Identification of quantitative trait loci that regulate obesity and serum lipid levels in MRL/MpJ × SJL/J inbred mice

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Abstract **The total body fat mass and serum concentration of total cholesterol, HDL cholesterol, and triglyceride (TG) differ between standard diet-fed female inbred mouse strains MRL/MpJ (MRL) and SJL/J (SJL) by 38–120% ($P < 0.01$). To investigate genetic regulation of obesity and serum lipid levels, we performed a genome-wide linkage analysis in 621 MRL× SJL F_2 female mice. Fat mass was affected by two significant loci, *D11Mit36* [43.7 cM, logarithm of the odds ratio (LOD) 11.2] and *D16Mit51* (50.3 cM, LOD 3.9), and one suggestive locus at *D7Mit44* (50 cM, LOD 2.4). TG levels were affected by two novel loci at *D1Mit43* (76 cM, LOD 3.8) and *D12Mit201* (26 cM, LOD 4.1), and two suggestive loci on chromosomes 5 and 17. HDL and cholesterol concentrations were influenced by significant loci on chromosomes 1, 3, 5, 7, and 17 that were in the regions identified earlier for other strains of mice, except for a suggestive locus on chromosome 14 that was specific to the MRL × SJL cross. In summary, linkage analysis in MRL × SJL F_2 mice disclosed novel loci affecting TG, HDL, and fat mass, a measure of obesity. Knowledge of the genes in these quantitative trait loci will enhance our understanding of obesity and lipid metabolism.**—Srivastava, A. K., S. Mohan, G. L. Masinde, H. Yu, and D. J. Baylink. **Identification of quantitative trait loci that regulate obesity and serum lipid levels in MRL/MpJ × SJL/J inbred mice.** ***J. Lipid Res.*** **2006.** 47: **123–133.**

Supplementary key words body fat mass • HDL cholesterol • cholesterol • triglyceride

Cardiovascular disease (CVD) is currently the leading cause of morbidity and mortality world wide, and its incidence is likely to increase. Elevated cholesterol, especially LDL cholesterol and triglyceride (TG) levels, low HDL cholesterol levels, hypertension, type 2 diabetes, and obesity modulate risk for CVD (1, 2). Such risk factors are present in 80–90% of CVD patients. Current understanding supports a complex etiology involving both environmental and genetic determinants. Environmental risk factors for CVDs identified in humans include diet, physical activity, cigarette smoking, high blood pressure, uncontrolled diabetes, obesity and overweight, stress, and adverse lipid profile (2). However, little is known about the genetic regulation of blood lipid levels, with only some of the genes that regulate production or blood levels of lipids having so far been identified (3–9). Because of inherent difficulties in carrying out linkage analyses for complex traits in humans, inbred strains of mice have been used as a powerful tool for identifying quantitative trait loci (QTLs) that contribute to variations in circulating levels of lipids (10–29). QTL studies in mice have not only revealed a large number of loci that regulate lipid levels in blood but also have shown that there is a high degree of concordance between human QTLs that regulate lipid levels and their corresponding mouse loci (30, 31). More than 60 QTLs on chromosomes 1, 2, 3, 5, 7, 8, 9, 14, 16, 17, 18, and 19 that affect plasma lipid levels, as well as body fat mass QTLs, have been identified in several strains of mice (10–29). The rationale for using different crosses is based on the fact that each parental mouse strain represents unique mapping panels for the identification of QTLs for a complex trait. The phenotypic differences between parental strains, the extent of allelic variation, and the strain background are critical factors that determine whether a given QTL can be detected in a particular cross. This implies that a best estimate of all genes that account for a total variation of lipid levels in humans and mice can only be achieved by comparing the results of multiple crosses.

Mouse strains MRL/MpJ (MRL) and SJL/J (SJL) display extreme rates of soft-tissue healing and regeneration, and our laboratory has used these two strains in the past to reveal linkage to several loci that control soft-tissue healing (32) and musculoskeletal phenotypes (33–37). We have observed that when MRL and SJL mice are fed a standard

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Abbreviations: apoA-II, apolipoprotein A-II; CVD, cardiovascular disease; LOD, logarithm of the odds ratio; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism; TG, triglyceride.

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diet, they also display large differences in serum levels of cholesterol, HDL, TG, and body fat mass. To examine the genetic basis of these differences, we measured serum levels of cholesterol, HDL, TG, and body fat mass in the F_2 progeny. The aim of this study was to identify loci that regulate body fat mass, cholesterol, HDL, and TG in the F_2 mice of MRL × SJL crosses. Because multiple phenotypes were measured in this study, an additional aim was to identify common loci that regulate obesity and lipid levels.

MATERIALS AND METHODS

Mice

Acquisition of MRL/MpJ (MRL) and SJL/J (SJL) mice, generation of F_1 and F_2 progeny, collection of DNA samples, and collection and processing of blood were performed as described previously (32). In brief, MRL females were mated with SJL males to produce F_1 mice. Brother-sister mating was established to produce F_2 mice. Only female F_2 mice were weaned onto standard diet (TD 99479; Harlan Teklad, Madison, WI) at 21 days and housed three to six animals per cage. Mice were fed standard diet up to 7 weeks of age, when they were euthanized and bled without fasting. Age-matched parent mice were purchased from the Jackson Laboratory for plasma lipid analyses. All mice were allowed free access to standard food and water throughout the course of the study.

All blood samples were collected in the afternoon at the same time of day (±2 h) under nonfasting conditions. Blood was collected directly into 1.5 ml plastic tubes (Eppendorf), and serum was separated from cells by centrifugation within 1 h of blood collection. Serum was stored at −70°C until analyzed. The experimental protocols were in compliance with animal welfare regulations and approved by the Institutional Animal Care and Use Committee, Jerry L. Pettis VA Medical Center.

Serum cholesterol, HDL, and TG measurements

Total cholesterol, HDL cholesterol, and TG were measured by direct enzymatic colorimetric assays using a fully automated Hitachi 912 Clinical Chemistry Analyzer (Roche Diagnostics; Indianapolis, IN). Measurements of cholesterol, HDL, and TG were validated in our laboratory for reproducibility and analytical recovery in mice serum. The inter-assay (n = 12) and intra-assay (n = 5) coefficients of variation for all three assays were <7%. The mean analytical recovery of the diluted mouse serum samples (n = 3; up to 4-fold) was 100.3%. The sensitivity of the cholesterol and HDL assay was 3 mg/dl, and that of the TG assay was 4 mg/dl. The measuring range for cholesterol and TG was 3–800 mg/dl. The measuring range for HDL was 3–200 mg/dl in mouse serum.

