Quantitative trait loci that determine plasma lipids and obesity in C57BL/6J and 129S1/SvImJ inbred mice

Naoki Ishimori,* Renhua Li,* Peter M. Kelmenson,* Ron Korstanje,* Kenneth A. Walsh,* Gary A. Churchill,* Kristina Forsman-Semb,† and Beverly Paigen[1,*]

The Jackson Laboratory,* Bar Harbor, ME; and AstraZeneca R&D Mölndal,† Mölndal, Sweden

Abstract The plasma lipid concentrations and obesity of C57BL/6J (B6) and 129S1/SvImJ (129) inbred mouse strains fed a high-fat diet containing 15% dairy fat, 1% cholesterol, and 0.5% cholic acid differ markedly. To identify the loci controlling these traits, we conducted a quantitative trait loci (QTL) analysis of 294 (B6 × 129) F_2 females fed a high-fat diet for 14 weeks. Non-HDL cholesterol concentrations were affected by five significant loci: *Nhdlq1* [chromosome 8, peak centimorgan (cM) 38, logarithm of odds [LOD] 4.4); *Nhdlq4* (chromosome 10, cM 70, LOD 4.0); *Nhdlq5* (chromosome 6, cM 0) interacting with *Nhdlq4*; *Nhdlq6* (chromosome 7, cM 10) interacting with *Nhdlq1*; and *Nhdlq7* (chromosome 15, cM 0) interacting with *Nhdlq4*. Triglyceride (TG) concentrations were affected by three significant loci: *Tgq1* (chromosome 18, cM 42, LOD 3.2) and *Tgq2* (chromosome 9, cM 66) interacting with *Tgq3* (chromosome 4, cM 58). Obesity measured by percentage of body fat mass and body mass index was affected by two significant loci: *Obq16* (chromosome 8, cM 48, LOD 10.0) interacting with *Obq18* (chromosome 9, cM 65). Knowing the genes for these QTL will enhance our understanding of obesity and lipid metabolism.—Ishimori, N., R. Li, P. M. Kelmenson, R. Korstanje, K. A. Walsh, G. A. Churchill, K. Forsman-Semb, and B. Paigen. **Quantitative trait loci that determine plasma lipids and obesity in C57BL/6J and 129S1/SvImJ inbred mice. *J. Lipid Res.* 2004.** 45: **1624–1632.**

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Cardiovascular disease is often coincident with dyslipidemia, obesity, hypertension, and diabetes, which are often clustered in some individuals and recognized as metabolic syndrome (1). These disorders are complex, multifactorial, and controlled by both environmental and genetic factors. The causal relationship between the risk of cardiovascular disease and either obesity or increased LDL cholesterol or triglyceride (TG) is definitively established. Much is known about the nature and effect of environmental factors, yet relatively little is known about the genetic basis of these disorders. Thus, knowledge of the primary genetic determinants of plasma lipoprotein levels and obesity will enhance our understanding of the pathophysiological background and may provide novel molecular targets for intervention.

Mouse crosses have helped to localize and identify genes underlying these complex traits (2–5). When exposed to a high-fat diet, mice of different inbred strains exhibit great variation in plasma lipoproteins and obesity (6). Our laboratory has used quantitative trait loci (QTL) analysis to investigate the genetics underlying lipoprotein metabolism and atherosclerosis (5, 7). The number of genetic loci that differ between C57BL/6J (B6) and 129S1/SvImJ (129) mice underscores the importance of strain background when evaluating the impact of a gene deficiency in targeted mutant mice. In most cases, targeted mutant mice are derived from embryonic stem cells of 129 mouse substrains. A target gene in these cells is "knocked out" by homologous recombination, and the resulting cells are microinjected into B6 blastocysts, which develop into B6/129 chimeras. These in turn are mated to B6 mice to produce mice heterozygous for B6 and 129 alleles at all loci for which these strains differ. These mice are intercrossed to generate mice homozygous for 129 alleles at the target locus (−/−) and a small region surrounding it, but the remainder of their genomes are a random mixture of B6 and 129 alleles (8). If littermates of such mixed-background targeted mutant stocks differ in their allelic combinations, they could yield different experimental results. Thus, to evaluate gene function in targeted mutant mice, the genetic background must be carefully controlled by constructing B6/129 congenic strains. This is carried out by successively backcrossing carriers of the targeted mutation to B6 mice until the only 129 alleles left

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Abbreviations: BMI, body mass index; cM, centimorgan; LOD, logarithm of odds; % fat, percentage of body fat mass; PLTP, phospholipid transfer protein; QTL, quantitative trait loci; SSLP, simple sequence length polymorphic; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: bjp@jax.org

on a nearly pure B6 background are the target locus (−/−) and the surrounding genetic materials (9).

We report here the results of our investigation of plasma non-HDL cholesterol levels, TG concentrations, and obesity among (B6 × 129) F_2 females that had for 14 weeks consumed a high-fat diet containing 15% dairy fat, 1% cholesterol, and 0.5% of the hydrophobic bile acid cholic acid, which promotes cholesterol absorption. Previously, we reported QTL detected with this intercross that determine plasma HDL-cholesterol levels and atherosclerosis susceptibility (10). In this study, we identified several QTL for non-HDL cholesterol, TG, percentage of body fat mass (% fat), and body mass index (BMI).

MATERIALS AND METHODS

Animals and diet

B6 and 129 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and mated to produce the (B6 × 129) F_1 progeny, which were intercrossed to produce an F_2 population of which the 301 female F_2 progeny were used in this investigation. Mice were maintained in a temperature- and humidity-controlled environment with a 14 h light/10 h dark cycle and given unrestricted access to food and acidified water. The cages were covered with polyester filters and contained pine shavings bedding. Six week old mice were fed a high-fat diet (11, 12) containing 15% dairy fat, 1% cholesterol, and 0.5% cholic acid for 14 weeks, after which they were killed by cervical dislocation. Experiments were approved by the Institutional Animal Care and Use Committee of The Jackson Laboratory.

