Characterization of Epistasis Influencing Complex Spontaneous Obesity in the BSB Model

Nengjun Yi,* Adam Diament,† Sally Chiu,†,‡ Kyoungmi Kim,* David B. Allison,* Janis S. Fisler‡ and Craig H. Warden†,§,[1]

*Department of Biostatistics, Section on Statistical Genetics, University of Alabama, Birmingham, Alabama 35294 and †Rowe Program in Genetics, ‡Department of Nutrition, §Department of Pediatrics and Section of Neurobiology, Physiology and Behavior, University of California, Davis, California 95616

Manuscript received December 5, 2003
Accepted for publication January 27, 2004

ABSTRACT

There is growing awareness that complex interactions among multiple genes and environmental factors play an important role in controlling obesity traits. The BSB mouse, which is produced by the backcross of (lean C57BL/6J × lean *Mus spretus*) × C57BL/6J, provides an excellent model of epistatic obesity. To evaluate potential epistatic interactions among six chromosomal regions previously determined to influence obesity phenotypes, we performed novel Bayesian analyses on the basis of both epistatic and nonepistatic models for four obesity traits: percentage of body fat, adiposity index, total fat mass, and body weight, and also for plasma total cholesterol. The epistatic analysis detected at least one more QTL than the nonepistatic analysis did for all obesity traits. These obesity traits were variously influenced by QTL on chromosomes 2, 7, 12, 15, and 16. Interaction between genes on chromosomes 2 and 12 was present for all obesity traits, accounting for 3–4.8% of the phenotypic variation. Chromosome 12 was found to have weak main effects on all obesity traits. Several different epistatic interactions were also detected for percentage of body fat, adiposity index, and total fat mass. Chromosomes 6 and 12 have not only main effects but also strong epistatic effects on plasma total cholesterol. Our results emphasize the importance of modeling epistasis for discovery of obesity genes.

THE underlying biological causes of obesity are complex, including genes with large effects that are independent of environment and epistasis, genes whose alleles interact with the environment to produce obesity in some individuals and not others, and genes that interact with each other (Allison *et al.* 2002). Understanding the overall biological etiology of obesity will require identification of genes responsible for each of these different mechanisms. Discrete combinations of alleles of genes, or gene products, may interact with each other in markedly different ways to influence complex diseases (Cordell 2002; Moore 2003). These epistatic interactions clearly influence obesity (Brockmann *et al.* 2000; Dong *et al.* 2003). The observation that obesity is influenced by epistasis has implications for gene discovery and becomes particularly important if epistasis underlies a significant fraction of human obesity as appears to be true for mice (Brockmann *et al.* 2000; Cheverud *et al.* 2001; Corva *et al.* 2001). The practical implication of epistasis for experimental work is that some quantitative trait loci (QTL) that have no independent effects may be identified, but that these loci may significantly influence the trait if combined with a specific allele of another gene. Evidence that epistasis is common in obesity has been derived from statistical analyses (Segal and Allison 2002), but is also apparent from the almost universal observation that obesity phenotypes of knockout and spontaneous mutant mice are dependent on the background mouse strain on which the mutations are placed (Hummel *et al.* 1972; Coleman and Hummel 1973; Harris *et al.* 2001; Hofmann *et al.* 2001). That is, different alleles of genes other than the knockout or mutant gene present in different mouse strains interact with (are epistatic with) the knockout or mutation.

In the BSB mouse model, mice are produced by a backcross of the lean female *M. musculus domesticus* (inbred C57BL/6J) with lean male *M. spretus* (either outbred SPRET/Pt or inbred SPRET/Ei). The lean F_1 females are then backcrossed to C57BL/6J to produce the BSB model. Each mouse is genetically unique and body fat can range from 1 to >50%. Since both parental strains and F_1's are lean on low-fat chow diets, then obesity in BSB mice may be due to interactions between alleles from the two strains (Fisler *et al.* 1993). Two previous studies, using either outbred SPRET/Pt or inbred SPRET/Ei, reported obesity and/or cholesterol quantitative trait loci (QTL) in BSB mice on chromosomes 6, 7, and 15 (Warden *et al.* 1993, 1995). A QTL on chromosome 12 was observed in one cross, but not in another (Warden *et al.* 1995). In addition, a chromosome 16 QTL was observed in the wild-type cross (C. H. Warden, personal communication) and a chromosome 2

[1]*Corresponding author:* Rowe Program in Genetics, University of California, Davis, California 95616. E-mail: chwarden@ucdavis.edu

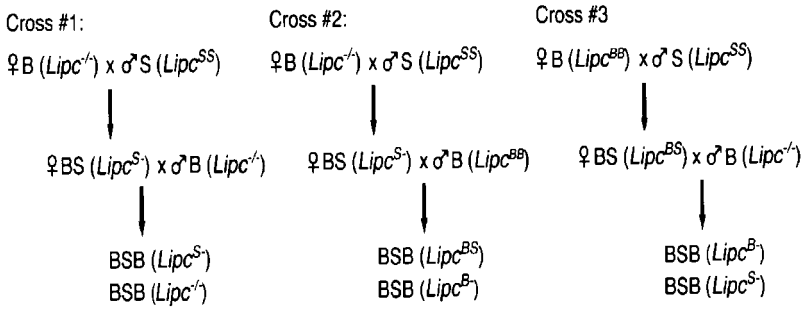

FIGURE 1.—Breeding scheme. A diagram of the breeding schemes for generating the mice used in this study is shown. C57BL/6J (B), either wild type ($Lipc^{+/+}$) or with knockout of *hepatic lipase* ($Lipc^{-/-}$), were bred with SPRET/Ei (S) to produce 743 BSB mice.

QTL was found in the crosses examined in the present study (DIAMENT *et al.* 2004).

Monogenic models such as spontaneous mutants, knockouts, gene traps, and mutagenized mice are inherently unable to survey genomes for those genes participating in complex gene × gene interactions. Thus, studies involving complex models are needed because they may identify genes that influence obesity only by interaction with other genes and because these studies may reveal novel mechanisms for causing obesity. The goal of this article is to evaluate evidence for interaction among six chromosomal regions with previous evidence for QTL in BSB mice, using recently described Bayesian statistical methods (YI *et al.* 2003).

MATERIALS AND METHODS

Animals: Breeding pairs of inbred *M. musculus domesticus* (C57BL/6J), or C57BL/6J mice carrying null *hepatic lipase* alleles ($Lipc^{-/-}$) on chromosome 9, and inbred *M. spretus* (SPRET/Ei) were obtained from the Jackson Laboratory (Bar Harbor, ME). BSB mice are the result of crossing male SPRET/Ei mice with female C57BL/6J mice and then backcrossing the female F_1 progeny to male C57BL/6J mice (FISLER *et al.* 1993). Male F_1 mice from this cross are infertile and an F_2 intercross is not possible. This study used mice from three BSB crosses as described in Figure 1. Cross 1 generated 361 BSB mice that carried either $Lipc^{-/-}$ or $Lipc^{S-}$ alleles, cross 2 resulted in 199 BSB mice with either $Lipc^{B-}$ or $Lipc^{BS}$ genotype and cross 3 generated 183 BSB mice that carried either $Lipc^{B-}$ or $Lipc^{S-}$ alleles. Although there were four potential alleles at *Lipc* on chromosome 9 in the resulting 743 BSB mice, there were no interactions between the data reported here and the *Lipc* genotype or the cross number. The QTL and interactions identified in this report are independent of *Lipc* (FARAHANI *et al.* 2004).

