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Quantitative trait locus mapping of genes that regulate HDL cholesterol in SM/J and NZB/B1NJ inbred mice

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Pitman, Wendy A., Ron Korstanje, Gary A. Churchill, Edwige Nicodeme, John J. Albers, Marian C. Cheung, Megan A. Staton, Stephen S. Sampson, Stephen Harris, and Beverly Paigen. Quantitative trait locus mapping of genes that regulate HDL cholesterol in SM/J and NZB/B1NJ inbred mice. *Physiol Genomics* 9: 93–102, 2002. First published March 26, 2002; 10.1152/physiolgenomics.00107.2001.—To investigate the quantitative trait loci (QTL) regulating plasma cholesterol, the female progeny of an (SM×NZB/B1NJ)×NZB/B1NJ backcross were fed an atherogenic diet. After 18 wk, plasma total cholesterol and high-density lipoprotein cholesterol (HDL-C) was measured. HDL-C concentrations were greater in NZB than in SM mice. For standard chow-fed mice, QTL were found near *D5Mit370* and *D18Mit34*. For mice fed an atherogenic diet, a QTL was found near *D5Mit239*. The QTL for chow-fed and atherogenic-fed mice on chromosome 5 seem to be two different loci. We used a multitrait analysis to rule out pleiotropy in favor of a two-QTL hypothesis. Furthermore, the HDL-C in these strains was induced by the high-fat diet. For inducible HDL-C, one significant locus was found near *D15Mit39*. The gene for an HDL receptor, *Srb1*, maps close to the HDL-C QTL at *D5Mit370*, but the concentrations of *Srb1* mRNA and SR-B1 protein and the gene sequence of NZB/B1NJ and SM/J did not support *Srb1* as a candidate gene. With these QTL, we have identified chromosomal regions that affect lipoprotein profiles in these strains.

high-density lipoprotein; cholesterol; genetics; *Srb1*

BECAUSE OF THE STRONG INVERSE correlation between plasma high-density lipoprotein cholesterol (HDL-C) concentration and the risk of atherosclerosis in human (14, 15), there is great interest in understanding the factors that regulate concentrations of plasma HDL-C. Several proteins that influence plasma HDL-C concentrations have been identified in recent studies. Variations in plasma HDL-C concentrations are associated with the apoA-I gene locus (10, 37), and low concentrations of HDL-C result from mutations (22, 30, 46, 51) or deletions (31) that cause decreased concentrations of Apo A-I. Plasma HDL-C concentrations are also affected by four proteins involved in lipid metabolism: *1*) lecithin:cholesterol acyltransferase (LCAT); *2*) cholesteryl ester transfer protein (CETP); *3*) hepatic lipase (HL) (for review see Ref. 41); and *4*) phospholipid transfer protein (PLTP) (2, 3, 12, 13, 20). Deficiency of LCAT is associated with reduced concentrations of HDL-C, whereas overexpression of LCAT leads to increased concentrations of HDL-C. Deficiency of CETP or HL results in elevated concentrations of HDL-C, whereas overexpression of either enzyme leads to decreased concentrations of apoA-I and HDL-C. PLTP modulates HDL concentration in vivo through its ability to transfer phospholipid to HDL and to remodel HDL size. The HDL receptor, SR-B1, affects HDL; a knockout of *Srb1* increases HDL concentration and size (45).

Although human studies and transgenic and knockout mice have provided valuable insights into the relationships between HDL-C concentrations and the known lipoproteins and apolipoproteins (7), our understanding of the factors that regulate HDL concentrations and the mechanisms responsible for the protective effect of HDL remain poorly understood. Furthermore, although these methodologies allow for the study of known genes, they do not provide opportunities for the discovery of new genes involved in HDL metabolism. The rapidly developing technique of quantitative trait locus (QTL) analysis can be used to discover new genes (11, 23, 27). QTL mapping has been important in the identification of several loci involved in the development of atherosclerotic lesions (35, 38, 39, 53) and in the regulation of plasma lipid concentrations (16, 25, 26, 40, 50, 55).

In this paper, we report the use of QTL mapping to identify several loci that regulate concentrations of plasma HDL and non-HDL lipoproteins [very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)] in the SM/J (SM) and NZB/B1NJ (NZB) inbred strains of mice. Our data confirm QTL identified in previous, independent studies using the same (40) and different (16, 25, 26, 50) strains of mice and identify

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additional loci involved in controlling plasma HDL and non-HDL cholesterol concentrations. We also present the results of tests carried out to determine whether *Srb1* is a viable candidate gene for one HDL-C QTL.