Quantitative phenotype measurements

After consuming the high-fat diet for 14 weeks, mice were fasted for 4 h before blood samples were collected in plasma separator tubes containing EDTA, placed on ice, and centrifuged. Plasma lipid concentrations were measured using an enzymatic assay (Beckman, Fullerton, CA) as previously described (12). Because mice were fasted before blood was collected and because chylomicrons display very short half-lives (13), non-HDL was presumed to comprise predominantly VLDL and LDL. Non-HDL concentrations were obtained by subtracting HDL from total cholesterol concentrations. The % fat of each mouse was measured using peripheral dual-energy X-ray absorptiometry (PIXImus; GE-Lunar, Madison, WI), a method that has been validated in mice as an accurate measure (14). The BMI of each mouse was calculated by dividing its body weight (grams) by the square of its anal-nasal length (meters).

Genotyping

We genotyped 294 F_2 progeny initially with 88 simple sequence length polymorphic (SSLP) markers (Research Genetics, Huntsville, AL) spaced ~20 centimorgan (cM) apart and later added 23 additional SSLP markers in the QTL regions as previously described (10). The average spacing between these markers (±SD) was 14 ± 12 cM. DNA isolation, PCR amplification, and subsequent gel electrophoresis have been described previously (15). Reported genetic map positions were retrieved from the Mouse Genome Informatics database (http://www.informatics.jax.org).

Statistics

One-way ANOVAs with Tukey's correction for multiple pairwise compositions were used to determine statistically significant differences in plasma lipid levels, BMI, and % fat between mouse groups. Data were analyzed using Graphpad Prism (Windows version 3.00; GraphPad Software, San Diego, CA). Phenotypes were associated using Pearson's correlation. As described previously (16, 17), a three-step QTL analysis was conducted to search for main effects and pairwise gene interactions and then to integrate all of the main and interacting QTL phenotype associations into a multiple regression. In the regression analysis, we combined all significant and suggestive QTL and interactions in a multiple regression model. Terms that did not meet the nominal 0.02 level in the regression were eliminated in a backward stepwise manner with the exception that main effect terms involved in a significant interaction were retained. Final models were reported for each trait. Some traits were log transformed before analysis. This resulted in approximate normality for non-HDL and BMI. QTL were deemed significant if they either met or exceeded the 95% genome-wide adjusted threshold, which was assessed by permutation analysis for each trait [logarithm of odds (LOD) ≥ 3.0 for log-transformed non-HDL, LOD ≥ 3.1 for TG and log-transformed BMI, and LOD ≥ 3.2 for % fat]; they were deemed suggestive if they either met or exceeded the 37% genome-wide adjusted threshold (LOD ≥ 1.8 for TG and LOD ≥ 1.9 for other traits) but were not significant. QTL confidence intervals were calculated according to the posterior probability density of QTL locations, as described previously (16). Variance indicates the percentage of the total F_2 phenotypic variance associated with each marker. Analyses were carried out using Pseudomarker 0.9 software (Sen and Churchill; http://www.jax.org/staff/churchill/labsite).

Naming QTL

In accordance with the International Committee on Standardized Genetic Nomenclature for Mice (http://www.informatics.jax.org/mgihome/nomen) and the Complex Traits Consortium (18), we have named QTL as follows. QTL are named if significant or if suggestive but confirm a QTL reported previously. If a QTL substantially overlaps a previously discovered QTL, it is given the same name if the crosses share at least one parent in common (i.e., B6 × 129 and CAST × 129) and it is given a new name if the strains are all different.

TABLE 1. Plasma lipid concentrations, % fat, and BMI of 10 female B6, 10 female 129, 8 female F_1, and 294 female F_2 progeny fed a high-fat diet for 14 weeks

		Plasma[a]			
Mice	n	Non-HDL	TG	% Fat	BMI
		mg/dl		*%*	*$\times 10^{-4}$ g/m^2*
B6	10	140 ± 9[b,c]	45 ± 2[d]	19 ± 0[b,e]	23 ± 1[b,c]
129	10	71 ± 3[c]	64 ± 5	36 ± 1[c]	29 ± 0
F_1	8	107 ± 6	38 ± 1[b]	29 ± 2	27 ± 1
F_2	294[f]	123 ± 5	51 ± 1	25 ± 0	27 ± 0

BMI, body mass index; % fat, percentage of body fat mass; TG, triglyceride. Data are presented as means ± SEM.

[a] Plasma lipid concentrations were measured in mice fasted for 4 h.

[b] Significant difference ($P < 0.001$, by ANOVA) versus 129.

[c] Significant difference ($P < 0.01$, by ANOVA) versus F_1.

[d] Significant difference ($P < 0.01$, by ANOVA) versus 129.

[e] Significant difference ($P < 0.001$, by ANOVA) versus F_1.

[f] The number of F_2 mice is 292 for plasma lipid concentrations and 291 for % fat. Because it is the distribution and not the mean among the F_2 population that is most important for detecting genetic linkage to a phenotype, we did not test for significant differences between F_2 progeny and either the parental strains or F_1 progeny.