Analysis of fat mass

The percent fat mass was measured using peripheral dual-energy X-ray absorptiometry (PIXIMUS densitometer; Lunar Corp., Madison, WI), a method that has been validated for mice (14). The precision of PIXIMUS for measurement of total body fat mass is coefficient of variance <2%. The body mass index (BMI) was calculated as described previously (14).

Construction of linkage map

Extraction of genomic DNA and PCR-based genotyping with 137 microsatellite markers have been described previously (32–36). Alleles derived from the MRL/MpJ parent were designated A, SJL/J-derived alleles were designated B, and MRL/SJL heterozygotes were designated H in data analyses. Marker orders were estimated from the publicly available Mouse Genome Database. A total of 132 markers distributed across the 19 autosomes were used for statistical analyses (excluding the X-chromosome). A list of markers and the resulting linkage map are available from the authors.

Statistical analyses

Data were analyzed using GraphPad Prism (Windows version 4.02; GraphPad Software, San Diego, CA). The Shapiro-Wilk test was used to test the normality of the F_2 population (32–37). One-way ANOVAs with Newman-Keuls test were used to compare pairs of data in order to determine statistically significant differences in plasma lipid levels, BMI, and percent fat between mouse groups.

Genotype data were analyzed using the Pseudomarker MAINSCAN algorithm (38) written for the MATLAB (Mathworks, Inc.; Natick, MA) programming environment (available from www.jax.org/research/churchill). Thresholds for logarithm of the odds ratio (LOD) scores for different QTLs were determined by genome-wide 1,000-permutation test for 1% genome-wide error ($P < 0.01$) and 5% genome-wide error ($P < 0.05$). Linkage analyses were also performed using MapQTL 5.0 (DLO Center for Plant Breeding and Reproduction Research; Wageningen, the Netherlands) as described for F_2 intercrosses. Pseudomarker and MapQTL 5.0 analyses yielded comparable results. Percent variance explained by each locus was calculated for peak interval by MapQTL software.

To study genome-wide interactions between QTLs, we used the Pseudomarker PAIRSCAN algorithm. This program analyzes the phenotypic effect of each marker or marker interval taken singly (MAINSCAN) and also the phenotypic effects of pairs of markers or intervals taken jointly (PAIRSCAN) for their effects on the trait. The PAIRSCAN allows a genome-wide search for epistasis. For PAIRSCAN, we tested the combined (or full model) effects on trait of a marker pair, which reflects the main effects of both markers plus their interaction (38). The threshold for genome-wide significance was set at 5%, which was estimated by a 200-permutation test carried out on F_2 data.

RESULTS

Serum lipoprotein cholesterol profiles of MRL, SJL, F_1, and F_2 intercross mice

The fat mass calculated as percent body weight was 38% ($P < 0.001$) higher in MRL mice as compared with SJL mice (**Table 1**). The MRL mice had 60% ($P < 0.001$) higher body weight as compared with SJL mice (details not shown). However, there was a moderate but highly significant correlation between percent fat mass and body weight (Pearson r value = 0.25; $P < 0.001$), indicating that about 6.2% of the body weight was explained by variance in percent fat mass. Serum concentrations of cholesterol and HDL were higher in MRL mice by 82% ($P < 0.001$), and 120% ($P < 0.001$), respectively, as compared with SJL mice (Table 1). The TG levels were 58% ($P < 0.001$) higher in SJL mice compared with MRL mice. The percent fat mass, cholesterol, and HDL levels were intermediate (Table 1) and significantly different in F_1 mice ($P < 0.01$ by ANOVA) compared with those of the parental strains.

TABLE 1. Percent body fat mass and lipid levels in standard diet-fed female MRL/MpJ and SJL/J mice

Mice	Body Weight	Fat Mass	Cholesterol	HDL	Triglyceride
	g	*%*		*mg/dl*	
MRL/MpJ (n = 20)	29.8 ± 1.7	16.9 ± 2.7	155.2 ± 18.7	152.2 ± 18.7	140.9 ± 32.8
SJL/J (n = 10–20)	18.1 ± 0.8[b]	12.3 ± 1.8	89.1 ± 7.5[b]	69.1 ± 5.1[b]	235.8 ± 61.8[b]
F_1 (n = 34)	26.8 ± 1.9[a]	14.4 ± 2.1[a]	131.8 ± 18.9[a]	115.5 ± 14.9[a]	202.7 ± 53.1[c]

[a] $P < 0.01$ vs MRL and SJL.
[b] $P < 0.01$ vs MRL.
[c] $P < 0.01$ from MRL but not significantly different from SJL.

These data suggest that high cholesterol, HDL, and fat mass were inherited in an additive manner. The TG levels in F_1 mice were significantly higher than in MRL mice but comparable to those of SJL mice ($P > 0.05$ by ANOVA), suggesting that TG levels were inherited in a dominant fashion (Table 1).

The distributions of fat mass, cholesterol, HDL, and TG among standard diet-fed female F_2 mice are shown in **Fig. 1A, C, E, G**. The distributions of fat mass did not pass the normality test (Shapiro Wilk $W = 0.9$; $P < 0.05$; n = 621; mean ± SD, 12.9 ± 3.1). As expected for mice fed a standard diet, there was a highly significant correlation between cholesterol and HDL levels in the F_2 mice (n = 518; Pearson correlation coefficient, $r = 0.96$; $P < 0.0001$). The distributions of cholesterol (mean ± SD, 133 ± 27 mg/dl), HDL (mean ± SD, 112 ± 25 mg/dl), and TG (mean ± SD, 207 ± 75 mg/dl) were continuous (Fig. 1C, E, G) but did not pass the normality test (Shapiro Wilk $W = 0.95$–0.98; $P < 0.01$). However, the range of F_2 values exceeded mean ± 2 SD parental intervals for each trait. The correlation between fat mass and lipid levels was very weak (Pearson r values 0.006, 0.021, 0.05 for HDL, cholesterol, and TG) and nonsignificant ($P > 0.05$). Cholesterol levels showed a highly significant correlation with TG (Pearson