Following weaning at 21 days of age, BSB mice were housed individually in wire cages, maintained in a 14-hr light, 10-hr dark cycle, and had *ad libitum* access to water and a standard chow diet (Purina Rodent Chow 5001; Research Diets, New Brunswick, NJ) containing 12% of energy as fat. Conditions of housing and care met the standards of AAALAC accreditation and the Animal Welfare Act PL85-544.

Phenotypic measures: At ~5 months of age, mice were fasted overnight and bled from the retro-orbital plexus under isoflurane anesthesia ~3 hr after the beginning of the light cycle. Blood was collected in plasma separator tubes, placed on ice, and centrifuged to prepare plasma. Total cholesterol was measured as previously described (CASTELLANI *et al.* 1997; WARNICK and REMALEY 2001) by a Centers for Disease Control and Prevention-certified laboratory, guaranteeing that the results were stable over time. Body weight was recorded and

TABLE 1

Markers, physical position on each chromosome, consensus linkage position, and intermarker distances calculated in BSB mice

Locus	Physical location (Mb)	Linkage (cM)	BSB (cM)
	Chromosome 2		
D2Mit106	Not mapped	75.6	0.0
D2Mit109	147.2	81.7	7.5
Agouti (a)	156.9	89.0	5.5
D2Mit55	159.8	91.0	0.2
Lpin3	162.7	No data	1.1
D2Mit229	170.7	99.0	5.5
D2Mit502	175.3	87.4	5.2
D2Mit74	180.3	107.0	4.7
	Chromosome 6		
D6Mit1	Not mapped	2.8	0.0
D6Mit222	38.9	15.4	13.2
D6Mit355	128.4	58.8	10.0
	Chromosome 7		
D7Mit185	87.7	50.0	0.0
D7Mit8	Not mapped	60.0	10.3
D7Mit103	118.0	63.5	5.3
D7Mit332	Not mapped	65.6	4.7
D7Mit12	125.5	66.0	3.5
D7Mit259	133.8	72.0	6.1
	Chromosome 12		
D12Mit27	81.4	42.0	0.0
D12Mit51	100.7	52.0	16.0
	Chromosome 15		
D15Mit13	32.9	6.7	0.0
D15Mit152	45.4	20.2	17.4
D15Mit183	55.3	23.0	5.9
	Chromosome 16		
D16Mit210	30.0	21.0	0.0
D16Mit134	Not mapped	23.5	2.3

Physical location is the position in megabases of the marker in the Ensembl public mouse genome assembly. Linkage is the location in Kosambi centimorgans of the microsatellite marker in the Mouse Genome Database at The Jackson Laboratory (http://www.informatics.jax.org/). BSB (cM) is the measured marker-to-marker distance in the BSB mice used for analysis of the present data. The Ensembl genome assembly and the BSB cross linkage data place Microsatellite D2Mit502 between D2Mit229 and D2Mit74. Thus, we conclude that the Mouse Genome Database position is incorrect.

TABLE 2

Bayesian posterior probability distribution for the number of QTL

Trait	Model	Estimated probability distribution for l = 0	1	2	3	4	5	6	7	Estimated expectation
% body fat	Nonepistasis	0	0	0.419	0.400	0.155	0.023	0.002	0	2.398
	Epistasis	0	0	0.019	0.379	0.424	0.157	0.024	0	3.788
Adiposity index	Nonepistasis	0	0.004	0.270	0.420	0.246	0.055	0.004	0	3.091
	Epistasis	0	0.005	0.134	0.363	0.279	0.169	0.051	0	3.625
Total fat mass	Nonepistasis	0.067	0.409	0.361	0.138	0.023	0.002	0.001	0	1.649
	Epistasis	0.004	0.031	0.132	0.706	0.115	0.011	0.001	0	2.933
Body weight	Nonepistasis	0	0.359	0.429	0.175	0.031	0.004	0	0	1.894
	Epistasis	0	0.07	0.044	0.656	0.204	0.024	0.0020	0	3.074
Total cholesterol	Nonepistasis	0	0.109	0.469	0.338	0.074	0.008	0	0	2.405
	Epistasis	0.00	0.0035	0.454	0.423	0.105	0.014	0	0	2.674

mice were killed by cervical dislocation. Kidneys, liver, brain, and gastrocnemius muscle were dissected and frozen for DNA isolation and other analyses reported elsewhere. Four fat pads, consisting of three intraabdominal (mesenteric, bilateral retroperitoneal, and gonadal) fat pads and a subcutaneous fat pad from the outer thigh (bilateral femoral) were dissected, weighed, and returned to the carcass. Adiposity index was calculated as summed fat pad weights divided by live body weight. Measurement of percentage of body fat was based on the remaining carcass. Lipid was extracted using the Soxhlet apparatus (Kimble Glass, Vineland, NJ) and total fat mass was determined gravimetrically (Bell and Stern 1977). Percentage of body fat was calculated as total fat mass ÷ carcass weight × 100.

Genotyping: Genomic DNA was extracted from mouse kidney (QIAamp blood and tissue kit; QIAGEN, Valencia, CA). Simple sequence length polymorphism (SSLP) markers, polymorphic between C57BL/6J and SPRET/Ei alleles, on chromosomes 2, 6, 7, 12, 15, and 16 (Table 1), were genotyped by amplification (Invitrogen, Carlsbad, CA). PCR used 30 cycles of 94° for 30 sec, 55° for 30 sec, and 68° for 30 sec. PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining and UV illumination. We genotyped chromosomes where there was previous evidence for QTL in BSB mice.

Statistical analyses: Five phenotypes, percentage of body fat, adiposity index, total fat mass, body weight, and plasma total cholesterol, were analyzed in this study. Two multiple-QTL models, an epistatic model and a nonepistatic model, were used to analyze the data. The multiple-QTL models fit all putative QTL simultaneously and jointly estimate the number, genomic positions, and genetic effects of QTL. We assume that an obesity trait is affected by l QTL. Under the epistatic model, observed phenotypic value of the ith mouse, y_i, can be described by the following linear model,

$$y_i = \mu + \sum_{q=1}^{l} \gamma_q x_{iq} a_q + \sum_{q_1<q_2}^{l} \gamma_{q_1q_2} x_{iq_1} x_{iq_2} b_{q_1q_2} + e_i, \quad i = 1, 2, \ldots, n, \tag{1}$$

where n is the number of animals in the mapping population, μ is the overall mean, l is the number of putative QTL, a_q is the main effect of putative QTL q, $b_{q_1q_2}$ is the epistatic effect between QTL q_1 and q_2, x_{iq} is the indicator variable denoting the genotype of putative QTL q for individual i and is defined by 0.5 or −0.5 for the two genotypes in the mapping population, e_i is the residual error assumed to follow $N(0, \sigma_e^2)$, γ_q is a binary indicator variable for the main effect of putative QTL q, taking value one if QTL q has main effect and zero otherwise, and $\gamma_{q_1q_2}$ is a binary indicator variable for epistatic effect between QTL q_1 and q_2, taking value one if QTL q_1 and q_2 interact and zero otherwise. Hereafter, we call these binary indicator variables effect indicators.