METHODS

Mice and diets. SM/J and NZB/BlNJ inbred strains of mice were obtained from The Jackson Laboratory, Bar Harbor, ME. SM females were mated to NZB males to produce the F_1 progeny, and F_1 females were mated to NZB males to produce 89 female backcross progeny. Mice were housed in a climate-controlled facility with a 14:10-h light/dark cycle. After weaning, mice were maintained on a standard chow diet (Old Guilford 234A, Guilford, CT) and offered free access to food and water throughout the experiment. The atherogenic diet contained (wt/wt) 15% dairy fat, 50% sucrose, 20% casein, 0.5% cholic acid, 1.0% cholesterol, as well as cellulose, vitamins, and minerals. The source of chemicals and the diet have been described previously (32, 33). For all experiments female mice were fed a standard chow diet until 6–8 wk of age, at which time they were bled for the 0-wk time point. Mice were then fed the atherogenic diet for specific time periods as indicated in the figure legends. All experiments were approved by the Jackson Laboratory's Animal Care and Use Committee.

Lipid measurements. At 0 and 18 wk of diet consumption, mice were fasted for 4 h before blood was collected for lipid determinations. Blood was collected by retro-orbital bleeding into EDTA-coated tubes, and plasma was separated by centrifugation at 1,500 rpm for 5 min at 4°C. Plasma total cholesterol (TC) concentrations were measured by commercial colorimetric enzymatic assay as described previously (4). HDL cholesterol (HDL-C) was measured after selective precipitation of apo B-containing lipoproteins with polyethylene glycol (18). The results are means ± SE in millimoles per liter.

HDL size determinations. HDL size was measured by nondenaturing polyacrylamide gel electrophoresis (PAGE) and fast performance liquid chromatography (FPLC). For the PAGE analysis, 20 μl of plasma and 10 μl of high-molecular-weight standards (Pharmacia Biotech, Piscataway, NJ) were electrophoresed on preformed 4–30% polyacrylamide gels (Alamo Gels, San Antonio, TX) in 0.09 M Tris, 0.08 M boric acid, 0.003 M EDTA, pH 8.35, at 200 V for 20 h at 4°C. Lipoproteins and molecular weight standards were visualized with Sudan Black B and Coomassie G250, respectively, and scanned with a laser densitometer (LKB Ultroscan XL) as described previously (8). For the FPLC analysis, two Superose columns, Superose 6 and Superose 12 (Pharmacia Biotech), were connected in series and equilibrated in 10 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 7.4. Lipoprotein separation was performed on 250 μl of pooled plasma from 10 animals, at a flow rate of 0.3 ml/min in the previously described buffer. Fractions of 500 μl were collected, and 50 μl of each fraction was mixed with 200 μl of direct cholesterol determination reagent PAP250 (Biomerieux-France). After a 15-min incubation at 37°C, optical density was measured at 492 nm, and the cholesterol concentration in each fraction was calculated using a cholesterol standard curve ranging from 0 to 1.0 g/l. Cholesterol concentrations were plotted, and determination of the VLDL, LDL, and HDL cholesterol concentrations was realized after integration of the three corresponding peaks.

Immunoblot and RNA blot analysis of livers for Srb1. To determine whether SR-B1 protein and mRNA concentrations differ between the parental strains, we performed Western blot and total liver mRNA Northern blot analyses. Mouse livers were frozen in liquid N_2 immediately after harvesting and stored at −80°C. Membrane fractions for immunoblotting were prepared from pulverized livers as previously described (24). For Northern blot analysis, 15-μg aliquots of total cellular mRNA were electrophoresed on a 1% (wt/vol) agarose, 0.59% (vol/vol) formaldehyde gel in the following buffer: 20 mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7.0. The fractionated mRNA was transferred in 10× SSC to Hybond N^+ nylon membrane (Amersham, Arlington Heights, IL), ultraviolet (UV) cross-linked, and then probed for SR-B1 as previously described (24).

Isolation and sequencing of Srb1 cDNA. Total RNA isolated from SM and NZB liver was reverse-transcribed using Messagemaker cDNA Synthesis System (GIBCO-BRL). A series of overlapping primers (Table 1), designed to span the *Srb1* mouse sequence (1) (GenBank accession number U37799), was used for PCR amplification with the following conditions: 94°C for 45 s, 58°C for 45 s, and 72°C for 2 min. Most PCR products were sequenced directly, but the PCR product amplified by primers 1 forward and 147 reverse was cloned into the TA-Cloning vector system (Invitrogen). Cycle sequencing was performed using M13 forward (CAGGAAACAGCTATGAC) and reverse (CAGCACTGACCCTTTTG) universal primers and analyzed on an Applied Biosystems 373 machine.