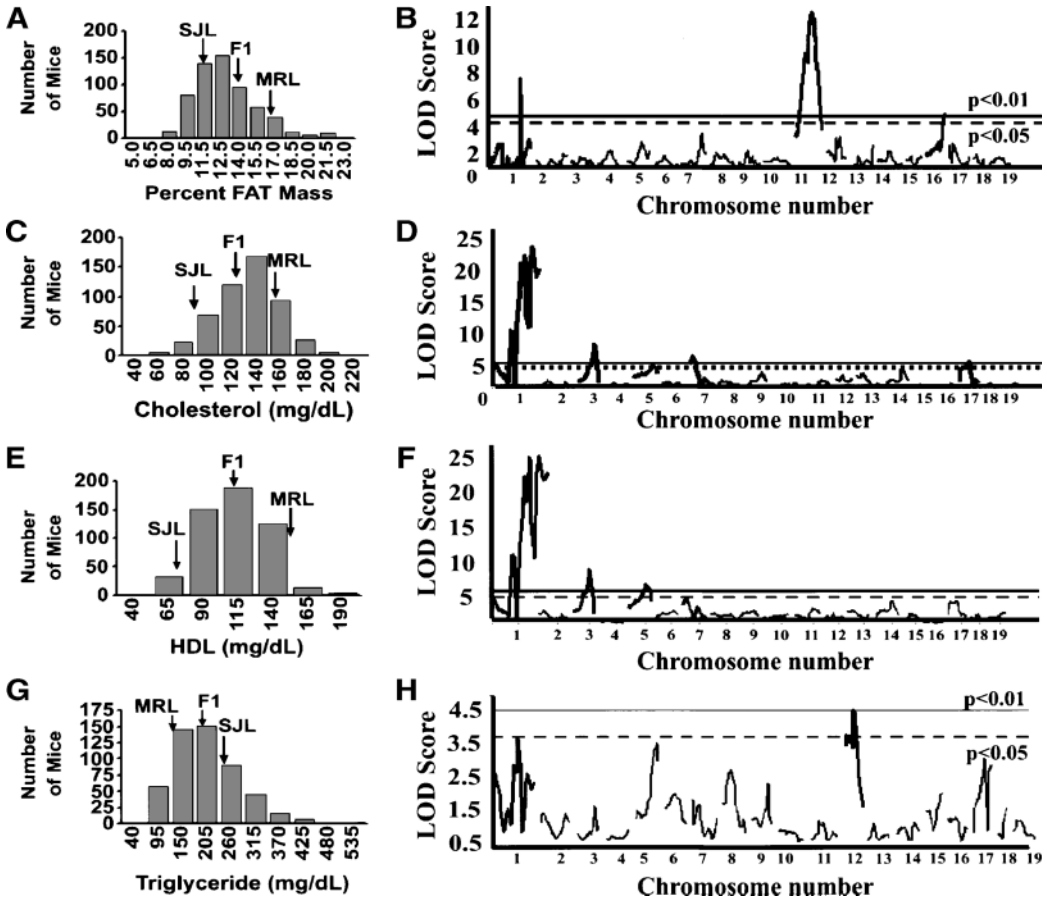

Fig. 1. Genome-wide linkage map of percent fat mass in MRL × SJL F_2 female mice. A, C, E, and G: Distribution of percent fat mass, cholesterol, HDL cholesterol, and triglyceride (TG) in MRL × SJL female F_2 mice (n = 518–621). B, D, F, H: Linkage maps of percent fat mass, cholesterol, HDL cholesterol, and TG in MRL × SJL female F_2 mice. Solid horizontal lines in B, D, F, and H indicate the threshold for genome-wide significance for $P < 0.01$. Broken horizontal lines in B, D, F, and H indicate the threshold for genome-wide significance for $P < 0.05$.

$r = 0.34$; $P < 0.0001$). Taken together, these data also suggest a complex inheritance of serum lipid levels and fat mass in this cross.

Identification of genetic loci affecting obesity or body fat mass

The linkage map for body fat mass was constructed using a panel of 621 MRL × SJL F_2 mice. Two significant QTLs were identified on chromosomes 11 and 16 (Fig. 1B), with strongest linkage (LOD score 11) on chromosome 11 (**Table 2**). Because single-marker D16Mit51 indicated the linkage on chromosome 16, mapping of D16Mit51 should be considered provisional; the locus is located at the end of a chromosome and confirmatory polymorphic markers flanking the linked marker (D16Mit51) could not be identified. A suggestive QTL was identified on chromosome 7 at 50 cM with an LOD score of 2.4 (genome-wide significance; $P < 0.68$). In addition, we observed a QTL on chromosome 1 at 82 cM linked to the marker D1Mit33 with an LOD score of 6.6 ($P < 0.0001$); however, this QTL was not supported by flanking markers, and hence was excluded from any further analysis. **Figure 2** shows posterior probability plots, likelihood statistics that give rise to the 95% confidence interval for a locus. Taken together, the three QTLs (excluding chromosome 1 QTL) explained approximately 18% of the phenotypic variance in F_2 female mice. We also calculated LOD scores for the BMI trait, which failed to pass the threshold of significance for suggestive linkage ($P < 0.63$) for any loci (data not shown), indicating that linkage of fat mass was largely independent of BMI in this cross.

Localization of cholesterol, HDL, and TG QTLs

The linkage maps for lipids were generated using a panel of 518 (MRL × SJL) F_2 mice for which blood was available. The results of interval mapping of these traits are shown in Table 2 and Fig. 1. Five statistically significant cholesterol QTLs were identified on chromosomes 1, 3, 5, 7, and 17 (the three strongest linkages are shown in **Fig. 3**). The highest LOD score was observed for the chromosome 1 locus, with peak LOD score of 22 at *D1Mit453* (Fig. 3). As expected for mice fed a standard diet, the HDL loci were coincident with loci underlying cholesterol levels, but only three QTLs on chromosomes 1, 3, and 5 reached the threshold of genome-wide significance (shown in **Figs. 3**, **4** along with the alleles contributing to closest peak marker). The QTL on chromosome 1 exerted the strongest effect on cholesterol and HDL, explaining 28% and 30%, respectively, of the variance (Table 2) in F_2 mice. Significant linkage for TG was observed on two loci on chromosomes 1 and 12 (**Fig. 5**). Two suggestive linkages were observed for TG on chromosomes 5 and 17 (Table 2).

The LOD scores for cholesterol were comparable to analogous scores for HDL levels at chromosomes 1, 3, and 5 QTLs, whereas LOD scores for cholesterol and HDL for chromosomes 7 and 17 appear to be differentially regulated, suggesting additional QTL(s) that may regulate non-HDL cholesterol levels. To investigate this further, we performed linkage analysis using non-HDL cholesterol levels (calculated by subtracting HDL from total cholesterol) in female F_2 mice (Table 2). Linkage to non-HDL cholesterol was detected in F_2 mice on chro-

TABLE 2. Major QTLs that influence body fat mass and lipid levels in standard diet-fed female MRL/MpJ and SJL/J mice