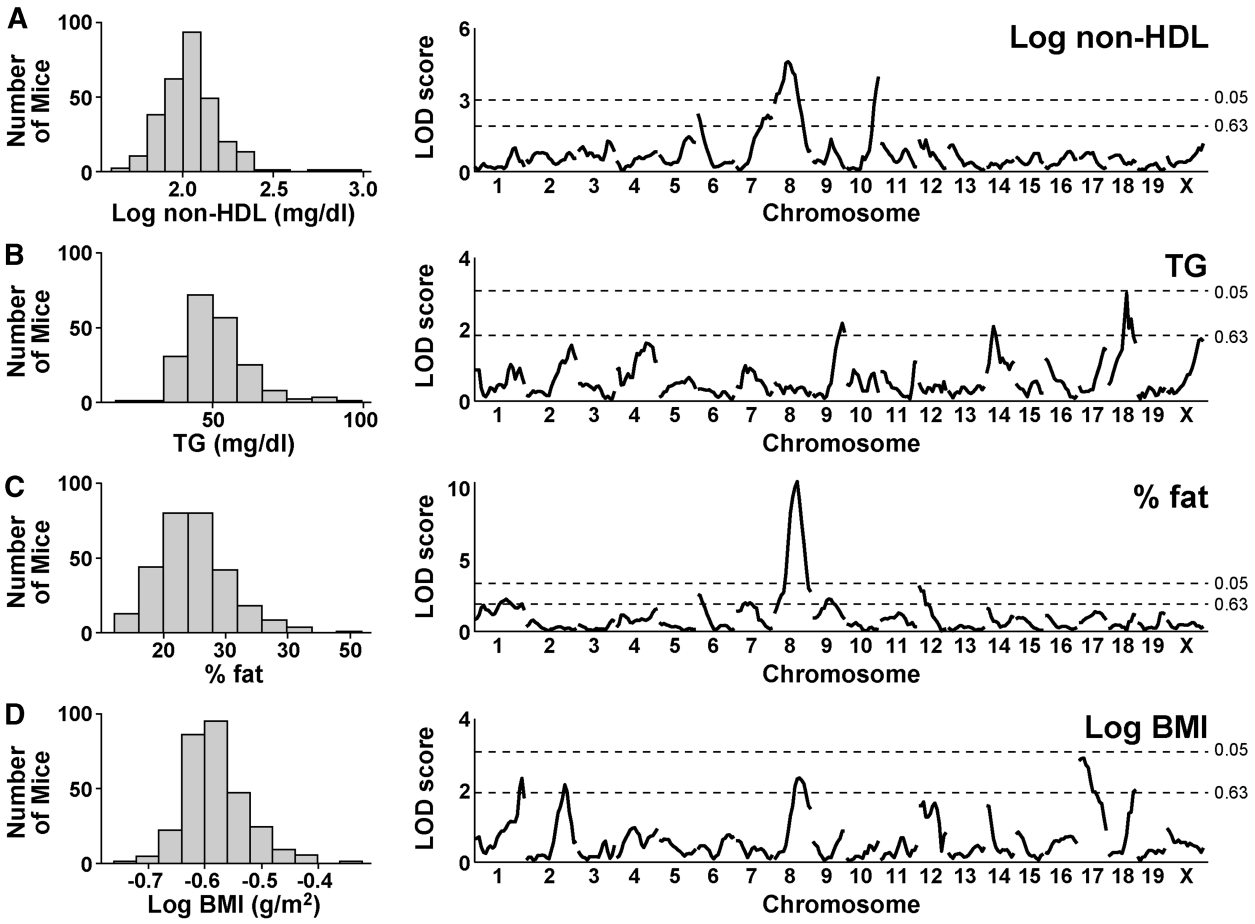

Fig. 1. Distributions and genome-wide scans for the traits. A–D: Log-transformed plasma non-HDL concentrations, plasma triglyceride (TG) concentrations, percentage of body fat mass (% fat), and log-transformed body mass index (BMI), respectively, in (B6 × 129) F_2 progeny fed a high-fat diet for 14 weeks. The number of mice is 292 for plasma lipid concentrations, 291 for % fat, and 294 for BMI. Chromosomes 1 through X are represented numerically on the ordinate. The relative width of the space allotted for each chromosome reflects the relative length of each chromosome. The abscissa represents the logarithm of odds (LOD) score, the traditional metric of genetic linkage. The significant ($P < 0.05$) and suggestive ($P < 0.63$) levels of linkage were determined by permutation testing (17).

RESULTS

Inheritance of plasma non-HDL and TG levels, % fat, and BMI

Plasma non-HDL and TG concentrations, % fat, and BMI were measured after animals had been fed the high-fat diet for 14 weeks (**Table 1**). Compared with 129, B6 mice displayed significantly increased non-HDL levels. The F_1 mice displayed non-HDL levels intermediate between and significantly different from those of the parental strains; thus, high non-HDL cholesterol levels were inherited in an additive manner. Compared with 129, B6 mice displayed significantly decreased plasma TG levels. The F_1 mice displayed TG levels comparable to those of strain B6 and significantly lower than those of strain 129; thus, high TG levels were inherited in a recessive manner. B6 mice displayed significantly lower % fat than did 129 mice, and the F_1 mice displayed intermediate values between those of the parental strains. The BMI distribution was similar to that for % fat; B6 mice displayed significantly lower BMI compared with 129 mice. The F_1 mice displayed BMI values intermediate between the parents but closer in value to those of strain 129. We started with 301 F_2 females and quantified each trait of 294 females after the 14 week high-fat diet. Log-transformed non-HDL, TG, % fat, and log-transformed BMI were normally distributed among the F_2 progeny (**Fig. 1A–D**). The log BMI was positively correlated with TG and % fat but negatively correlated with log-transformed non-HDL (**Table 2**).

Identification of genetic loci affecting non-HDL and TG concentrations, % fat, and BMI

The genome-wide scans for single QTL are presented in Fig. 1 and summarized in **Table 3**, which provides the QTL peak, 95% confidence interval, LOD score, allele conferring the high value, nearest SSLP marker to QTL peak, overlapping QTL reported previously, and candidate genes for each QTL. The QTL were named if they were significant either as single QTL or interacting QTL. Suggestive QTL in this cross that were found previously were also named. We named the loci *Nhdlq* for non-HDL QTL, *Tgq* for TG QTL, and *Obq* for obesity QTL, in each

TABLE 2. Pearson correlation coefficients among plasma lipid concentrations, % fat, and BMI in the F_2 progeny fed a high-fat diet for 14 weeks

Variable	Log Non-HDL	TG	% Fat
TG	0.14[a]		
% fat	−0.23[b]	0.21[c]	
Log BMI	−0.23[b]	0.22[c]	0.40[b]

The number of mice for each analysis was from 289 to 292.
[a] $P < 0.05$.
[b] $P < 0.0001$.
[c] $P < 0.001$.

case followed by a number. **Figure 2** shows the allele effects, which demonstrate the magnitude of the effect and the inheritance pattern (dominant, recessive, or additive).