DNA isolation and genotyping. DNA was isolated from tails or spleens. One centimeter of tail or ~⅓ to ½ of one spleen were digested overnight in 500 μl of 1× digestion buffer (50 mM Tris·HCl, pH 8.0, 100 mM EDTA pH 8.0, 100 mM NaCl, 1% SDS, 1 mg/ml proteinase K) in a 55°C water bath. Digests were mixed with 1 volume of 25:24:1 phenol:chloroform:isoamyl alcohol and centrifuged for 5 min at 14,000 rpm at room temperature. DNA was precipitated by adding 2 volumes of 100% ethanol to the isolated aqueous phase. Strands of DNA were wound around a glass capillary pipette and air-dried. The dried DNA pellets were resuspended in 1 ml TE (10 mM Tris·HCl, 1 mM EDTA, pH 7.5–8.0). Genotyping by PCR using mouse MIT MapPairs primers (Research Genetics, Huntsville, AL) was carried out under standard conditions at an annealing temperature of 55°C. To detect polymorphisms as small as 6 bp, PCR products were electrophoresed on 8% horizontal polyacrylamide gels in 1× Tris-borate-EDTA running buffer for 2 h at 180 V. Gels were stained with ethidium bromide and photographed over short-wave UV light.

QTL analysis. From blood samples collected at 0 and 18 wk time points, both TC and HDL-C were measured. From these measurements, VLDL and LDL cholesterol values were cal-

Table 1. *Primer sequences used for Srb1*

Primer Designation	Sequence (5′-3′)	Nucleotide Start Position
146 Forward	CCTTCAGGTCCTGAGCCC	10
3 Forward	GAGATCCTGTGGGGCTATGA	222
4 Forward	TTCTTCGAAGTGGTCAACCC	255
2 Forward	ACAAATGGAACGGACTCAGC	754
5 Forward	GAACCCTAACCCAAAGGAGC	1113
1 Forward	CACTACGCGCAGTATGTGCT	1374
4 Reverse	TCTCCATCAATATCGAGCCC	498
3 Reverse	TCGAGTTGTTCATCCCAACA	693
2 Reverse	CCCGTTGGCAAACAGAGTAT	983
5 Reverse	CTCTGTTCGAACCACAGCAA	1306
1 Reverse	TGAATGGCCTCCTTATCCTG	1513
147 Reverse	CTATCAGGTTTGGGGGGC	1590

culated by subtracting HDL-C from TC. In the mouse, the major atherogenic lipoprotein is VLDL, but small quantities of LDL are also present (17); these lipoproteins are therefore referred to collectively as non-HDL-C. Additionally, we calculated an inducible HDL-C value (Ind HDL-C) and an inducible non-HDL-C value from the ratio of the lipid values at 18 wk to that at 0 wk. Therefore, six phenotypic traits were obtained: HDL-C 0 wk, HDL-C 18 wk, non-HDL-C 0 wk, non-HDL-C 18 wk, Ind HDL-C, and Ind non-HDL-C. For QTL analysis, 15 animals at the high and low extremes for each trait were selected to be genotyped. Since some mice were at the extremes for more than one trait, 53 of the 89 backcross mice were genotyped. The genome-wide scan was conducted using 78 simple sequence length polymorphism (SSLP) markers with 3–5 markers per chromosome (Table 2), except for additional markers typed on those chromosomes with a significant QTL. Because the percentage of polymorphic markers between NZB and SM is only ~30% compared with 50% for most other strains, the number of useful markers for our cross was limited. More markers were added later on chromosomes 5 and 6 to refine QTL in those regions. The average distance between markers was 20–25 centimorgans (cM), except on chromosomes 2, 10, and X where gaps of more than 30 cM exist due to a lack of polymorphic markers in these regions. Centimorgan positions listed throughout this paper are from the Mouse Genome Informatics database (**http://www.informatics.jax.org**).

Statistical analysis. Genome scans for main effect QTL were performed using the method of Sen and Churchill (47). This analysis is equivalent to the interval mapping procedure of Lander and Botstein (23) but uses a different computational algorithm. Logarithm of the odds ratio (LOD) scores were computed at 2-cM increments along the entire genome, and significance was assessed by permutation analysis (9). Significant QTL meet or exceed the 95% genome-wide threshold, and suggestive QTL meet the 80% genome-wide threshold. Confidence intervals for QTL locations were computed using a Baysian method at 95% (47).

In addition to QTL with main effects, we wished to identify pairs of QTL that might make significant contributions to the phenotypic variance through epistatic interactions. We carried out simultaneous genome scans for all pairs of loci using the method of Sen and Churchill (47). The search strategy employed has been described by Sugiyama et al. (49). Briefly, the genome scan searches through all pairs of loci fitting a full two-way ANOVA model with an interaction term. A LOD score contrasting the full model to a null model (with no genetic effects) is computed for each pair, and genome-wide significance is established by permutation testing. A secondary test for the significance of the interaction term is computed only for those pairs that pass the genome-wide screening. The interaction test is carried out using a stringent nominal significance level (0.005), and only those locus pairs passing both tests are deemed to be interacting. In this study, no significant interactions were identified; however, one significant QTL (on chromosome 19) was identified in the pairwise analysis that was not detectable in the main effects genome scans. To assess the combined effects of all QTL on a trait, we carried out a multiple QTL analysis for each trait including all suggestive and significant QTL. The percent of variance explained by each QTL is reported based on this model. The multiple regression was fit using all of the data in the pseudomarker software package to properly account for missing genotypes in the calculation of percent variance explained. A new function "panova" was created for this purpose, and it is available in pseudomarker release version 9.1 (**http://www.jax.org/research/churchill**). Comparisons of cholesterol and *Srb1* mRNA and protein concentrations were performed using Statview II (Abacus Concepts, Berkeley, CA). Between-group comparisons were analyzed by one-way ANOVA, using Fisher's least significant difference test