Phenotype	Chromosome	Peak Marker (cM)	LOD Score	*P*	Variance Explained by Peak Interval
Fat mass	7	50	2.42[b]	0.003780	1.9
	11[a]	43.0	11.51[c]	<0.000001	12.0
	16	50.0	3.99[c]	0.000102	3.6
Cholesterol	1	95	22.00[c]	<0.000001	27.7
	3	40	6.61[c]	<0.000001	11.2
	5	50	3.32[c]	0.000479	3.3
	7	10	4.74[c]	0.000018	4.9
	14	40	2.62[b]	0.002415	2.9
	17	25	3.73[c]	0.000180	5.1
HDL	1	95	23.29[c]	<0.000001	30.0
	3	40	7.02[c]	<0.000001	11.7
	5	45	4.86[c]	0.000014	4.8
	7	10	2.95[b]	0.001116	2.2
	14	45	2.43[b]	0.003747	2.6
	17	25	2.5[b]	0.003170	3.5
Triglyceride	**1**	76.2	3.80[c]	0.000152	3.4
	5	55.0	3.05[b]	0.000900	3.7
	12	26.0	4.10[c]	0.000079	4.6
	17	30.0	2.59[b]	0.002547	3.6
Non-HDL cholesterol	7	15	3.69[c]	0.000206	4.0
	12	5	5.09[c]	0.000008	6.2
	9	40	2.73[b]	0.001847	3.0

LOD, logarithm of the odds ratio; QTL, quantitative trait locus.
[a] QTL identified earlier in same cross (Reference #33) also corresponds to body length (43.7 cM, LOD 4.8) and muscle mass (43.7 cM, LOD 2.7). QTLs in bold letters indicate novel QTL finding.
[b] Suggestive QTLs genome-wide significance calculated for cutoff <0.68.
[c] Kruskal-Wallis test results of significant markers $P < 0.001$.

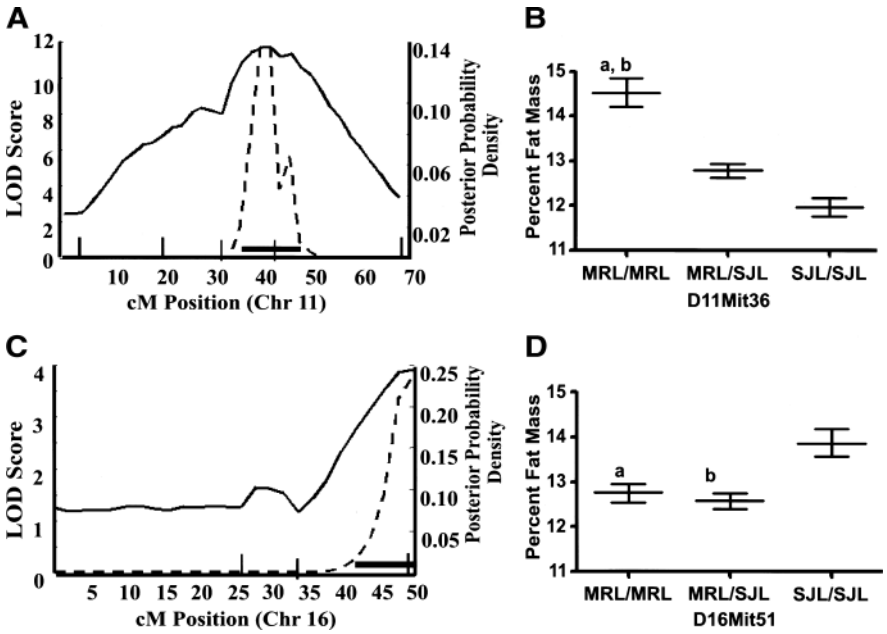

Fig. 2. A, B: Logarithm of the odds ratio (LOD) score and posterior probability density plots for the major quantitative trait loci (QTLs) influencing percent fat mass. C, D: Allelic contribution of closest marker located on major QTL peak. MRL/MRL represents homozygosity for MRL/MpJ alleles; SJL/SJL represents homozygosity for SJL/J alleles; and heterozygosity at a locus is represented by MRL/SJL. Chromosome 11 was analyzed using five markers, and chromosome 16 was analyzed using three markers. Locations of these markers are shown as vertical lines on the x axis. Posterior probability density is a likelihood statistic that gives rise to the 95% confidence intervals indicated by black horizontal bars. Error bars represent SEM. a = P 0.05 vs SJL/SJL; b = $P < 0.05$ vs MRL/SJL (by ANOVA). Chr, chromosome; cM, centimorgan.

mosome 7 (D7Mit76), accounting for 4.0% variance. Additional linkages were observed for chromosome 12 (D12Mit182), accounting for 6.2% variance, and chromosome 9 (D9Mit208), accounting for 3.0% variance (inherited in an additive manner; data not shown). The peaks on chromosome 7 and chromosome 17 were coincident with the peaks for cholesterol and HDL levels (Table 2). Although the above data provide genetic evidence for differential regulation of non-HDL cholesterol, however, the results on non-HDL cholesterol should be interpreted cautiously because the blood levels of non-HDL cholesterol are very low.

Nonparametric mapping with the Kruskal-Wallis Test to assess fat mass and lipid QTLs

The interval-mapping approach for QTL analysis is based on the assumption that the residual environmental variation follows a normal distribution. However, the fat mass and lipid data showed slight deviation from normal distribution. Therefore, the Kruskal-Wallis rank sum test, which makes no assumptions about the probability distributions of the phenotypic data, was also employed to determine trait-marker association. The Kruskal-Wallis analysis, performed with fat mass and serum lipid level phenotype data in the MapQTL program, confirmed all loci identified by interval mapping. The Kruskal-Wallis K values ranged between 15 and 90 for different loci; most importantly, the significance level for all significant loci identified by interval mapping was $P < 0.001$ (Table 2).

Allelic variation for QTLs affecting lipid levels and body fat mass

At the peaks of linkage for cholesterol, HDL, TG, and fat mass, the genotypic means in F_2s, represented by the closest markers, were calculated for F_2 females and are shown in Figs. 2–5. For fat mass locus on chromosome 11, the homozygotes for the MRL allele had 21.3% ($P < 0.0001$) higher fat mass, as compared with the homozygotes for the SJL allele. At the chromosome 7 locus, the mean fat mass for the homozygous MRL and SJL alleles were 5–8% lower than that of heterozygotes (data not shown). For the loci on chromosome 16, homozygotes for the SJL allele had 8.7% ($P < 0.05$) higher fat mass than the homozygotes for the MRL allele. At the chromosome 11 locus, the phenotypic effect of the MRL allele best fit an additive model, whereas for the chromosome 16 locus, the phenotypic effect of the SJL allele best fit a recessive mode of inheritance.