Under the nonepistatic model, the epistatic effects are not included in the model. The nonepistatic model can be expressed as

$$y_i = \mu + \sum_{q=1}^{l} z_{iq} a_q + e_i, \quad i = 1, 2, \ldots, n. \tag{2}$$

In Equation 2, the residual error e_i includes epistatic effects and environmental error. Other terms are defined as above. Under the nonepistatic model, the l putative QTL are chosen only on the basis of their significant main effects. Using the epistatic model, however, one is able to find QTL with either significant main effects or epistatic effects.

The multiple-QTL models were analyzed using Bayesian methods. In a Bayesian framework, the statistical inference is based on the joint posterior distribution of all unknowns in the model given the observed data. The observed data include the phenotypic values and the marker genotypes. The unknowns include the number of QTL, genomic locations of QTL, effect indicators, main effects, epistatic effects, overall mean, residual variance, genotypes of missing markers, and genotypic indicators of putative QTL. Calculation of the joint posterior distributions is analytically intractable, and thus a Markov chain Monte Carlo (MCMC) approach is utilized to obtain posterior samples from the joint posterior distribution. We used the Bayesian method and MCMC algorithm developed by Yi *et al.* (2003) to generate posterior samples from the joint posterior distribution and then estimate the number, locations, main effects, and epistatic effects of QTL simultaneously using the posterior samples. Briefly, the MCMC algorithm consisted of the following steps: (a) update the model parameters (main effects, epistatic effects, overall mean, and residual variance); (b) update the genotypic indicators of QTL and the genotypes of missing markers; (c) update the locations of QTL; (d) update the effect indicator, add or delete a main or epistatic effect; and (e) update the number of QTL. Two different steps were used to update the number of QTL: (1) add a QTL with main effects or epistatic effects between existing QTL or delete an existing QTL and (2) add two new QTL with only epistatic effect between themselves or delete two existing QTL. The detailed algorithms for each of these steps were described in Yi and Xu (2002) and Yi *et al.* (2003).

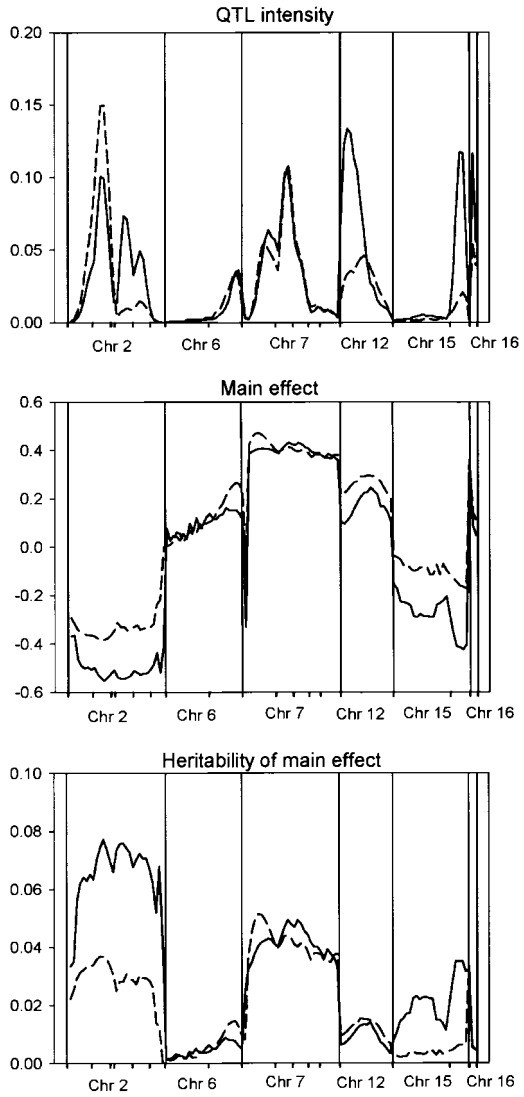

FIGURE 2.—Percentage of body fat. Profiles of posterior QTL intensity, location-wise main effect, and heritability explained by the main effect under the nonepistatic (dashed lines) and epistatic models (solid lines) are shown. Ticks on the *x*-axis represent genetic markers.

For each trait, the phenotypic values were standardized using $y_i = (y_i - \bar{y})/s$, where $\bar{y}$ is the mean and s is the standard deviation of y. The standardized records were subject to Bayesian analysis. For all analyses, the MCMCs were started with no QTL in the model. The prior for the overall mean was $N(0, 10)$. The residual variance took uniform$(0, 1)$, where the upper bound is the variance of the standardized records. The priors for all main and epistatic effects were chosen to be $N(0, 1)$. The prior distribution for the number of QTL was taken as uniform$(0, 10)$. The prior for each effect indicator was taken as uniform at two states of 0 and 1. The tuning parameter of proposals in the random-walk Metropolis-Hastings algorithm for updating QTL positions was chosen to be 0.5 cM. The prior of the QTL position was uniform over the genome region.

Bayesian QTL analyses were executed with a C program (YI *et al.* 2003). In each analysis, the MCMC sampler was run for 4×10^5 cycles after discarding the first 2000 cycles for the burn-in period. The chain was thinned (one iteration saved in every 20 cycles) to reduce serial correlation in the stored samples so that the total number of samples kept in the post-Bayesian analysis was 2×10^4. The stored samples are called posterior samples.

The posterior samples were used to obtain inferences about the parameters of interest. The posterior probability distribution of the number of QTL, $p(l = x|\mathbf{y}, \mathbf{M})$ $(x = 0, 1, 2, \ldots)$, was obtained by counting the number of samples in which the number of QTL is l, divided by the total of number of samples. The posterior probability that a chromosomal region contains at least one QTL was calculated as the number of samples with at least one QTL in this region over the total number of samples. QTL locations were estimated using the posterior QTL intensity function (SILLANPAA and ARJAS 1998). The posterior QTL intensity was depicted via plotting the frequency of hits by the QTL in a short interval against the genome location of the interval. The regions frequently hit by the QTL are candidate locations of the QTL. The location-wise estimates for main effect and proportion of variance explained by the main effect were obtained by calculating the mean of the estimates for these parameters in each short interval. The main effect and proportion of variance explained by the main effect at a chromosomal region were obtained similarly. Inference for the epistatic effects and the proportion of phenotypic variance explained by each epistatic effect was obtained conditional on the estimated loci falling into the corresponding chromosomal regions. The posterior probability that each epistatic effect is included in the model was calculated as the number of samples containing this epistatic effect over the total number of samples.

RESULTS

Percentage of body fat: The posterior probability distribution of the number of QTL is given in Table 2. Under the nonepistatic model, the variation of percentage of body fat was likely contributed by two or three loci with an equal chance (~40%) in these genomic regions. However, the epistatic model analysis showed that percentage of body fat was most likely affected by four loci. The QTL intensity curves in Figure 2 show that QTL activity was found in chromosomes 2 and 7 in the nonepistatic model analysis. The posterior probabilities that the loci on these two chromosomes were included in the nonepistatic model were ~1.0 (Table 3), showing that these two chromosomal regions almost certainly affect percentage of body fat. In the epistatic model analysis, QTL activity was detected in chromosomes 2, 7, 12, and 15. The probabilities that these chromosomes included QTL were 1.00, 1.00, 0.969, and 0.567, respectively (Table 4). Therefore, two additional QTL were discovered by the epistatic model analysis.