Table 2. *List of microsatellite markers typed in the (SM × NZB)NZB backcross*

Chr	Marker	Position, cM	Chr	Marker	Position, cM	Chr	Marker	Position, cM
1	*D1Mit20*	19	6	*D6Mit50*	4	13	*D13Mit34*	21
	D1Mit22	33		*D6Mit3*	34		*D13Mit102*	44
	D1Mit92	64		*D6Mit44*	48		*D13Mit73*	45
	D1Mit34	82		*D6Mit15*	78		*D13Mit35*	72
	D1Mit17	103	7	*D7Mit25*	15	14	*D14Mit15*	13
2	*D2Mit1*	1		*D7Mit27*	28		*D14Mit37*	28
	D2Mit88	30		*D7Mit71*	50		*D14Mit7*	44
	D2Mit35	45		*D7Nds4*	70		*D14Mit97*	58
	D2Mit49	95	8	*D8Mit4*	14	15	*D15Mit18*	18
3	*D3Mit12*	34		*D8Mit45*	43		*D15Mit31*	41
	D3Mit11	53		*D8Mit56*	69		*D15Mit39*	59
	D3Mit38	70	9	*D9Mit2*	13	16	*D16Mit3*	21
4	*D4Mit2*	11		*D9Mit21*	30		*D16Mit5*	38
	D4Mit17	29		*D9Mit8*	42		*D16Mit70*	57
	D4Mit9	43		*D9Mit15*	61	17	*D17Mit50*	15
	D4Mit11	64	10	*D10Mit126*	17		*D17Mit20*	30
	D4Mit312	70		*D10Mit11*	51		*D17Mit76*	55
5	*D5Mit228*	14		*D10Mit24*	64	18	*D18Mit34*	10
	D5Mit114	31	11	*D11Mit19*	14		*D18Mit24*	25
	D5Mit7	34		*D11Mit4*	37		*D18Mit9*	42
	D5Mit10	37		*D11Mit41*	49	19	*D19Mit16*	17
	D5Mit239	40		*D11Mit11*	69		*D19Mit27*	43
	D5Mit316	44	12	*D12MIt2*	18		*D19Mit71*	56
	D5Mit209	51		*D12Mit5*	38	X	*DXMit89*	3
	D5Mit65	58		*D12Mit7*	50		*DXMit1*	32
	D5Mit370	60		*D12Mit8*	58			
	D5Mit99	75						

Chr, chromosome.

to determine statistical significance. All values are expressed as means ± SE. The numbers of mice used for each experiment are specified in the individual Figs. 1–5 and Tables 1–6.

RESULTS

HDL-C concentrations and size in parental strains and F_1 progeny. The SM and NZB inbred strains of mice differ in plasma HDL-C and VLDL-C as previously described (38, 40). HDL-C concentrations were significantly ($P < 0.0001$) higher in strain NZB than in SM or the F_1 progeny when mice were fed standard chow or the atherogenic diet (Table 3). The HDL-C concentrations in F_1 progeny were intermediate and significantly different from both parental strains. Although HDL-C concentrations in many strains of mice decrease or remain stable in response to consuming an atherogenic diet (34), they were significantly ($P < 0.03$) increased in NZB, SM, and the F_1 progeny. Non-HDL-C concentrations were also higher in NZB than in SM mice, and in this case the F_1 progeny resembled the SM parent.

Strains NZB and SM also differ from one another in HDL particle size as determined by analysis of pooled samples (Fig. 1). The peak of the HDL particle size as determined by PAGE (Fig. 1*A*) lay further from the origin in NZB (9.86 nm) than in SM (9.32 nm) when mice were fed the chow diet; this difference became more pronounced after 6 wk on the atherogenic diet, as the HDL peak moved in NZB to 10.46 nm while it remained unchanged in SM (9.29 nm). Consistent with these results, the FPLC profiles (Fig. 1*B*) demonstrated that the shift toward larger particle size in NZB became more pronounced as the time on the atherogenic diet was extended. Thus both methods demonstrate that HDL particles are larger in NZB than SM mice, regardless of diet.