For cholesterol and HDL, the phenotypic effects of the MRL allele at loci on chromosomes 1, 3, 5, 7, and 17 are inherited in an additive manner. For cholesterol, HDL, and TG, the loci on chromosome 1 of the MRL allele were 27, 31, and 19%, respectively (all $P < 0.001$), higher than the those of the homozygotes for the SJL allele. For the locus on chromosome 14, the homozygous SJL alleles have 9% higher cholesterol and HDL levels as compared with MRL homozygotes (details not shown). At the locus on chromosome 12 for TG, homozygotes for the SJL allele had 19.2% ($P < 0.001$) higher TG levels than the

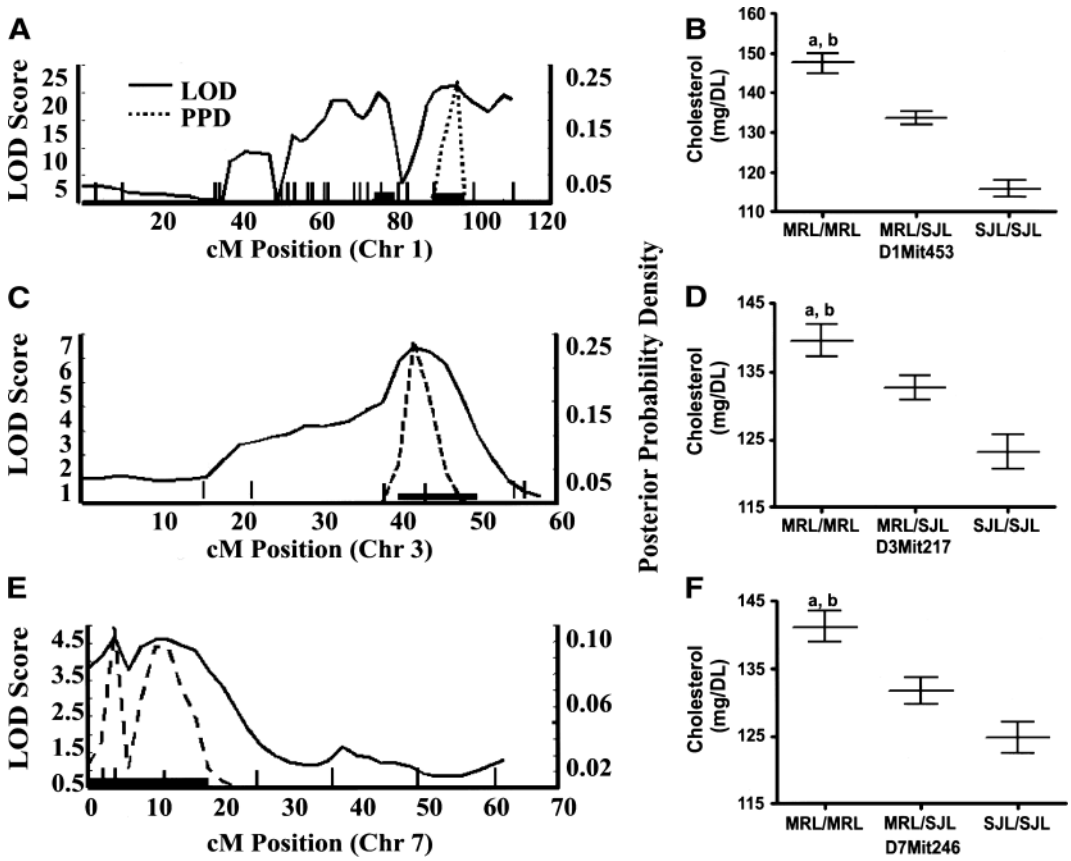

Fig. 3. A, C, E: LOD score and posterior probability density plots for the major QTLs influencing cholesterol. B, D, F: Allelic contribution of closest marker located on major QTL peak. MRL/MRL represents homozygosity for MRL/MpJ alleles; SJL/SJL represents homozygosity for SJL/J alleles; and heterozygosity at a locus is represented by MRL/SJL. Chromosome 1 was analyzed using 22 markers, chromosome 3 was analyzed using 5 markers, and chromosome 7 was analyzed using 7 markers. Locations of these markers are shown as vertical lines on the x axis. Posterior probability density is a likelihood statistic that gives rise to the 95% confidence intervals indicated by the black horizontal bars. Error bars represent SEM. a = $P < 0.05$ vs SJL/SJL; b = $P < 0.05$ vs MRL/SJL (by ANOVA). Chr, chromosome; cM, centimorgan; PPD, posterior probability density.

homozygotes for the MRL allele, and the SJL allele are inherited in an additive manner.

Although the fat mass, cholesterol, and HDL levels were higher in the MRL strain, it is noteworthy that recessive SJL alleles at chromosome 16 increased fat mass (Fig. 2), and a dominant chromosome 14 SJL allele increased HDL and cholesterol levels (data not shown). Similarly, parental SJL strains have higher TG levels than do parental MRL strains, yet QTL analysis in the F_2s revealed that dominant MRL alleles at chromosome 1 increase TG levels (Fig. 5). This type of finding has been observed in other QTL analyses (39), and presumably means that the MRL or SJL alleles at these loci are phenotypically silent (39) in the context of the MRL and SJL genomes, respectively, but the increase fat mass or lipid levels in the presence of one or more SJL alleles.

QTL-QTL interactions

Two loci showed interactions when the genotype at one locus affected the effect of the other locus. However, the LOD scores for all locus interactions, determined using the Pseudomarker PAIRSCAN software program, were suggestive in nature (data not shown).

Pleiotropic effects on fat mass and lipid levels

Our results show that the two loci on chromosome 1 and chromosome 11 have pleiotropic effects on multiple phenotypes. The chromosome 1 locus at 82 cM has a pleiotropic effect on cholesterol, HDL, and TG levels. In addition to phenotypes described in this study, we have also obtained QTLs for body weight, body length, and several musculoskeletal phenotypes (33–36). Interestingly, loci on chromosome 1 and chromosome 11 showed concordance with QTLs that regulate some skeletal phenotypes. The chromosome 1 locus (D1Mit33) was concordant with the total body bone density and volumetric bone density at tibia midshaft described previously in the same F_2 female mice (35). The chromosome 11 locus (D11Mit36) was concordant with QTLs identified for fat mass, body weight, muscle mass, and body length (QTLs for these phenotypes have been described previously) (33). In addition, the chromosome 11 locus showed concordance with the QTL that affects radial bone size (periosteal circumference at midshaft tibia).