The profiles of the location-wise main effect and the heritability explained by the main effect show that SPRET/Ei alleles promote leanness on chromosomes 2 and 15 and obesity on other chromosomes (Figure 2). Under the nonepistatic analysis, the main effects on chromosome 2 and 7 were estimated to account for 3.4 and 4.3% of the phenotypic variance, respectively (Table 3). The epistatic analysis estimated that these two chromosomes explain 7.2 and 4.4% of the phenotypic variance, respectively (Table 4). Other chromosomes

TABLE 3

Nonepistatic model

Trait	Posterior summary	Chr 2	Chr 6	Chr 7	Chr 12	Chr 15	Chr 16
% body fat	Posterior probability	0.999	0.204	1.000	0.472	0.112	0.101
	Main effect	−0.369 (0.075)	0.213 (0.120)	0.414 (0.078)	0.269 (0.110)	−0.136 (0.095)	0.121 (0.109)
	Heritability	0.034 (0.013)	0.011 (0.009)	0.043 (0.015)	0.013 (0.009)	0.005 (0.005)	0.005 (0.005)
Adiposity index	Posterior probability	0.995 (0.076)	0.054	1.000	0.081	0.154	0.273
	Main effect	−0.378 (0.084)	0.138 (0.123)	0.481 (0.048)	0.178 (0.112)	−0.198 (0.094)	0.261 (0.103)
	Heritability	−0.036 (0.013)	0.006 (0.007)	0.052 (0.013)	0.007 (0.007)	0.009 (0.007)	0.014 (0.011)
Total fat mass	Posterior probability	0.333 (0.076)	0.082	0.828	0.271	0.038	0.097
	Main effect	−0.219 (0.089)	0.152 (0.122)	0.313 (0.102)	0.242 (0.115)	−0.023 (0.109)	0.107 (0.165)
	Heritability	0.013 (0.009)	0.007 (0.007)	0.025 (0.014)	0.011 (0.009)	0.002 (0.003)	0.007 (0.007)
Body weight	Posterior probability	0.051 (0.076)	0.082	1.00	0.414	0.267	0.065
	Main effect	−0.086 (0.076)	−0.120 (0.100)	0.444 (0.085)	0.275 (0.113)	−0.192 (0.085)	0.089 (0.121)
	Heritability	0.003 (0.004)	0.005 (0.006)	0.049 (0.017)	0.014 (0.010)	0.009 (0.007)	0.004 (0.005)
Total cholesterol	Posterior probability	0.0427 (0.076)	0.848	0.434	0.911	0.055	0.113
	Main effect	−0.054 (0.089)	0.392 (0.132)	0.230 (0.095)	0.471 (0.142)	−0.070 (0.120)	0.165 (0.114)
	Heritability	0.002 (0.003)	0.030 (0.017)	0.014 (0.011)	0.032 (0.018)	0.003 (0.004)	0.006 (0.007)

Averaged main effect for each chromosome, heritability explained by the main effect, and posterior probability that each chromosome is included in the model are shown. Posterior standard deviations of the estimates are given in parentheses. Chr, chromosome.

had weak main effects and thus were not detected in the nonepistatic analysis. The QTL on chromosome 12, which has a weak main effect, interacts with the QTL on chromosome 2 and the QTL on chromosome 15. The epistatic genetic variance was approximately equal to the genetic variance explained by the main effects. The posterior probabilities that these two epistatic effects are included in the model were 0.964 and 0.879, respectively.

Adiposity index: There was a posterior prediction of one additional QTL when allowing for epistasis (Table 2). The profiles of QTL intensity given in Figure 3 show that chromosomes 2 and 7 affected adiposity index on both nonepistatic and epistatic analyses. The posterior probabilities that the loci on these two chromosomes were included were ~1.0 in both model analyses (Tables 3 and 4). The posterior probabilities that chromosomes 12 and 16 include QTL were 0.509 and 0.629, respectively, under the epistatic model (Table 4), whereas these posterior probabilities were 0.081 and 0.273 under the nonepistatic model (Table 3), suggesting that the loci on these two chromosomes are involved mainly in epistatic interactions.

As for percentage of body fat, SPRET/Ei alleles pro-

TABLE 4

Epistatic model

Trait	Posterior summary	Chr 2	Chr 6	Chr 7	Chr 12	Chr 15	Chr 16
% body fat	Posterior probability	1.00	0.175	1.00	0.969	0.567	0.176
	Main effect	−0.529 (0.110)	0.137 (0.130)	0.411 (0.108)	0.155 (0.189)	−0.391 (0.142)	0.069 (0.145)
	Heritability	0.072 (0.028)	0.006 (0.008)	0.044 (0.022)	0.009 (0.010)	0.032 (0.020)	0.005 (0.006)
Adiposity index	Posterior probability	0.984	0.139	1.000	0.509	0.363	0.629
	Main effect	−0.359 (0.105)	0.117 (0.152)	0.469 (0.120)	0.032 (0.197)	−0.350 (0.166)	0.286 (0.119)
	Heritability	0.034 (0.019)	0.006 (0.009)	0.056 (0.027)	0.005 (0.005)	0.027 (0.022)	0.018 (0.013)
Total fat mass	Posterior probability	0.956	0.057	0.869	0.948	0.036	0.066
	Main effect	−0.406 (0.112)	0.105 (0.122)	0.279 (0.105)	0.318 (0.124)	−0.107 (0.153)	0.055 (0.175)
	Heritability	0.0434 (0.023)	0.005 (0.006)	0.021 (0.014)	0.024 (0.013)	0.007 (0.009)	0.006 (0.007)
Body weight	Posterior probability	0.873	0.048	0.999	0.907	0.166	0.080
	Main effect	−0.326 (0.095)	−0.117 (0.101)	0.441 (0.088)	0.322 (0.112)	−0.220 (0.112)	0.108 (0.128)
	Heritability	0.028 (0.015)	0.005 (0.006)	0.049 (0.019)	0.019 (0.012)	0.012 (0.012)	0.005 (0.005)
Total cholesterol	Posterior probability	0.049	0.995	0.441	0.997	0.060	0.130
	Main effect	−0.071 (0.104)	0.384 (0.133)	0.229 (0.102)	0.469 (0.146)	−0.011 (0.152)	0.047 (0.145)
	Heritability	0.003 (0.006)	0.029 (0.018)	0.015 (0.012)	0.031 (0.013)	0.004 (0.007)	0.003 (0.005)

Averaged main effect for each chromosome, heritability explained by the main effect, and posterior probability that each chromosome is included in the model are shown. Posterior standard deviations of the estimates are given in parentheses. Chr, chromosome.