QTL analysis of factors affecting lipoprotein concentrations. To investigate genetic factors regulating plasma lipoproteins, we carried out a backcross and collected 90 female backcross progeny. The distribution of HDL-C concentrations for the backcross progeny at both 0 and 18 wk were unimodal (Fig. 2, *A* and *B*), suggesting that multiple loci of small to moderate effect determine these phenotypes. The mean (±SE) HDL-C concentrations at 0 (2.56 ± 0.04 mmol/l) and 18 (4.0 ± 0.1 mmol/l) wk fell between those of the parental strains but more closely resembled those of the NZB parent than those of the SM parent. The distribution

Table 3. *Comparison of plasma cholesterol concentrations in NZB, SM, and F1 progeny*

	Diet	NZB	SM	F_1
HDL-C	chow	3.4 ± 0.3*	1.2 ± 0.1	1.9 ± 0.3
	high fat	5.5 ± 0.3*	1.9 ± 0.1	3.2 ± 0.4
Non-HDL-C	chow	0.8 ± 0.3*	0.3 ± 0.1	0.4 ± 0.1
	high fat	4.0 ± 0.3*	2.5 ± 0.2	2.0 ± 0.4
Induced HDL-C		1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2

Values are means ± SE. Female mice were fed chow or the atherogenic high-fat and high-cholesterol ("high fat") diet for 26 wk as described previously (38). cholesterol concentrations expressed (in mmol/l) were determined by commercial colorimetric enzymatic assay and non-HDL cholesterol calculated as TC minus HDL-C. Induced HDL-C was calculated as the ratio of the concentration after 26 wk on the atherogenic diet to the concentration at 0 wk on diet. TC, plasma total cholesterol; HDL-C, high-density lipoprotein cholesterol. *Significantly different from SM and F_1 ($P < 0.0001$); $n = 6$ for NZB and SM, and $n = 5$ for F_1.

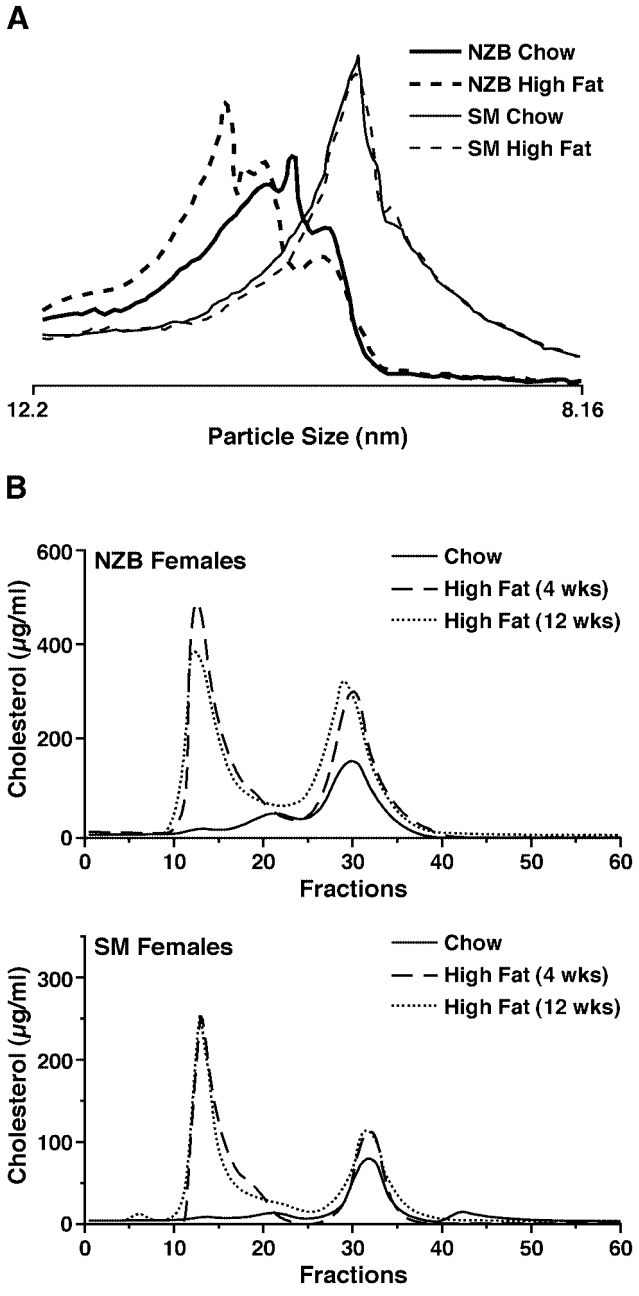

Fig. 1. SM/J (SM) and NZB/B1NJ (NZB) strain differences in high-density lipoprotein (HDL) size in mice fed either a standard chow or an atherogenic diet. *A*: HDL size distribution as determined by nondenaturing 4–30% PAGE. Mice were bled prior to and after 6 wk of consuming the atherogenic diet. Each line represents a pool of 6 female and 6 male mice. *B*: distribution of total cholesterol by fraction as determined by fast performance liquid chromatography (FPLC). Female mice were bled prior to and after 12 wk of consuming the atherogenic diet. Plasma from 10 mice was pooled for each time point.