For plasma non-HDL concentrations, the genome scan is shown in Fig. 1A. The significant chromosome 8 QTL (Fig. 2A; peak LOD 4.4), named *Nhdlq1*, had a dominant B6 allele for increased non-HDL concentrations (Fig. 2B). This locus confirmed a QTL identified earlier using strains CAST and 129 (19). *Nhdlq4*, on chromosome 10 (Fig. 2C; peak LOD 4.0), caused higher non-HDL when homozygous for a recessive 129 allele (Fig. 2D). Two suggestive QTL were discovered at the *D6Mit86* locus and the *D7Mit141* locus. The pairwise genome scan revealed three significant interactions. *Nhdlq1* interacted with the *D7Mit294* locus, which we named *Nhdlq6*. *Nhdlq6* did not affect non-HDL concentrations by itself, but its effect in combination with *Nhdlq1* on non-HDL was strong (**Fig. 3A**). When the *Nhdlq1* genotype was B6/B6, homozygosity for a recessive allele from strain 129 at *Nhdlq6* contributed significantly to increase non-HDL. A second significant interaction was found between *Nhdlq4* and the *D6Mit86* locus, named *Nhdlq5*, which was suggestive as a single QTL (Fig. 3B). When the *Nhdlq4* genotype was 129/129, the contribution of a recessive B6 allele for increased non-HDL at *Nhdlq5* became significant. A third interaction was found between *Nhdlq4* and the *D15Mit13* locus, which we named *Nhdlq7*. *Nhdlq7* did not affect non-HDL by itself, but its combined effect with *Nhdlq4* on non-HDL was dramatic (Fig. 3C). When the *Nhdlq4* genotype was 129/129, homozygosity for a recessive B6 allele at *Nhdlq7* significantly increased plasma non-HDL.

For plasma TG concentrations, we found a significant locus on chromosome 18, which we named *Tgq1* (Fig. 2E; peak LOD 3.2) and two suggestive loci on chromosomes 9 and 14. At *Tgq1*, the heterozygous B6/129 genotype was associated with significantly increased TG concentrations (Fig. 2F). The pairwise genome scan revealed that an interaction at *D9Mit281* and *D4Mit308*, which we named *Tgq2* and *Tgq3*, respectively, affected plasma TG concentrations with statistical significance (Fig. 3D). When the *Tgq3* genotype was 129/129, homozygosity for a recessive strain B6 allele at *Tgq2* contributed significantly increased TG.

For obesity measured by % fat, the genome scan is shown in Fig. 1C. The significant chromosome 8 QTL named *Obq16* (Fig. 2G; peak LOD 10.0) had an additive 129 allele for higher % fat (Fig. 2H). Three suggestive QTL were discovered on chromosomes 1, 6, and 12. The *D6Mit86* locus confirmed a QTL, *Mob2*, identified earlier using strains B6 and SPRET (20). We named this locus *Mob2* in the present cross, which shared the parental strain B6 in common with the earlier cross. The *D1Mit495* locus confirmed adjacent QTL, *Obq8* and *Obq9*, identified earlier using strains NZO and SM (21). We named this lo-

TABLE 3. QTL identified for single gene or pairwise genome-wide scans of 294 (B6 × 129) F_2 females

Traits	Chromosomal (Chr) Location	95% Confidence Interval	Locus Name	Logarithm of Odds Score	High Allele	Nearest Marker	Overlapping QTL[a] (Reference)	Candidate Genes (cM)
		cM						
Non-HDL	Chr 8 (38)[b]	15–52	*Nhdlq1*	4.4	B6	*D8Mit248*	*Nhdlq1* (19)	*Cpe* (32.6), *Lpl* (33.0)
	Chr 10 (70)[b]	65–70	*Nhdlq4*	4.0	129	*D10Mit35*	*Pltp2* (25)	*Apof* (73.0)
	Chr 6 (0)[b]	0–24	*Nhdlq5*	2.4[c]	B6	*D6Mit86*		
	Chr 7 (70)	50–80	–[d]	2.4[c]	129	*D7Mit141*		
	Chr 7 (10)[b]	5–20	*Nhdlq6*	—	—	*D7Mit294*	Unnamed QTL (19)	*Apoc2* (4.0)
	Chr 15 (0)[b]	0–20	*Nhdlq7*	—	—	*D15Mit13*	Unnamed QTL (19)	
TG	Chr 18 (42)	37–44	*Tgq1*	3.2	B6/129	*D18Mit50*		
	Chr 9 (66)[b]	44–68	*Tgq2*	2.2[c]	B6	*D9Mit281*	Unnamed QTL (28)	
	Chr 14 (14)	6–48	–[d]	2.0[c]	B6	*D14Mit60*		
	Chr 4 (58)[b]	30–90	*Tgq3*	—	—	*D4Mit308*		*Lepr* (46.7), *Angptl3* (48.5), *Cpt2* (54.4)
% fat	Chr 8 (48)[b]	42–53	*Obq16*	10.0	129	*D8Mit248*		
	Chr 12 (2)	0–16	–[d]	2.9[c]	129	*D12Mit182*		*Pomc1* (4.0)
	Chr 6 (0)	0–10	*Mob2*	2.6[c]	129	*D6Mit86*	*Mob2* (20)	*Lep* (10.5)
	Chr 1 (74)	48–108	*Obq17*	2.3[c]	129	*D1Mit495*	*Obq8* (21), *Obq9* (21)	
	Chr 9 (65)[b]	0–75	*Obq18*	—	—	*D9Mit281*	*Dob2* (30), *Mob8* (31), *Obq5* (32), *Adip5* (33)	
BMI	Chr 17 (8)	0–25	*Obq19*	2.9[c]	B6	*D17Mit143*	Unnamed QTL (21), *Obq4* (22)	*Igf2r* (7.35), *Acat2* (7.5), *Ppard* (13.5)
	Chr 8 (52)	38–72	*Obq16*	2.5[c]	129	*D8Mit248*	*Obq16* (this study)	
	Chr 1 (102)	56–108	*Obq17*	2.4[c]	129	*D1Mit406*	*Obq8* (21), *Obq9* (21)	