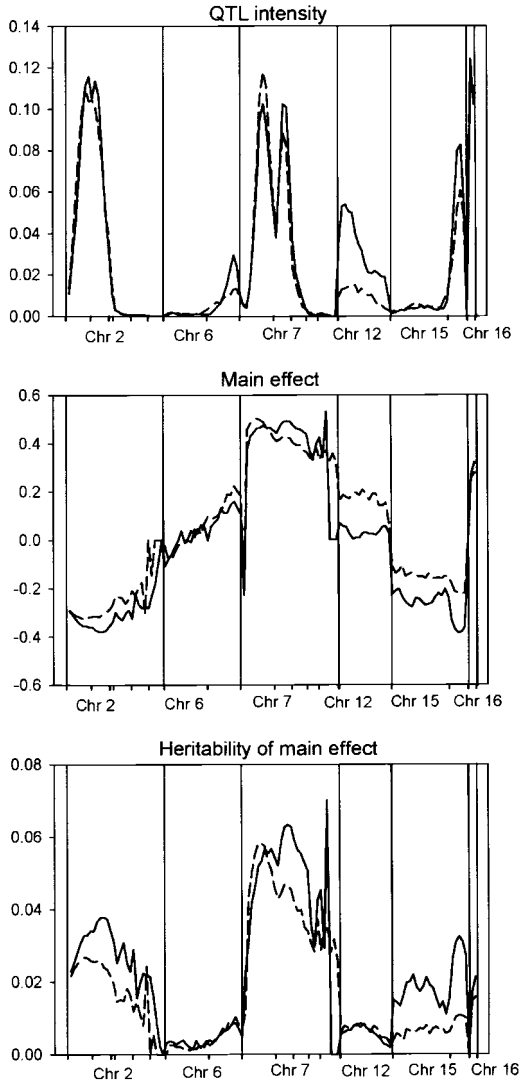

FIGURE 3.—Adiposity index. Profiles of posterior QTL intensity, location-wise main effect, and heritability explained by the main effect under the nonepistatic (dashed lines) and epistatic models (solid lines) are shown. Ticks on the *x*-axis represent genetic markers.

mote leanness on chromosomes 2 and 15 and obesity on other chromosomes (Figure 3). The main effect on chromosomes 2 and 7 explained 3.5 and 5.4% of the phenotypic variance, respectively (Tables 3 and 4). Other chromosomes had weak main effects and thus were not detected in the nonepistatic analysis. Four chromosomes were involved in epistatic interactions (Table 5). The interaction of chromosome 2 with chromosome 12 was similar to that for percentage of body fat. The interaction between chromosomes 12 and 15, which was significant for percentage of body fat, did not occur for adiposity index. However, two additional epistatic effects, chromosome 12 with 7 and 7 with 16, were observed. Compared with those for percentage of body fat, these interactions had smaller effects and each accounted for only ~1.7% of the phenotypic variance.

Total fat mass: The posterior probability showed that there is most likely one QTL in these genomic regions affecting the variation of total fat mass when excluding epistasis, whereas there are three QTL when allowing for epistasis (Table 2). The profile of the posterior QTL intensity on chromosome 7 under the nonepistatic model is fairly similar to that under the epistatic model, indicating that the QTL on chromosome 7 affects fat mass primarily through its main effect (Figure 4). The two models also gave similar posterior probabilities that chromosome 7 includes one QTL (Tables 3 and 4). Chromosomes 2 and 12 have significant peaks in the epistatic plot and are essentially invisible in the nonepistatic plot (Figure 4). Under the epistatic model, the posterior probabilities that the QTL on chromosomes 2 and 12 are included in the model were 0.956 and 0.948, respectively. Therefore, we found two additional QTL in the epistatic analysis.

As for percentage of body fat and adiposity index, SPRET/Ei alleles promote increased fat mass on chromosomes 7 and 12 and reduced fat mass on chromosome 2. The main effect on chromosome 7 in the nonepistatic and epistatic analyses was estimated to account for 2.5 and 2.1% of the phenotypic variance, respectively (Tables 3 and 4). Under the nonepistatic model, the averaged main effects of other chromosomes were estimated to be small. As seen for percentage of body fat and adiposity, chromosome 2 strongly interacts with chromosome 12 (Table 5): the epistatic effect between these chromosomes is −0.849, which accounts for 4.8% of the phenotypic variance, and this epistatic effect was always included in the model. Chromosome 2 also interacts with chromosome 7, but this epistatic effect explains only 1.2% of the phenotypic variance and was included in the model with probability of 0.67.

Body weight: Under the nonepistatic model, the variation of body weight was likely contributed by one or two loci in these genomic regions (Table 2). However, the epistatic model analysis showed that body weight was most likely affected by three loci. As with the three obesity traits aforementioned, the QTL activity for body weight was detected in chromosome 7 in both the nonepistatic and epistatic models (Figure 5) and was always selected into the model (Tables 3 and 4). The QTL on chromosomes 2 and 12 were included in the model with high probabilities of 0.873 and 0.907, respectively, under the epistatic model. Therefore, the QTL on chromosomes 2 and 12 were discovered only in the epistatic analysis.

As for the aforementioned obesity traits, SPRET/Ei alleles promote higher body weight on chromosomes 7 and 12, and reduced body weight on chromosome 2 (Figure 5). The main effect of the QTL on chromosome 7 was estimated to be similar under both models, ~0.44, and accounts for 4.9% of the phenotypic variance in the two model analyses. Under the nonepistatic model, the main effects of other chromosomes are small

TABLE 5

Epistatic effect, heritability, and posterior probability that the epistatic effect is included in the model

Trait	Loci pair	Posterior probability	Epistasis	Heritability
% body fat	Chr 2 and Chr 12	0.964	−0.667 (0.211)	0.031 (0.017)
	Chr 12 and Chr 15	0.879	−0.804 (0.261)	0.041 (0.020)
Adiposity index	Chr 2 and Chr 12	0.510	−0.441 (0.241)	0.016 (0.011)
	Chr 12 and Chr 7	0.500	−0.477 (0.288)	0.018 (0.012)
	Chr 7 and Chr 16	0.539	0.484 (0.200)	0.017 (0.010)
Total fat mass	Chr 2 and Chr 12	0.992	−0.849 (0.226)	0.048 (0.024)
	Chr 2 and Chr 7	0.667	0.379 (0.188)	0.012 (0.008)
Body weight	Chr 2 and Chr 12	0.980	−0.847 (0.196)	0.047 (0.021)
Total cholesterol	Chr 6 and Chr 12	0.982	−0.919 (0.26)	0.039 (0.020)

Posterior standard deviations of the estimates are given in parentheses. Chr, chromosome.

(Table 3). As with total fat mass, however, the main effects of chromosomes 2 and 12 in the epistatic analysis were much higher, −0.326 and 0.322, and account for 2.8 and 1.9% of the phenotypic variance, respectively (Table 4). As seen for the other three obesity traits, chromosome 2 strongly interacts with chromosome 12.

Plasma total cholesterol: The posterior probabilities of the number of QTL show that two or three QTL in these regions were detected to influence the variation of total cholesterol (Table 2). The QTL activity was found in chromosomes 6 and 12 in both the nonepistatic and epistatic model analyses (Figure 6). In the nonepistatic analysis, the posterior probabilities that these two chromosomes include QTL were ~85 and 91%, respectively (Table 3). In the epistatic analysis, these two chromosomes were always included into the model (Table 4). The QTL on chromosome 7, which was found to influence all four obesity traits, was detected with small probability of 0.4 and, thus, chromosome 7 does not influence total cholesterol. These results indicate largely separate genetic control for obesity and total cholesterol.

