney was removed for use in other experiments, and the thawed carcass was scanned by using a small animal DEXA densitometer (PIXImus; Lunar, Madison, WI). Whole-body densitometry was performed with the head outside of the ROI to measure the LBM, FTM, PBF, BMD, BA,

and BMC phenotypes, and the abdominal area also was examined by placing the ROI from the lower rib margin to the superior aspect of the pelvis to measure the ABFM and percent abdominal fat phenotypes. The omental, mesenteric, gonadal, and retroperitoneal fat pads were dissected and weighed on a laboratory scale to the nearest one-thousandth of a gram.

Linkage Analysis. The linkage analysis used 255 markers with an average spacing of 5.9 cM. The origin of the markers and the details of the genotyping process are described in ref. 30. Linkage and interval mapping was performed by using R/qtl software (version 0.97) (31) with the scanone function, employing the maximum likelihood algorithm. Gender was used as an additive covariate, and males and females also were analyzed separately. Fat pad weights were analyzed alone and with total abdominal fat pad weights (MOF plus GFF plus RF) as an additive covariate to explore for regions that were associated with fat pad distribution. Some of the F_2 phenotype data (BDLN, FTM, AFM, and PBF in males, GF, and RF) were skewed and not normally distributed. Interval mapping was performed with permutation testing; 1,000 permutations produced genomewide LOD thresholds of 3.60 for BW, BDLN, PBF, FTM, LTM, AFM, BMD, and BMC and 3.61 for BA, BMI, GF, MOF, and RF (both 95th percentile).

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