cM, centimorgan; QTL, quantitative trait loci. The number of F_2 mice is 292 for plasma lipid concentrations and 291 for % fat.
[a] Overlapping QTL identified in previous studies.
[b] Interacting QTL.
[c] Suggestive QTL.
[d] We did not name this QTL because it is below the statistically significant level and overlaps no previously discovered QTL.

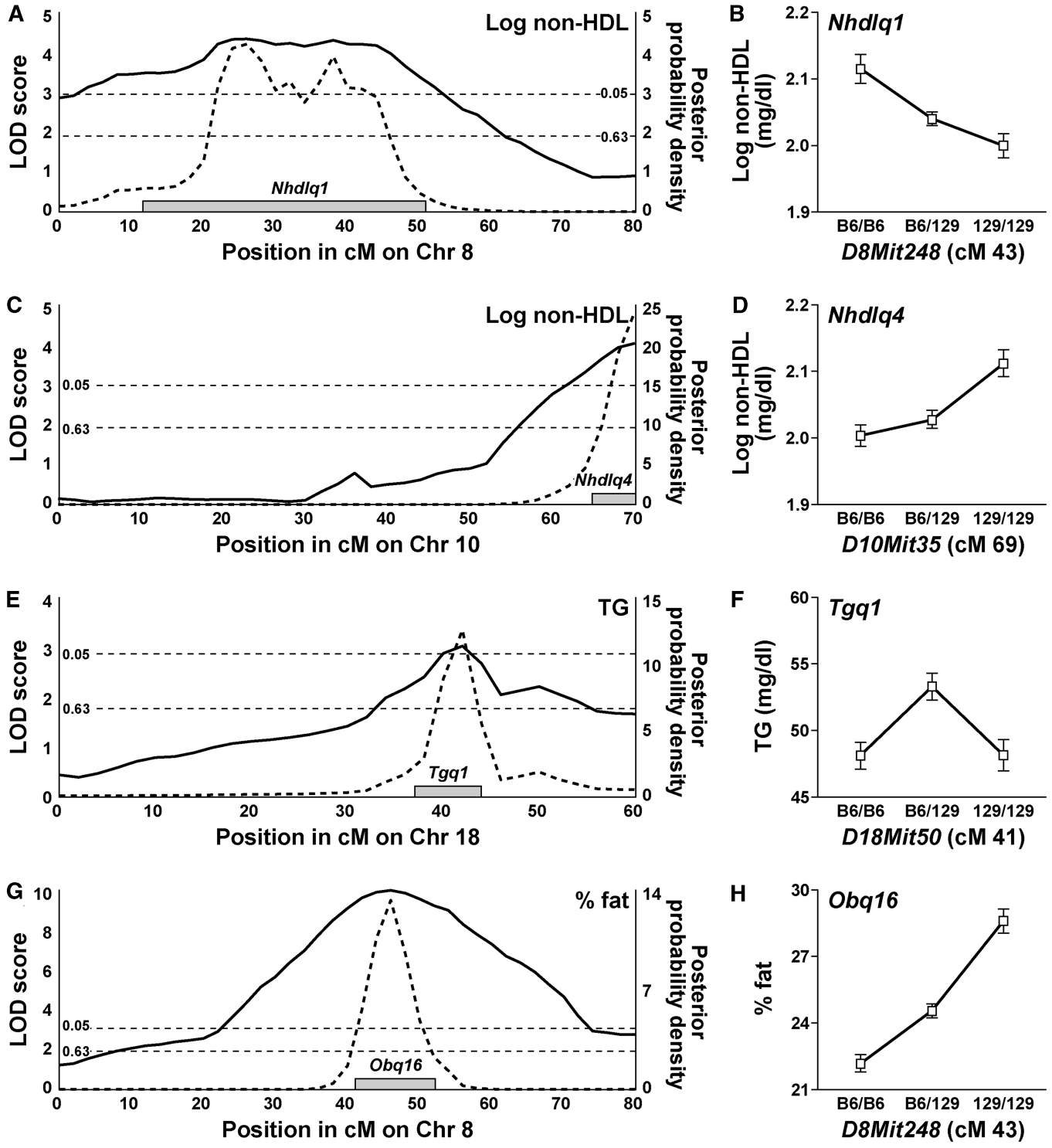

Fig. 2. Genome-wide scans (solid lines) and posterior probability densities (broken lines) for the quantitative trait loci (QTL). A, C, E, and G represent the QTL, posterior probability densities, and 95% confidence intervals; B, D, F, and H provide the contributions at the peak of each QTL. Posterior probability density is a likelihood statistic that gives rise to the 95% confidence intervals indicated by gray bars (16). Homozygosity for B6 alleles is represented by B6/B6, homozygosity for 129 alleles is represented by 129/129, and heterozygosity at a locus is represented by B6/129. Marker locations for each QTL are in parentheses. Error bars represent SEM. Chr, chromosome; cM, centimorgan.

cus *Obq17* in the present study. The pairwise genome scan revealed a significant interaction between *Obq16* and the *D9Mit281* locus, which we named *Obq18*. *Obq18* was not shown to affect % fat by itself, but its combined effect with *Obq16* on % fat was dramatic (Fig. 3E). When the *Obq16* genotype was 129/129, an additive/codominant allele for higher % fat from strain B6 at *Obq18* contributed a significant effect.