The nonepistatic and epistatic analyses gave similar profiles of the main effect and the heritability explained by the main effect (Tables 3 and 4). The SPRET/Ei alleles lead to a higher value of total cholesterol. Chromosomes 6 and 12 have strong interaction, which accounted for 3.9% of the phenotypic variance (Table 5).

Sensitivity analysis: In a Bayesian analysis, as used in this study, it is important to check for sensitivity to the choice of prior distributions. It has been shown that posterior inferences are influenced mainly by prior specifications of the number and genetic effects of QTL (Satagopan *et al.* 1996; Gaffney 2001; Yi *et al.* 2003). Therefore, we investigated sensitivity to the choice of prior distributions for these two types of parameters.

We took Poisson distribution with different prior means as priors of the number of QTL and normal distribution with different prior variances as priors of additive and epistatic effects. Under the epistatic model, the estimated

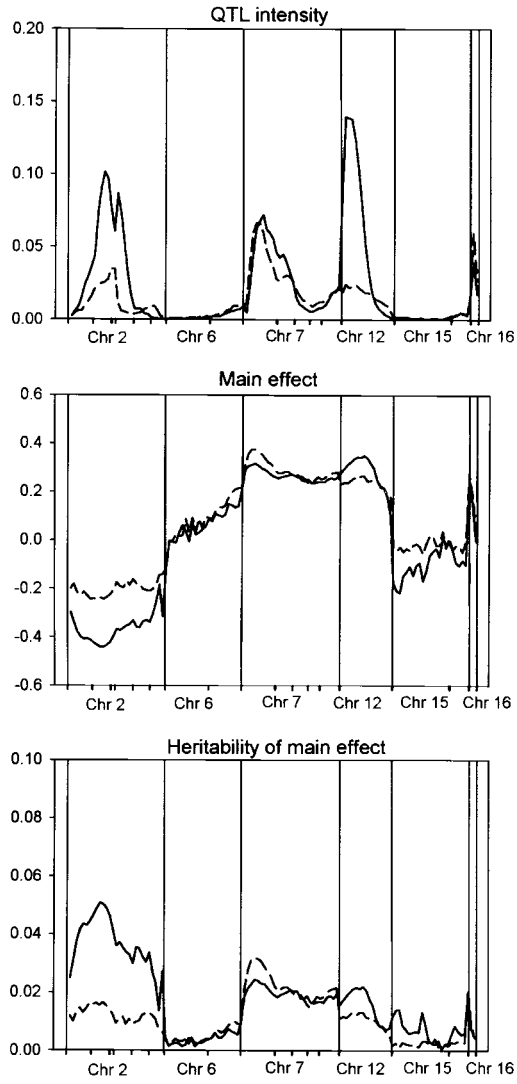

Figure 4.—Total fat mass. Profiles of posterior QTL intensity, location-wise main effect, and heritability explained by the main effect under the nonepistatic (dashed lines) and epistatic models (solid lines) are shown. Ticks on the *x*-axis represent genetic markers.

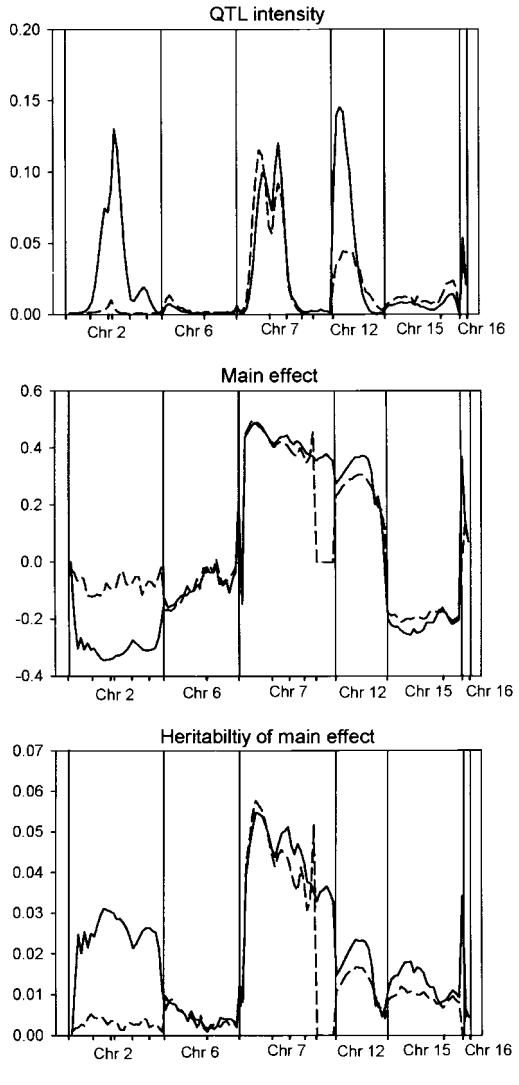

FIGURE 5.—Body weight. Profiles of posterior QTL intensity, location-wise main effect, and heritability explained by the main effect under the nonepistatic (dashed lines) and epistatic models (solid lines) are shown. Ticks on the *x*-axis represent genetic markers.

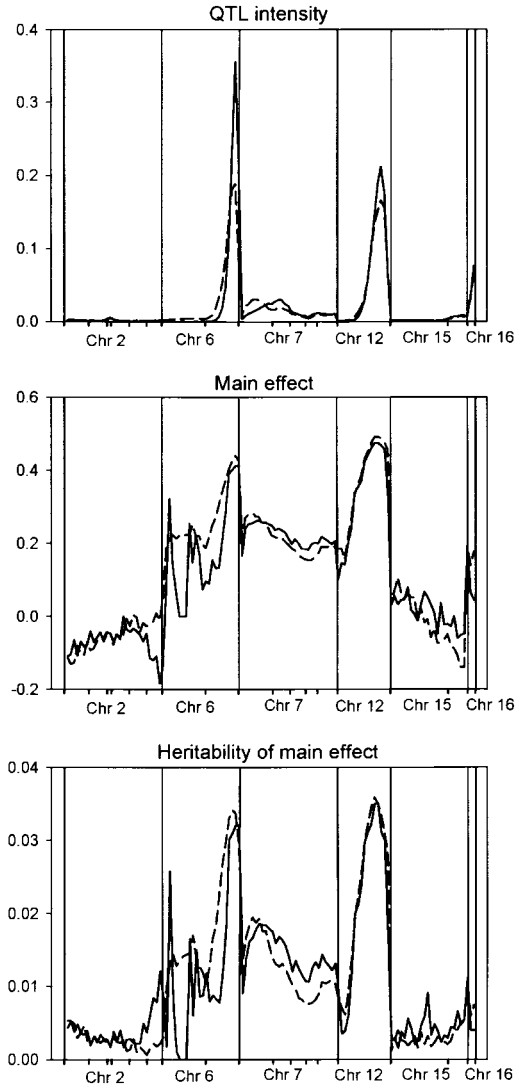

FIGURE 6.—Total cholesterol. Profiles of posterior QTL intensity, location-wise main effect, and heritability explained by the main effect under the nonepistatic (dashed lines) and epistatic models (solid lines) are shown. Ticks on the *x*-axis represent genetic markers.

posterior probability distributions of the number of QTL for percentage of body fat are given in Table 6. In all analyses, the mode of the number of QTL was estimated to be 3 or 4. Increasing the prior mean of the number of QTL favored a higher number of QTL. Similarly, reducing the prior variance for genetic effects also favored a higher number of QTL. Table 6 also gives Bayes' factor $B(3, 2)$ for all analyses. The Bayes factor appeared to be less sensitive to the prior on the number of QTL, but was greatly affected by the choice of prior variance of genetic effects. The profiles of the QTL intensity are depicted in Figure 7. These profiles are fairly similar and thus these priors did not seem to affect the posterior inference of QTL locations. We also found that the posterior inference about the genetic effects and the effect indicators were not sensitive to the priors of the number and genetic effects of QTL.