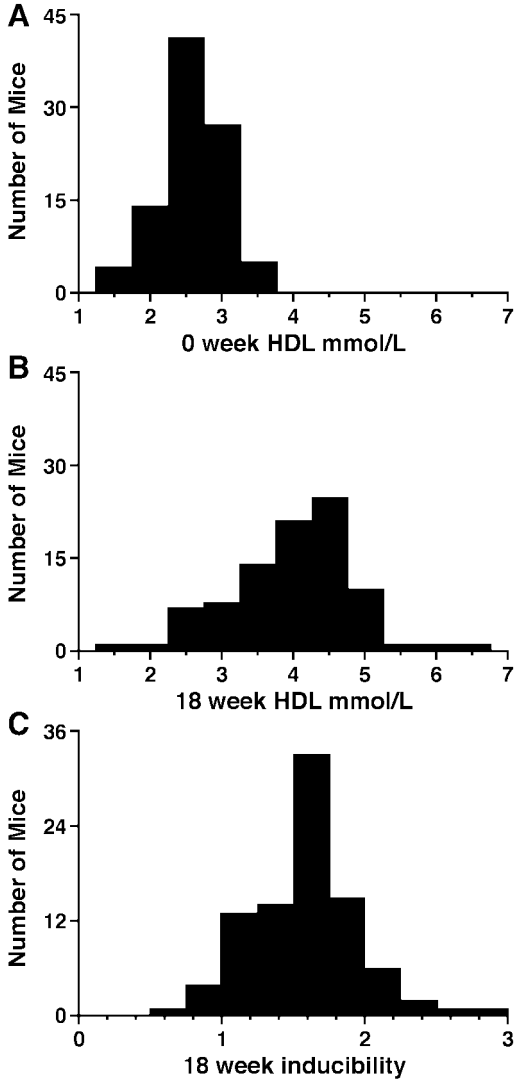

Fig. 2. Distribution of HDL cholesterol (HDL-C) concentrations among the (SM×NZB)×NZB backcross progeny. HDL-C concentrations were determined for 91 individual mice prior to and after consuming the atherogenic diet for 18 wk as described in METHODS. *A*: distribution of mice by HDL-C concentration after 0 wk on the atherogenic diet. *B*: distribution of mice by HDL-C concentration after 18 wk on the atherogenic diet. *C*: Distribution of mice by HDL inducibility index. HDL inducibility index is calculated as the ratio of HDL-C after 18 wk on the atherogenic diet to HDL-C after 0 wk on the atherogenic diet, with a value of 1.0 representing no change.

for inducible HDL-C in the backcross progeny was also unimodal (Fig. 2*C*), and the mean inducible HDL-C for the backcross progeny was 1.6 ± 0.04, similar to both parental strains.

The genotyping of mice and statistical analysis for QTL identified several loci associated with these traits. Figure 3 shows the genome-wide scans for HDL-C at 0 wk, HDL-C at 18 wk, induced HDL-C, non-HDL-C at 0 wk, non-HDL-C at 18 wk, and induced non-HDL-C. The significant ($P < 0.05$) and suggestive ($P < 0.2$) loci identified are shown in Table 4. For chow-fed mice, the NZB alleles near *D5Mit370* and *D18Mit34* are associated with high HDL-C concentration; heterozygosity at either locus is associated with a reduction in HDL-C. Both loci are highly significant (Table 4) and assigned as *Hdlq1* and *Hdlq3*, respectively. The fitted model using these two QTL explains 24% of the total variance (Table 5). In mice fed the high-fat diet, the NZB alleles near *D5Mit239* and *D19Mit71* are associated with high HDL-C concentrations. The QTL on chromosome 19 did not achieve significance in the scan for single QTL but did achieve significance in the pairwise QTL ge-