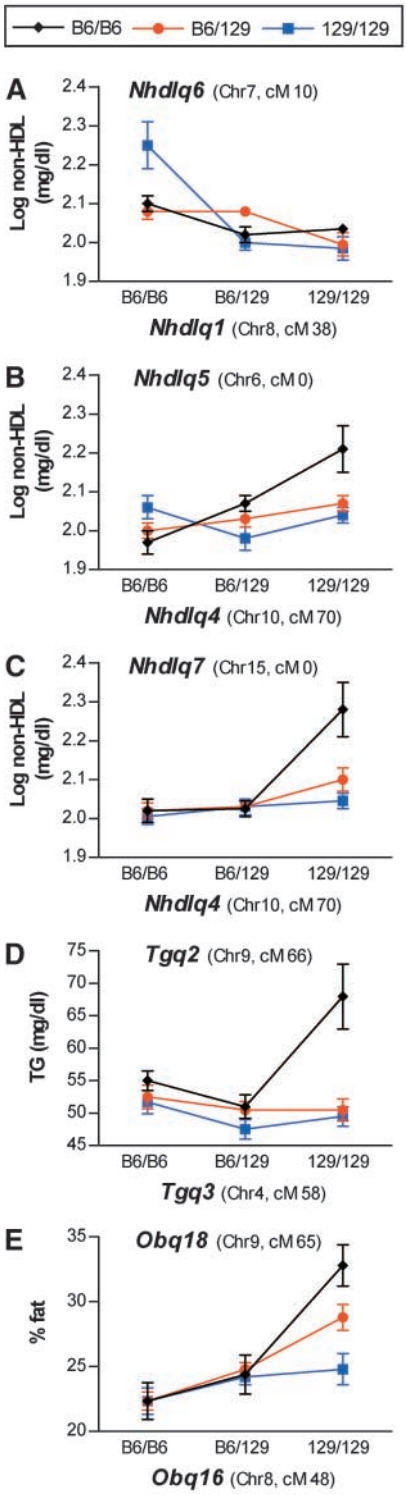

Fig. 3. The effects of gene interactions detected by the pairwise genome scan. Homozygosity for B6 alleles is represented by B6/B6, homozygosity for 129 alleles is represented by 129/129, and heterozygosity is represented by B6/129. Y axes show mean values of log non-HDL (A, B, and C), TG (D), and % fat (E). Error bars represent SEM.

For obesity measured by BMI, the genome scan is shown in Fig. 1D. Three suggestive QTL were discovered on chromosomes 1, 8, and 17. The *D17Mit143* locus confirmed QTL identified earlier using either strains AKR/J and C57L/J (22) or strains NZO and SM (21). The *D8Mit248* locus confirmed a significant QTL, *Obq16*, identified for % fat in this cross. Thus, we gave this locus the same name, *Obq16*. The *D1Mit406* locus colocalized a QTL, *Obq17*, identified for % fat in this cross and was given the same name. Two loci, the *D2Mit285* locus and the *D18Mit4* locus, exceeded the 37% genome-wide adjusted threshold (LOD $\geq$ 1.9), but their terms did not meet the nominal level in the regression, so we did not report these loci as suggestive QTL (Fig. 1D).

The multiple regression analyses (**Tables 4** and **5**) show the effect of each QTL and interactions when considered together. The percent of the total phenotypic variance in F_2 mice is best estimated by a multiple regression analysis. For non-HDL, this multiple regression analysis confirmed six QTL and three interactions identified for single gene or pairwise genome-wide scans. Taken together, these QTL and their interactions explained 49.9% of the total F_2 phenotypic variance; *Nhdlq1* and *Nhdlq4* contributed ~10% each and the others each contributed 2–5% of the total variance. For TG, four single QTL and one interaction explained 24.3% of the total variance. For % fat, five single QTL and one interaction explained 34.3% of the total variance; *Obq16* contributed approximately half of the genetic variance. For BMI, three QTL explained 11.1% of the total variance, with each QTL contributing 3–4% of the total variance.

DISCUSSION

In the present study, we describe two inbred mouse strains, B6 and 129, that display different plasma levels of non-HDL cholesterol and TG and degrees of obesity when fed a high-fat diet. Three-step QTL analyses on 294 (B6 × 129) F_2 progeny resulted in the localization of six QTL for non-HDL, four QTL for TG, five QTL for % fat, three QTL for BMI, and five gene interactions.

For plasma non-HDL concentrations, we identified four main-effect QTL (*Nhdlq1*, *Nhdlq4*, *Nhdlq5*, and the *D7Mit141* locus) and two additional QTL by gene interactions (*Nhdlq6* and *Nhdlq7*). Previously, our group mapped a chromosomal locus in a (CAST × 129) F_2 intercross that determines non-HDL levels to chromosome 8 (cM 20–60) and named it *Nhdlq1* (19). Because the present study confirmed the previously reported QTL using a cross having a parental strain, 129S1/SvImJ, in common with the cross used in the earlier study, we named the locus *Nhdlq1* in accordance with the International Committee on Standardized Genetic Nomenclature for Mice. Potential candidate genes for *Nhdlq1* are the gene (*Lpl*; cM 33.0) coding for LPL and the gene (*Cpe*; cM 32.6) coding for carboxypeptidase E, which produces biologically active forms of proinsulin and proopiomelanocortin. Mice possessing the *fat* mutation (a spontaneous mutation in the *Cpe* gene) exhibited prominent obesity and higher plasma non-HDL concentrations relative to controls after consuming a high-fat diet (23). *Nhdlq1* coincidentally colocalizes with a