DISCUSSION

In this study, we adopted a Bayesian model selection method recently developed by YI *et al.* (2003) to evaluate potential epistatic interactions in the BSB backcross for four obesity traits and plasma total cholesterol. The statistical approach used here models multiple QTL, their main effects, and epistatic effects simultaneously. With this statistical method, we can jointly infer the genetic architecture of a complex trait and estimate the associated genetic parameters, including the number, positions, main and epistatic effects of the identified QTL, and the genetic variance and heritability explained by each genetic effect. Therefore, this Bayesian mapping method can detect multiple QTL with any combination of main and epistatic effects.

A variety of other statistical methods for mapping epistatic QTL have been developed. The simplest method is

TABLE 6

Bayesian posterior probability distribution of the number of QTL for percentage of body fat under different prior distributions for the number of QTL and genetic effects

Priors	Estimated probability distribution for l =								Estimated expectation	Bayes' factor $B(3, 2)$
	0	1	2	3	4	5	6	>7		
Poisson(1), $N(0, 1)$	0	0	0.087	0.666	0.224	0.021	0.001	0	3.181	22.929
Poisson(3), $N(0, 1)$	0	0	0.025	0.479	0.411	0.078	0.006	0	3.561	19.160
Poisson(5), $N(0, 1)$	0	0	0.011	0.346	0.467	0.151	0.024	0	3.830	19.873
Uniform(0, 10), $N(0, 0.5)$	0	0	0.005	0.244	0.451	0.230	0.077	0	4.101	40.800
Uniform(0, 10), $N(0, 1.5)$	0	0	0.023	0.380	0.458	0.125	0.013	0	3.627	16.520

to carry out two-way variance analyses for all marker pairs. We used this method to test interactions between markers close to the QTL detected by the Bayesian epistatic analysis, but found that some of the epistatic effects were not significant even under an unadjusted threshold value, *e.g.*, 5% (see Table 7). The marker data used in this study include missing marker genotypes of 30%. The large proportion of missing values largely reduces the sample size used in variance analysis. Furthermore, the two-locus model approaches may ignore other potential associated QTL effects, which increases the residual variance and thus reduces the statistical power on detecting epistasis.

Using the interval-mapping method (Lander and Botstein 1989), two previous studies searched for BSB obesity QTL (Warden *et al.* 1993, 1995). In those BSB crosses, the central region of chromosome 7 was significant for body weight, percentage of body fat, and body mass index as well as for plasma total cholesterol. The central portion of chromosome 7 was discovered to be especially rich in QTL associations for obesity in several other studies as well (Warden *et al.* 1995; Vaughn *et al.* 1999; Cheverud *et al.* 2001). A chromosome 12 QTL for percentage of body fat was present in a BSB cross using outbred SPRET/Pt (Warden *et al.* 1993), but was absent in a later cross using inbred SPRET/Ei (Warden *et al.* 1995), leading us to conclude that the main effect of chromosome 12 QTL was weak. QTL were previously identified for femoral and mesenteric fat depots, but not for percentage of body fat, on chromosomes 6 and 15, respectively.

We have now performed three additional crosses, all of which involve a strain of C57BL/6J that is congenic for knockout of *hepatic lipase* on chromosome 9 (B6-LIPC^{null}) and inbred SPRET/Ei (Farahani *et al.* 2004). BSB mice of these three crosses were maintained in a different mouse facility in different caging from that of the previous studies, although all mice in all crosses were maintained in individual cages. As found previously in the cross involving inbred SPRET/Ei (Warden *et al.* 1995), in these crosses involving B6-LIPC^{null} and SPRET/Ei mice there is no independent QTL on chromosome 12. The chromosome 7 QTL was reproduced, a new QTL was observed on chromosome 2, and there were no independent QTL on chromosomes 6 and 15. The QTL on chromosome 7 was found to have significant positive main effects on the obesity traits analyzed, with the SPRET/Ei allele on chromosome 7 resulting in greater obesity. In the nonepistatic analyses, the region of chromosome 2 was found to influence only percentage of body fat and adiposity index, both of which are ratios of body fat mass to total body weight, but when allowing for epistasis, chromosomes 2 and 12 were found to influence fat mass and body weight as well. The main effects of chromosome 2 for the four obesity traits were all negative, and thus the SPRET/Ei allele led to lower phenotypes of obesity on chromosome 2. The previous crosses had observed

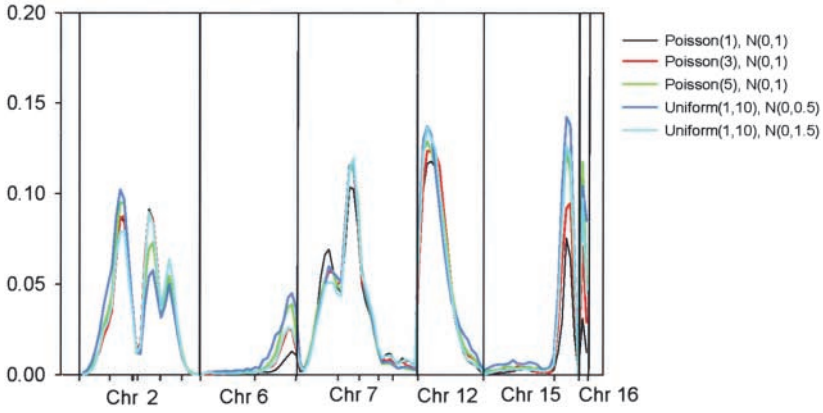

Figure 7.—Profiles of posterior QTL intensity under the epistatic model for percentage of body fat under different prior distributions for the number of QTL and genetic effects are shown. Ticks on the *x*-axis represent genetic markers.

TABLE 7

Two-way variance analysis of two markers close to the QTL detected in Bayesian epistatic analyses

Trait	Marker pair	Sample size	*P* value
% body fat	D2Mit109, D12Mit51	347	0.0302
	D12Mit51, D15Mit152	170	0.1474
Adiposity index	D2Mit109, D12Mit51	347	0.0275
	D12Mit51, D7Mit103	324	0.5315
	D7Mit103, D16Mit210	286	0.2355
Total fat mass	D2Mit109, D12Mit51	442	0.0035
	D2Mit109, D7Mit8	316	0.1115
Body weight	D2Mit55, D12Mit51	489	0.0053
Total cholesterol	D6Mit355, D12Mit27	202	0.1523

only marginally significant effects for chromosome 2, whereas the B6-LIPC^{null} cross has a highly significant QTL. This chromosome 2 QTL has already been confirmed by production of congenic mice carrying the SPRET/Ei chromosome 2 on the B6-LIPC^{null} background (Diament *et al.* 2004) and is thus a confirmed QTL. The chromosome 12 QTL had weak main effects on the obesity traits and thus was not detected when ignoring the epistatic effects.