To investigate how lipid QTLs affect fat mass, we compared the genotype influence of loci (*D11Mit36* and *D16Mit51*) that regulate fat mass in a subset of female F_2

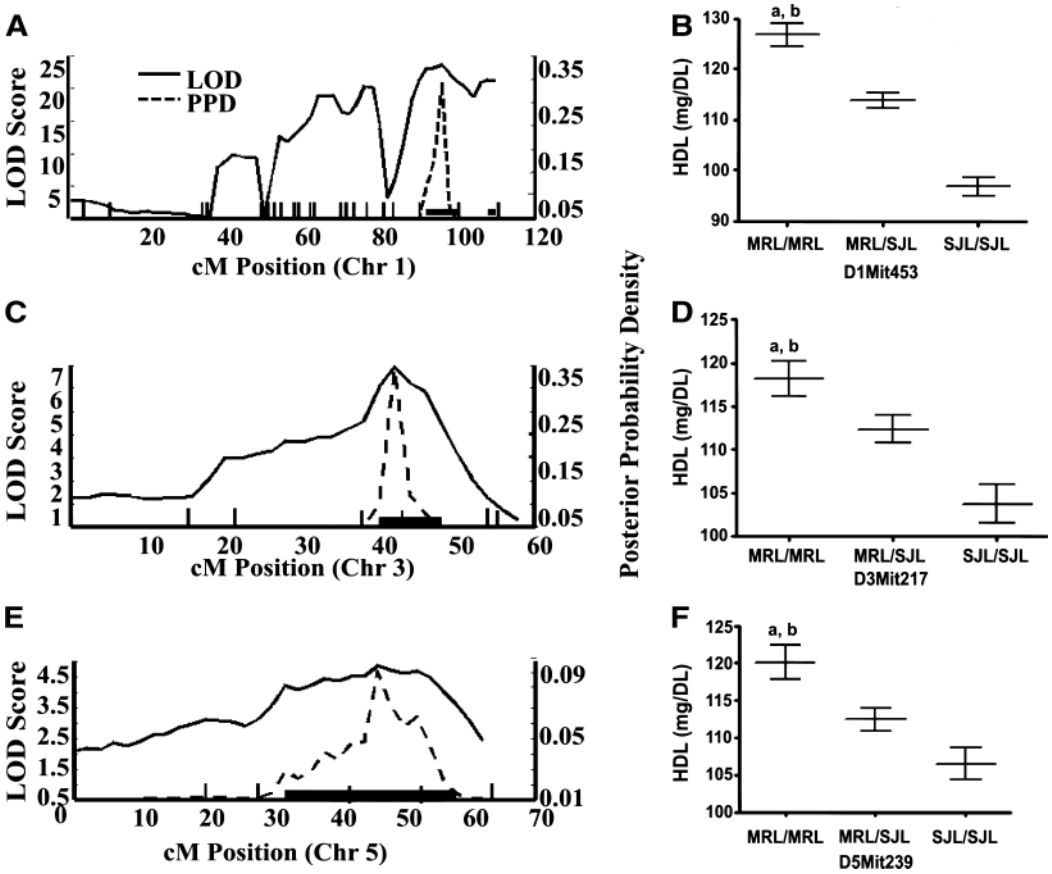

Fig. 4. A, C, E: LOD score and posterior probability density plots for the major QTLs influencing HDL. B, D, F: Allelic contribution of closest marker located on major QTL peak. MRL/MRL represents homozygosity for MRL/MpJ alleles; SJL/SJL represents homozygosity for SJL/J alleles; and heterozygosity at a locus is represented by MRL/SJL. Chromosome 1 was analyzed using 22 markers; chromosomes 3 and 5 were analyzed using 5 markers each. Locations of these markers are shown as vertical lines on the x axis. Error bars represent SEM. Posterior probability density is a likelihood statistic that gives rise to the 95% confidence intervals indicated by the black horizontal bars. a = $P < 0.05$ vs SJL/SJL; b = $P < 0.05$ vs MRL/SJL (by ANOVA). Chr, chromosome; cM, centimorgan.

mice grouped by genotype of loci that regulate cholesterol and HDL (*D1Mit453*, *D3Mit217*, *D7Mit246*, *D5Mit136*, and *D12Mit201*). Genotype groups that showed significant differences (by ANOVA) between homozygous MRL and SJL alleles are shown in **Fig. 6**. For genotype *D1Mit453*, F_2 mice homozygous for the MRL-derived allele for *D11Mit36* (Fig. 6A) exhibited 7% higher fat mass ($P < 0.05$ by ANOVA), as compared with those derived from homozygous SJL alleles. For genotype *D1Mit453*, F_2 mice homozygous for the SJL-derived allele for *D16Mit51* (Fig. 6B) exhibited 10% higher fat mass ($P < 0.05$ by ANOVA) as compared with those derived from homozygous MRL alleles. For female F_2 mice grouped by genotype for *D1Mit453*, F_2 mice homozygous for the SJL-derived allele for genotype *D12Mit201* exhibited 20–28% higher levels of TG ($P < 0.05$) as compared to those derived from homozygous MRL alleles (Fig. 6C). The relative effects of *D1Mit453* alleles on fat mass among the F_2 mice indicate that locus *D1Mit453*, in addition to regulating cholesterol and HDL levels, represents an important determinant of the fat mass variation between the MRL and SJL strains. Taken together, these data suggest that the genes underlying loci of chromosomes 1, 11, 12, and 16 could play critical roles in determining both fat mass and lipid levels.

DISCUSSION

The genome-wide scans of MRL × SJL F_2 mice for associations between marker genotypes and the quantitative phenotypes of total body fat mass and serum lipid levels resulted in the localization of several novel QTLs, particularly for percent fat mass and TG levels. Fat mass has a significant heritability (40) and previous studies have identified >20 loci (**Table 3**) that regulate total body fat mass (12, 14, 17, 26, 40–44). However, only two major loci, located on chromosome 2 (17) and chromosome 8 (14) have been concordant in more than one cross. A body fat QTL identified on chromosome 2 in NZB/BINJ × SM/J mice (17) was concordant with a QTL in a C57BL/6J × CAST/Ei (29) cross. The region of chromosome 2 QTL in these two crosses is syntenic with a large region of human chromosome 20, which shows linkage to body fat mass. Recently Ishimori et al. (14) have shown a locus on chromosome 8 in the C57Bl/6J ×