TABLE 4. Multiple regression analyses of variance for non-HDL and TG in 292 (B6 × 129) F_2 females

Traits	Chromosomal (Chr) Location	Nearest Marker	DF[a]	Type III SS[b]	Variance (%)[c]	F Value	*P* Value	Locus Name
	cM							
Non-HDL	Chr 8 (38)[d]	*D8Mit248*	6	0.784	10.0	6.61	1.58×10^{-6}	*Nhdlq1*
	Chr 10 (70)[d]	*D10Mit35*	10	1.063	13.5	5.38	2.99×10^{-7}	*Nhdlq4*
	Chr 6 (0)[d]	*D6Mit86*	6	0.380	4.8	3.21	0.0047	*Nhdlq5*
	Chr 7 (70)	*D7Mit141*	2	0.178	2.3	4.51	0.0118	
	Chr 7 (10)[d]	*D7Mit294*	6	0.276	3.5	2.33	0.0329	*Nhdlq6*
	Chr 15 (0)[d]	*D15Mit13*	6	0.375	4.8	3.16	0.0051	*Nhdlq7*
	Chr 8 (38):Chr 7 (10)	*D8Mit248:D7Mit294*	4	0.251	3.2	3.17	0.0144	*Nhdlq1:Nhdlq6*
	Chr 10 (70):Chr 6 (0)	*D10Mit35:D6Mit86*	4	0.268	3.4	3.39	0.0101	*Nhdlq4:Nhdlq5*
	Chr 10 (70):Chr 15 (0)	*D10Mit35:D15Mit13*	4	0.344	4.4	4.35	0.0020	*Nhdlq4:Nhdlq7*
Totals			291	7.871	49.9			
TG	Chr 18 (42)	*D18Mit50*	2	1,351	3.4	5.59	0.0042	*Tgq1*
	Chr 9 (66)[d]	*D9Mit281*	6	2,850	7.1	3.93	0.0009	*Tgq2*
	Chr14 (14)	*D14Mit60*	2	1,055	2.6	4.37	0.0135	
	Chr 4 (58)[d]	*D4Mit308*	6	2,596	6.5	3.58	0.0019	*Tgq3*
	Chr 9 (66):Chr 4 (58)	*D9Mit281:D4Mit308*	4	1,900	4.7	3.93	0.0040	*Tgq2:Tgq3*
Totals			291	40,249	24.3			

[a] DF indicates degrees of freedom; it includes main effect and any interactions.
[b] SS, sum of squares.
[c] Variance indicates the percentage of the total F_2 phenotypic variance associated with each marker.
[d] Interacting QTL.

QTL for % fat, *Obq16*, in the present cross. *Nhdlq6* maps to the region containing the apolipoprotein gene cluster (*Apoc1*, *Apoc2*, *Apoc4*, and *Apoe*; cM 4.0). Interestingly, APOC2 is a cofactor for LPL (24). The gene interaction between *Nhdlq1* and *Nhdlq6* makes *Lpl* and *Apoc2* excellent candidates for genes underlying these QTL. Likewise, *Nhdlq4* interacted independently with *Nhdlq5* and *Nhdlq7*. These gene interactions may give clues to the candidate genes. *Nhdlq4* colocalized with a QTL for phospholipid transfer protein (PLTP) activity, *Pltp2*, found previously using an (SM × NZB) F_2 intercross (25). PLTP is responsible for the transfer of phospholipids from VLDL to HDL (26), suggesting that the gene underlying *Nhdlq4* might determine plasma non-HDL levels by regulating PLTP activity. An excellent candidate gene for *Nhdlq4* is the gene (*Apof*; cM 73.0) coding for a lipid transfer inhibitor protein, Apo F (27). Previously, our group reported a single nucleotide polymorphism that causes an amino acid change in the protein between B6 and 129 strains (25).

For TG levels, we identified three main-effect QTL (*Tgq1*, *Tgq2*, and the *D14Mit60* locus) and, by gene interactions, an additional QTL (*Tgq3*). Potential candidate genes for *Tgq3* are the gene (*Lepr*; cM 46.7) coding for the receptor of leptin; the gene (*Cpt2*, cM 54.4) coding for a mitochondrial fatty acid transporter, carnitine palmitoyltransferase 2; and the gene (*Angptl3*, cM 48.5) coding for angiopoietin-like 3. *Tgq2* colocalized with a QTL previously identified in a (B6 × KK-A^y) F_2 intercross (28) but did not map near any genes known to play a prominent role in lipoprotein or lipid metabolism. Alternatively, genes underlying *Tgq2* are entirely novel genes that might otherwise not have been considered. The interaction be-

TABLE 5. Multiple regression analyses of variance for % fat and BMI in 294 (B6 × 129) F_2 females

Traits	Chromosomal (Chr) Location	Nearest Marker	DF[a]	Type III SS[b]	Variance (%)[c]	F Value	*P* Value	Locus Name
	cM							
% fat	Chr 8 (48)[d]	*D8Mit248*	6	1,606	16.2	10.60	1.36×10^{-10}	*Obq16*
	Chr 12 (2)	*D12Mit182*	2	400	4.0	7.91	0.0005	
	Chr 6 (0)	*D6Mit86*	2	229	2.3	4.52	0.0117	*Mob2*
	Chr 1 (74)	*D1Mit495*	2	272	2.8	5.39	0.0050	*Obq17*
	Chr 9 (65)[d]	*D9Mit281*	6	504	5.1	3.32	0.0036	*Obq18*
	Chr 8 (48):Chr 9 (65)	*D8Mit248:D9Mit281*	4	355	3.6	3.52	0.0081	*Obq16:Obq18*
Totals			290	9,909	34.3			
BMI	Chr 17 (8)	*D17Mit143*	2	0.036	4.3	6.91	0.0012	*Obq19*
	Chr 8 (52)[d]	*D8Mit248*	2	0.027	3.2	5.19	0.0061	*Obq16*
	Chr 1 (102)[d]	*D1Mit406*	2	0.031	3.6	5.88	0.0031	*Obq17*
Totals			293	0.844	11.1			

The number of F_2 mice is 291 for % fat.
[a] DF indicates degrees of freedom; it includes main effect and any interactions.
[b] SS, sum of squares.
[c] Variance indicates the percentage of the total F_2 phenotypic variance associated with each marker.
[d] Interacting QTL.

tween *Tgq2* and *Tgq3* may give clues to the underlying genes' identities.