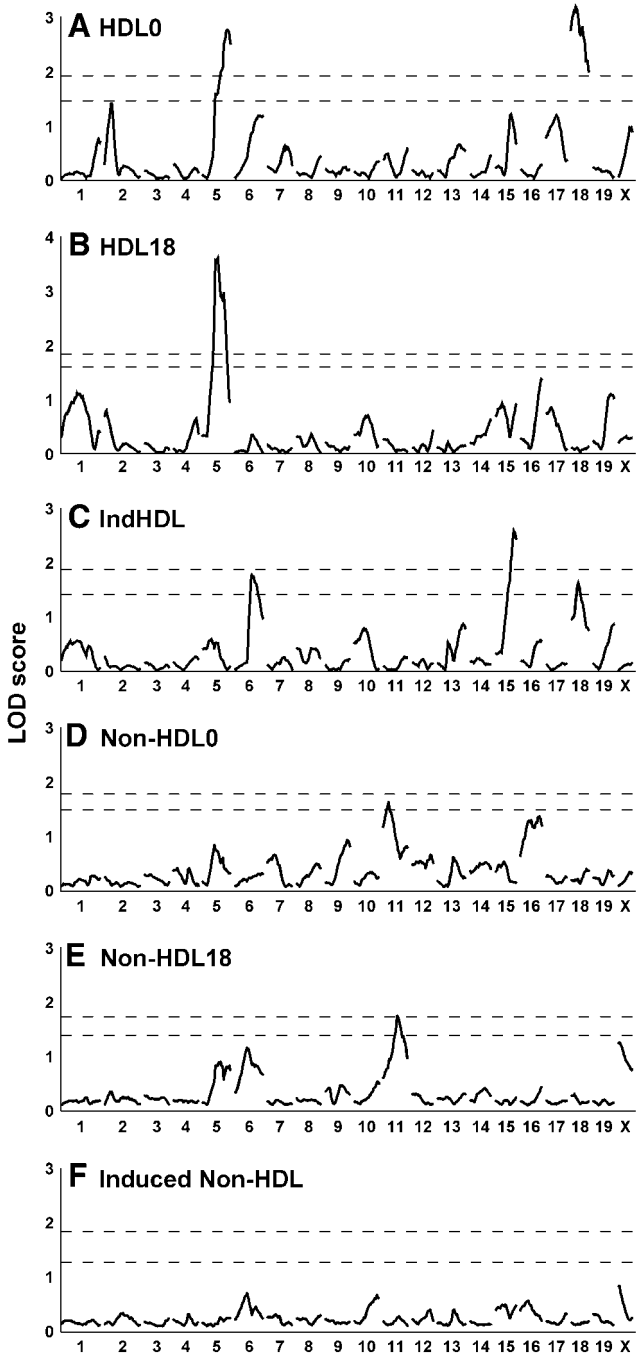

Fig. 3. Genome-wide scans of 0 wk HDL-C (*A*), 18 wk HDL-C (*B*), induced HDL-C (*C*), 0 wk non-HDL-C (*D*), 18 wk non-HDL-C (*E*), induced non-HDL-C (*F*). The *y*-axis indicates logarithm of the odds ratio (LOD) scores; the *x*-axis indicates the positions along the chromosomes. Suggestive ($P < 0.2$) and significant ($P < 0.05$) thresholds are indicated.

Table 4. *QTL analysis*

Trait	Diet	Chr	Position, cM	LOD Score	
HDL-C	chow	5	66	**2.7**	***Hdlq1***
		18	12	**3.2**	***Hdlq3***
HDL-C	high fat	5	42	**3.6**	***Hdlq2***
Induced HDL-C		6	48	1.8	
		15	56	**2.5**	***Hdlq4***
		18	18	1.6	
Non-HDL-C	chow	11	14	1.5	
Non-HDL-C	high fat	11	44	1.5	

LOD, logarithm of the odds ratio; QTL, quantitative trait locus. Significant QTL are in boldface.

Table 5. *Multiple QTL analysis*

Trait	Diet	QTL	*P* Value	Variance Explained
HDL-C	Chow	Chr 5	0.00068	24%
		Chr 18	0.00043	
HDL-C	High fat	Chr 5	0.000004	25%
		Chr 19	0.00170	
Induced HDL-C		Chr 6	0.01305	23%
		Chr 15	0.00457	
		Chr 18	0.02129	
Non-HDL-C	Chow	Chr 11	0.006333	8%
Non-HDL-C	High fat	Chr 11	0.011189	7%

nome scan (LOD = 6.4). Heterozygosity at either locus is associated with a reduction of HDL-C. The locus at *D5Mit239* is highly significant (Table 4) and assigned *Hdlq2*. The fitted model with the QTL on chromosomes 5 and 19 explains 25% of the total variance for HDL-C in high-fat diet fed mice (Table 5).

For inducible HDL-C, expressed as log(HDL-18 wk/HDL-0 wk), the locus near *D15Mit39* is associated with higher induction in heterozygotes (Table 4) and assigned *Hdlq4*. Near loci *D6Mit44* and *D18Mit24* are suggestive QTL. The model including all three loci explains 23% of the variance in this trait (Table 5). By itself, *D15Mit39* explains 12% of the variance. The absence of significant effects associated with loci on chromosome 5 is somewhat surprising and may be the result of undetected interactions with other loci.

Analysis of the 0 wk non-HDL-C concentrations identified suggestive association on chromosome 11. For 18 wk non-HDL-C there is a peak on chromosome 11 that reaches the suggestive level. SM alleles on chromosome 11 are associated with increased levels of non-HDL-C.

We derived a value for inducible non-HDL-C from the ratio of the value at 18 wk on diet to that at 0 wk on diet. No suggestive associations were noted for inducible non-HDL-C. However, very little non-HDL-C is present in the chow-fed mouse and these low concentrations are too close to the background of the assay, so measurement noise obscures the phenotype for inducible non-HDL-C.