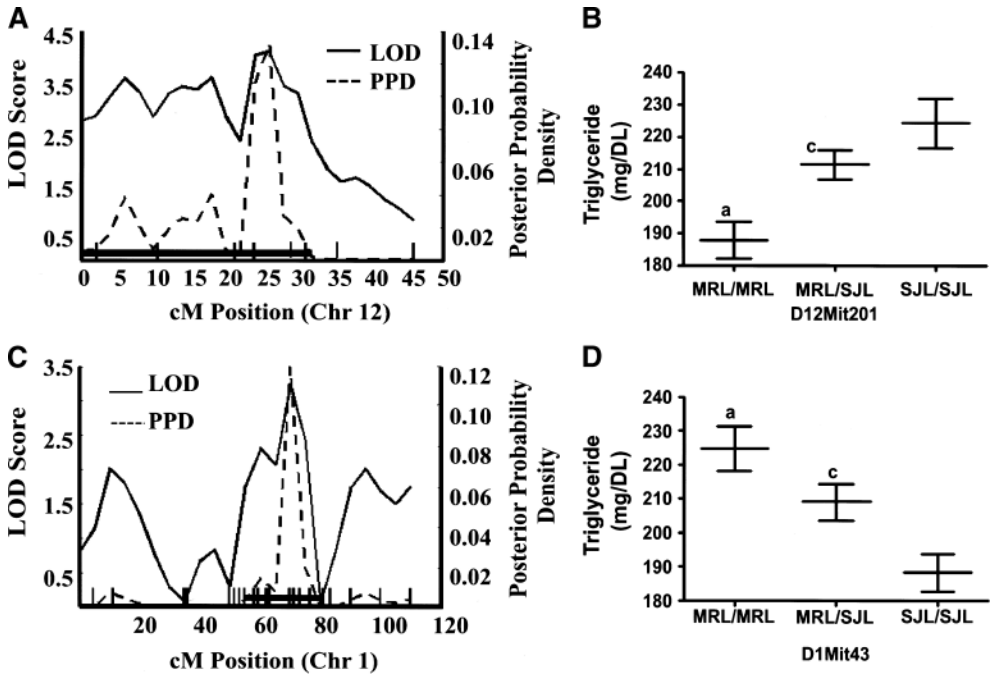

Fig. 5. A, C: LOD score and posterior probability density plots for the major QTLs influencing TG. B, D: Allelic contribution of closest marker located on major QTL peak. MRL/MRL represents homozygosity for MRL/MpJ alleles; SJL/SJL represents homozygosity for SJL/J alleles; and heterozygosity at a locus is represented by MRL/SJL. Chromosome 1 was analyzed using 22 markers, and chromosome 12 was analyzed using 8 markers. Locations of these markers are shown as vertical lines on the x axis. Error bars represent SEM. Posterior probability density is a likelihood statistic that gives rise to the 95% confidence intervals indicated by the black horizontal bars. a = $P < 0.05$ vs SJL/SJL; c = $P < 0.05$ vs MRL/MRL and SJL/SJL (by ANOVA). Chr, chromosome; cM, centimorgan.

129S1/SvlmJ cross that regulates fat mass similar to that identified previously in the C57Bl/6J × CAST/Ei cross (29). These findings suggest that the full repertoire of QTLs affecting fat mass has not yet been determined and that identification of candidate genes that regulate obesity is still in its early phase. QTLs observed in this study account for approximately 18% of the total variance in percent fat mass in F_2 mice. The lower estimates

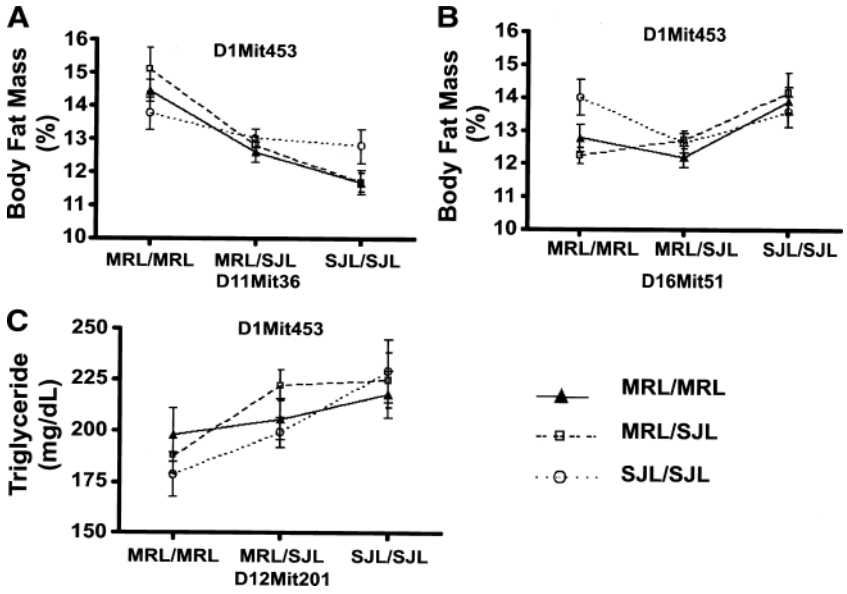

Fig. 6. A, B: Genotypic influence of three loci (D11Mit36 and D16Mit51) regulating fat mass in a subset of female F_2 mice grouped by genotype of locus (D1Mit453) regulating blood cholesterol and HDL levels. C: Genotypic influence of locus (D12Mit201) regulating TG in a subset of female F_2 mice grouped by genotype at locus (D1Mit453) regulating cholesterol. Data points and error bars represent mean ± SEM.

TABLE 3. Body fat mass or obesity QTLs detected in female mice in various genetic crosses

Cross	Chromosome	Peak cM or (Range in cM)	LOD Score	Reference
C57BL/6J × 129S1/J (F_2)	**8**[a]	48 (42–53)	10.0	14
	1[a]	74 (48–108)	2.3	
	12[a]	2 (0–16)	2.9	
	6[a]	0 (0–10)	2.6	
NZB/B1NJ × SM/J (F_2)	**2**[a]	15–30	4.3	17
C57BL/6J × SPRET (F_2)	6[b]	2.8	4.8	44
	7[a]	60	4.2	
	12[a]	52	4.8	
	15[b]	6.7	3.4	
129/Sv × Le/Suz (F_2)	1[b]	2–16	2.9	41
	7[b]	3–25	3.5	
C57BL/6J × AKR/J (F_2)	**2**[b]	50	5.1	12
	17[b]	5	4.6	
C57BL/6J × CAST/Ei (F_2)	**2**[a]	95.5	5.8	43
	8[b]	53.3	3.2	
	9[b]	42	4.7	
C57BL/6J × KK (F_2)	9[b]	15–23	6.3	42

Numbers in bold represent loci colocalized in more than one cross.

[a] Body fat mass.

[b] Fat pad weight or other measure of fat mass.

of total F_2 variance explained by the loci identified in this study could be due to the presence of QTLs that have a small effect on F_2 variance and hence are difficult to identify. In addition, the best estimate of total variance explained is obtained by identifying plieotropic interactions, which is a challenging task because of lower power to detect such interactions. We did not observe any significant interaction, which may partly explain the lower variance explained by the loci identified in this study. Taken together, our results and published findings indicate that results from several F_2 crosses may be necessary for identifying the majority of QTLs that affect fat mass. Out of three loci (chromosomes 7, 11, and 16) that regulate body fat mass, two QTLs on chromosomes 11 and 16 were specific to the MRL × SJL cross. Previous data published by our laboratory on the MRL × SJL F_2 mice (33) indicated that the chromosome 7 (LOD 2.4) locus was colocalized with a locus that regulates lean body mass (5.5 cM, LOD 2.9). In addition, the locus on chromosome 11 colocalizes with QTLs that regulate body weight, muscle mass, body length, and radial bone size. It could be speculated that the effect of the chromosome 11 locus on radial bone size is indirectly related to bone adaptive response to mechanical loading resulting from higher body weight. In mice, the major effect of mechanical loading is reflected in an increase in the periosteal perimeter of long bones. It is noteworthy that there was a significant correlation between body weight and percent fat mass ($r = 0.25$; $P < 0.0001$), indicating that the same gene(s) may regulate these phenotypes. Together, these findings suggest that the chromosome 7 and chromosome 11 loci that regulate fat mass have pleiotropic effects on body weight. It remains to be verified whether the same gene or independent genes under these loci regulate the multiple phenotypes.