To identify genetic loci that affect the development of obesity in response to the high-fat diet, we measured two different traits that reflect obesity, % fat and BMI. For % fat, we identified four main-effect QTL (*Obq16*, *Obq17*, *Mob2*, and the *D12Mit182* locus) and one additional QTL (*Obq18*) by its interaction with *Obq16*. For BMI, we identified three suggestive main-effect QTL (*Obq16*, *Obq17*, and *Obq19*). Because log BMI was positively correlated with % fat ($P < 0.0001$), two of three QTL for BMI, the *D8Mit248* locus and the *D1Mit406* locus, colocalized with QTL for % fat and for *Obq16* and *Obq17*, respectively. The discrepancy between QTL obtained from these traits might be reflected from variations in body length. The suggestive *D12Mit182* locus maps to the region of the gene (*Pomc1*; cM 4.0) coding for proopiomelanocortin-α, mutation of which causes monogenic obesity through the liptin-melanocortin signaling pathway (29). The suggestive QTL on chromosome 6 colocalized with a QTL for fat pad weight, *Mob2*, found previously using a (B6 × SPRET) × B6 backcross (20). This genetic locus maps to the region of the gene (*Lep*; cM 10.5) coding for leptin. The *Obq17* locus colocalized with an adjacent QTL for obesity, *Obq8* and *Obq9*, which were discovered previously using progeny of (SM × NZO) F_2 intercrosses (21). The *Obq18* locus has been found repeatedly using (SWR × AKR) F_2, (B6 × CAST) F_2, and (B6 × KK) F_2 intercross progeny fed a high-fat diet (30–32). *Obq19* colocalized with a QTL found previously using progeny of (AKR × C57L) and (SM × NZO) F_2 intercrosses fed a high-fat diet (21, 22). Potential candidate genes for *Obq19* are the gene *Ppard* (cM 13.5), coding for an important transcriptional factor that regulates glucose and fatty acid metabolism; the gene *Igf2r* (cM 7.35), coding for an insulin-like growth factor II receptor; and the gene *Acat2* (cM 7.5), coding for an acetyl-CoA acetyltransferase 2 that catalyzes the synthesis of cytosolic acetoacetyl-CoA, a precursor of cholesterol and other steroids. However, high-fat diets in the most previous reports do not contain substantial amounts of cholesterol or any cholate, and this difference in diets might affect the identities of colocalizing QTL between this study and previous studies.

Reed and colleagues (33) carried out a study of both male and female progeny of an F_2 intercross between mice of the C57BL/6ByJ and 129P3/J strains to identify QTL for body weight, body length, and adiposity. Of the QTL found in the present study, only *Obq18* colocalized with a QTL for adiposity, *Adip5*, reported by Reed et al. However, these investigators reported that the effect of *Adip5* is limited to F_2 males and is not found in females (peak LOD < 1.0). Whereas a QTL on chromosome 16, *Adip9*, was found to interact with *Adip5* by Reed et al., *Obq18* interacted with *Obq16* on chromosome 8 in the present study, suggesting that the gene underlying *Adip5* is not identical to the one underlying *Obq16*. The QTL identified by Reed et al. in the (C57BL/6ByJ × 129P3/J) F_2 cross are based on analyses of mice fed a normal chow diet, and it is unlikely that these loci would similarly affect the development of obesity in response to a high-fat diet. Indeed, obesity is a complex trait, reflecting the effect of a network of genes, and it is affected by diet, age, gender, and exercise (4).

Several spontaneous single-gene mutations causing obesity, such as agouti yellow (A^y), obese (Lep^{ob}), diabetes ($Lepr^{db}$), fat (Cpe^{fat}), tubby (Tub^{tub}), and mahogany ($Atrn^{mg}$), have been identified in inbred mice (34, 35). These mutations, however, do not account for the wide variation of obesity in the general human population. Some human pedigree studies provide clear genetic evidence of oligogenic or polygenic predisposition for obesity, indicating that obesity is a complex trait (36). Indeed, mice of different inbred strains exhibit wide variation in body weight and predisposition to spontaneous or diet-induced obesity. Multiple modifier genes likely contribute to the variation. Crosses between mice of various strains have identified ~100 chromosomal loci that contribute to obesity (4).

The present study discovered five epistatic interactions for plasma non-HDL and TG concentrations and % fat (Fig. 3). The pairwise genome scans revealed four significant QTL, *Nhdlq6*, *Nhdlq7*, *Tgq3*, and *Obq18*, that did not affect traits by a single locus but affected the traits with the counterpart locus. When a genotype of one locus was B6/B6, homozygosity for a 129 allele at the counterpart locus contributed significantly to the effect on the trait. This evidence might facilitate in vitro assays to test candidate genes.

In summary, by performing a QTL analysis of a (B6 × 129) F_2 female cohort, we identified chromosomal regions that affect plasma non-HDL and TG concentrations and obesity in mice with backgrounds that are a combination of B6 and 129. Knowledge of the primary genetic determinants of plasma lipid concentrations and obesity will enhance our understanding of lipoprotein metabolism and likely provide novel molecular targets for metabolic obesity.

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