Epistasis was found to play an important role in controlling the obesity traits analyzed in this study. The epistatic interaction, estimated to be negative and thus promoting leanness between chromosomes 2 and 12, had significant effects on all four obesity traits. For percentage of body fat, chromosome 12 also interacts with chromosome 15 and this epistatic effect resulted in detection of chromosome 15 in the epistatic model analysis. Except for the interaction between chromosomes 2 and 12, which is shared for all obesity traits, different epistatic interactions were detected for different traits, indicating largely separate genetic control for these different ways of measuring obesity.

Total cholesterol was influenced only by loci on chromosomes 6 and 12. Thus, a different genetic architecture was discovered for the obesity traits and total cholesterol. The main effects of these two chromosomes were estimated to be positive, and thus the SPRET/Ei allele led to higher phenotype on both chromosomes. A strong negative epistatic effect between chromosomes 6 and 12 led to lower total cholesterol. The proportion of the phenotypic variance explained by this epistatic interaction was actually higher than those explained by the main effects. We previously identified QTL for total cholesterol on chromosomes 6 and 7 in BSB mice (Warden *et al.* 1995), so the present study confirms the chromosome 6, but not chromosome 7, QTL.

This work has focused on epistasis between known obesity QTL in the BSB model. This means that there may be other sites of epistasis that remain undetected because we have not performed a whole-genome scan. Nevertheless, these results provide clear information about the presence of epistasis in the BSB model and provide information about the role of epistasis in the chromosome 12 QTL.

We thank Larry Castellani for plasma total- and HDL-cholesterol measurements. We are grateful to Susan Bennett and Noreene Shibata for the breeding and care of animals. N. Yi and D. B. Allison are supported by the National Institutes of Health (NIH RO1ES09912, NIH RO1 DK056366, and NIH P30DK056336). A. Diament, S. Chiu, J. S. Fisler, and C. H. Warden are supported by NIH RO1 DK52581, NIH training grant PHS DK07355, and by the University of California, Davis, Clinical Nutrition Research Unit (NIH DK35747).

LITERATURE CITED

Allison, D. B., A. Pietrobelli, M. S. Faith, K. R. Fontaine, E. Gropp *et al.*, 2002 Genetic influences on obesity, pp. 31–74 in *Obesity: Mechanisms and Clinical Management*, edited by R. H. Eckel. Elsevier, New York.

Bell, G. E., and J. S. Stern, 1977 Evaluation of body composition of young obese and lean Zucker rats. Growth **41** (1): 63–80.

Brockmann, G. A., J. Kratzsch, C. S. Haley, U. Renne, M. Schwerin *et al.*, 2000 Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F(2) variance of growth and obesity in DU6i × DBA/2 mice. Genome Res. **10** (12): 1941–1957.

Castellani, L. W., M. Navab, B. J. Van Lenten, C. C. Hedrick, S. Y. Hama *et al.*, 1997 Overexpression of apolipoprotein AII in transgenic mice converts high density lipoproteins to proinflammatory particles. J. Clin. Invest. **100** (2): 464–474.

Cheverud, J. M., T. T. Vaughn, L. S. Pletscher, A. C. Peripato, E. S. Adams *et al.*, 2001 Genetic architecture of adiposity in the cross of LG/J and SM/J inbred mice. Mamm. Genome **12** (1): 3–12.

Coleman, D. L., and K. P. Hummel, 1973 The influence of genetic background on the expression of the obese (Ob) gene in the mouse. Diabetologia **9** (4): 287–293.

Cordell, H. J., 2002 Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. Hum. Mol. Genet. **11** (20): 2463–2468.

Corva, P. M., S. Horvat and J. F. Medrano, 2001 Quantitative trait loci affecting growth in high growth (hg) mice. Mamm. Genome **12** (4): 284–290.

Diament, A. L., P. Farahani, S. Chiu, J. S. Fisler and C. H. Warden, 2004 A novel mouse chromosome 2 congenic strain with obesity phenotypes. Mamm. Genome (in press).

Dong, C., S. Wang, W. D. Li, D. Li, H. Zhao *et al.*, 2003 Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. Am. J. Hum. Genet. **72** (1): 115–124.

Farahani, P., J. S. Fisler, H. Wong, A. L. Diament, N. Yi *et al.*, 2004 Reciprocal hemizygosity analysis of mouse hepatic lipase reveals influence on obesity. Obesity Res. **12:** 292–305.

Fisler, J. S., C. H. Warden, M. J. Pace and A. J. Lusis, 1993 BSB: a new mouse model of multigenic obesity. Obes. Res. **1:** 271–280.

Gaffney, P. J., 2001 An efficient reversible jump Markov chain Monte Carlo approach to detect multiple loci and their effects in inbred crosses. Thesis, Madison, WI.

Harris, R. B., T. D. Mitchell, X. Yan, J. S. Simpson and S. M. Redmann, Jr., 2001 Metabolic responses to leptin in obese db/db mice are strain dependent. Am. J. Physiol. Regul. Integr. Comp. Physiol. **281** (1): R115–R132.

Hofmann, W. E., X. Liu, C. M. Bearden, M. E. Harper and L. P. Kozak, 2001 Effects of genetic background on thermoregulation and fatty acid-induced uncoupling of mitochondria in UCP1-deficient mice. J. Biol. Chem. **276** (15): 12460–12465.

Hummel, K. P., D. L. Coleman and P. W. Lane, 1972 The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains. Biochem. Genet. **7** (1): 1–13.

Lander, E. S., and D. Botstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

Moore, J. H., 2003 The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum. Hered. **56:** 73–82.

Satagopan, J. M., B. S. Yandell, M. A. Newton and T. C. Osborn, 1996 A Bayesian approach to detect quantitative trait loci using Markov chain Monte Carlo. Genetics **144:** 805–816.

Segal, N. L., and D. B. Allison, 2002 Twins and virtual twins: bases of relative body weight revisited. Int. J. Obes. Relat. Metab. Disord. **26** (4): 437–441.

Sillanpaa, M. J., and E. Arjas, 1998 Bayesian mapping of multiple quantitative trait loci from incomplete inbred line cross data. Genetics **148:** 1373–1388.

Vaughn, T. T., L. S. Pletscher, A. Peripato, K. King-Ellison, E. Adams *et al.*, 1999 Mapping quantitative trait loci for murine growth: a closer look at genetic architecture. Genet. Res. **74:** 313–322.

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** (2): 773–779.

Warden, C. H., J. S. Fisler, S. M. Shoemaker, P. Z. Wen, K. L. Svenson *et al.*, 1995 Identification of four chromosomal loci determining obesity in a multifactorial mouse model. J. Clin. Invest. **95** (4): 1545–1552.

Warnick, G. R., and A. T. Remaley, 2001 Measurement of cholesterol in plasma and other body fluids. Curr. Atheroscler. Rep. **3** (5): 404–411.

Yi, N., and S. Xu, 2002 Mapping quantitative trait loci with epistatic effects. Genet. Res. **79** (2): 185–198.

Yi, N., S. Xu and D. B. Allison, 2003 Bayesian model choice and search strategies for mapping interacting quantitative trait loci. Genetics **165:** 867–883.

Communicating editor: G. A. Churchill