Several candidate genes have been identified for all fat mass loci. The prominent candidates for the chromosome 11 locus include *Nos2* (nitric oxide synthase 2), *Hcrt* (hypocretin), *Alox12*, *Alox3*, *Alox15*, *Alox12b*, and *Alox12e* (all belong to a family of lipoxygenases that are a class of iron-containing dioxygenases that catalyze the hydroperoxidation of lipids), and Lpd1, a mouse insertional mutation lpd (lipid defect) whose phenotype includes elevated plasma TG (45) and is a potential candidate gene for the chromosome 16 QTL. Although the above-mentioned genes are obvious candidates, on the basis of their known functions, ultimately, identification of the gene(s) underlying a particular QTL will be necessary for determining which subset of genes contributes to the genetic variation of a trait across species.

Previous linkage studies have identified >20 loci regulating TG on chromosomes 4, 8, 9, 11, 12, 14, 18, and 19 (as shown in **Table 4**). However, only two loci on chromosome 2 and chromosome 8 have been colocalized in multiple crosses. In this study, four loci on chromosomes 1, 5, 12, and 17 were in linkage with serum levels of TGs. The chromosome 12 QTL (at 25 cM) identified in this study does not appear to be concordant with any previously known QTL on chromosome 12, which showed a peak at 39 cM. Therefore, the majority of TG QTLs identified in the MRL × SJL cross appear to be novel. The QTL on chromosome 12, however, incorporates QTLs identified earlier for fat mass (at 29 cM) (46) and HDL (at 17.2 cM) (31) in mice. For non-HDL cholesterol, the locus identified in this study on chromosome 7 was concordant with the cholesterol and HDL locus, suggesting that the candidate gene in this region may regulate both HDL and non-HDL cholesterol. The chromosome 12 locus was proximal to the TG QTL located on chromosome 12 and

TABLE 4. QTLs affecting triglyceride levels in various genetic crosses

Cross	Chromosome	Peak (cM)	LOD Score	Reference
C57BL/6J × 129S1/J (F_2)	**9**	**66**	**2.2**	14
	14	14	2.0	
	18	42	2.9	
RR × KK (F_2)	8	—	4.7	27
SM/J × A/J (F_2)	**4**	**35.4**	**2.4**	10
	8	8	3.4	
	9	**71**	**2.3**	
	11	57	2.8	
	12	39	2.5	
	19	50	2.2	
SMXA (RI)	4	4.3	2.5	11
	11	64	2.6	
C57BL/6J × RR (F_2)	1	94.8	4.4	28
MRL/lpr × Balb/C (F_2)	**4**	**66**	**2.9**	21
	15	70	2.1	
	15	45	2.7	
	19	3	4.0	

Numbers in bold represent loci colocalized in more than one cross.

could independently regulate non-HDL cholesterol. The locus on chromosome 9 appears to be novel for non-HDL cholesterol. The TG QTLs identified in this study have a limitation in that the blood samples were collected under nonfasting conditions and some of the QTLs could be influenced by nongenetic factors.

QTLs on chromosomes 1, 3, 5, 7, and 17 (14, 30, 31) that regulate HDL and cholesterol levels are concordant with linkages previously identified in standard diet-fed F_2 mice. Location and peaks of all the loci identified in this study, with the exception of the chromosome 14 locus, coincide with published HDL cholesterol peaks. These findings confirm the belief that, in general, we may have reached saturation as far as identification of HDL and cholesterol QTLs are concerned. Results of this study show that almost 70% (Table 2) of the total variance in cholesterol levels in F_2 mice was explained by the QTLs identified in this study. The strongest linkage for cholesterol was identified on chromosome 1 in MRL × SJL F_2 mice. This chromosome 1 locus contains the apolipoprotein A-II (apoA-II) gene, which has polymorphism that has been implicated as an HDL QTL gene in seven different crosses (47). The MRL strain carries the APOA2b haplotype allele (47) known to impart higher HDL levels, whereas the SJL strain carries the apoA-IIc allele, which is responsible for lower HDL levels. However, a high LOD score of 21–24 for two distinct peaks may indicate the possible involvement of other unknown gene(s) present in this region. The chromosome 14 locus observed in this study, although suggestive in nature, was located in the distal region of any known chromosome 14 QTL (30, 31) and therefore could represent a distinct linkage finding. Several candidate genes for cholesterol and HDL have been located within the QTL regions identified in this study. A comprehensive list of these genes is available in a recently published review (31).

Identifying the gene underlying a QTL is a complex task; therefore, the process of identifying genes for QTLs discovered to date has been slow. Recently it was shown that single-nucleotide polymorphisms (SNPs) can be used to narrow a QTL, because gene(s) underlying a QTL should be in the region where the parental strains have a haplotype divergence (47–49). Consequently, haplotype analysis of the chromosome 1 QTL (confirmed in multiple crosses) led to identification of a polymorphism in the *Apoa2* gene that affected HDL levels (47). In this regard, our findings on HDL and cholesterol QTLs could be important for future research directed toward the search for genes that regulate lipid levels. Results from each analysis were compared on the assumption that more common variants would be detected more frequently across the strains and that the QTL genes that are detected in these multiple crosses could be discovered quickly by analyzing their SNPs and haplotypes in multiple strains. The inferred haplotype data may also facilitate the refinement of QTL regions, such that candidate genes can be more easily identified and characterized.

In summary, we have identified several distinct linkages for TG and body fat mass and have confirmed the loci that regulate HDL and cholesterol that were identified in previous genetic crosses. The presence of similar QTLs in previous crosses suggests that for some genes, there are higher degrees of polymorphism. On the other hand, identification of novel loci may indicate the presence of additional genes or modifier genes that regulate a given trait. Thus, our data contribute to the growing knowledge of the genetic complexity of lipid metabolism and obesity and also underscore the importance of strain background in the evaluation of the linkage for a complex trait. It is hoped that the discovery of genes at these loci may help in explaining the variability in lipid levels in humans.

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