The chromosome 5 LOD curves for HDL-C on standard chow and high-fat diets have distinct peaks at 65 and 45 cM, respectively (Fig. 4). Posterior densities for the QTL locations on chromosome 5 are well separated, and the 95% confidence intervals have limited overlap. These observations raise the possibility that there may be two QTL on this chromosome, each having an effect on only one of the two HDL-C measurements. To test the hypothesis of two vs. one QTL, we computed the LOD scores for a multitrait analysis (5, 19) under the assumption of one QTL and two QTL. The difference in LOD scores is converted to a chi-square statistic with two degrees of freedom ($\chi^2_2 = 5.88$, $P = 0.053$). The marginal significance of this test provides modest support for the two-QTL hypothesis. We favor the conclusion that there are two distinct QTL rather than a single QTL with pleiotropic effects. However, further investigation of this region is needed to reach a definitive conclusion.

Srb1 as a possible candidate gene for Hdlq1. Previous genetic analyses have also identified QTL for HDL-C on chromosome 5 (25, 26, 40) and mapped the scavenger receptor, class B, type I, *Srb1*, to distal chromosome 5 near the QTL at *D5Mit370* (54). Since the SR-B1 protein has been shown to be an HDL receptor (1), we considered *Srb1* as a possible candidate gene for this QTL. This is a particularly attractive hypothesis because the HDL-C phenotype in strain NZB resembles the phenotype of the *Srb1* knockout mice (45) in the elevated concentrations and increased size of HDL. To determine whether *Srb1* is a viable candidate gene, we performed Western immunoblot analyses on liver membranes and Northern blot analyses on total liver mRNA from the parental strains fed either the standard chow or the atherogenic diet for 18 wk (Fig. 5; Table 6). Levels of *Srb1* mRNA were not significantly different in NZB than in SM when mice were consuming the chow diet. Since the QTL that mapped close to *Srb1* was observed in chow-fed mice, the failure to observe a significant difference in mRNA in these animals does not support the hypothesis that *Srb1* is the candidate gene. Levels of *Srb1* mRNA were upregulated significantly ($P < 0.002$) in SM but not in NZB after 18 wk on the atherogenic diet. This resulted

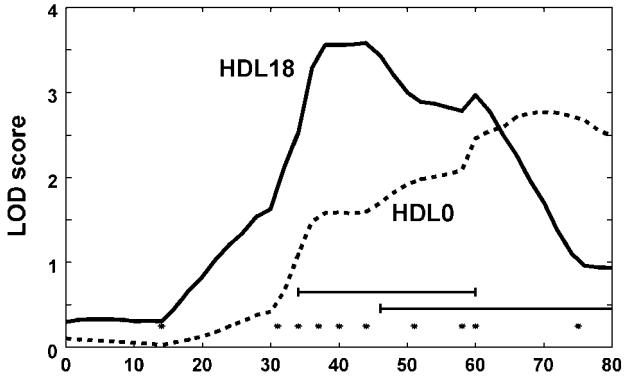

Fig. 4. LOD score plot for two traits on chromosome 5. The difference in profiles for HDL-C concentrations at 0 wk and 18 wk suggest that two distinct chromosome 5 loci exist. The *y*-axis indicates LOD scores; the *x*-axis indicates the positions along the chromosomes, and asterisks represent the markers that where typed. The 90% confidence regions are given for both quantitative trait loci (QTL).

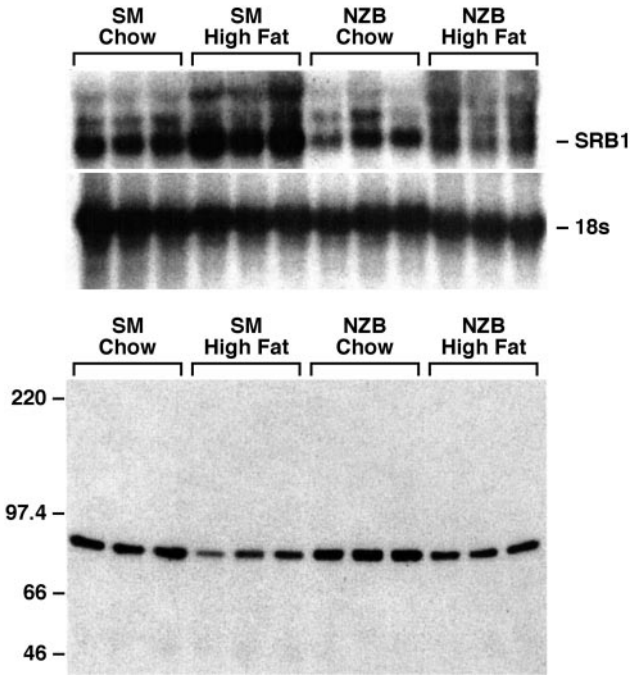

Fig. 5. *Srb1* mRNA and protein in strains SM and NZB fed standard chow or atherogenic diet. *Top*: a Northern blot of *Srb1* mRNA and 18S ribosomal RNA as a control. *Bottom*: a Western blot of SR-B1 protein. Each lane represents an individual mouse.

in a significantly higher level of *Srb1* mRNA in SM compared with NZB mice after 18 wk ($P < 0.001$). Concentrations of SR-B1 protein in the liver were similar in NZB and SM when mice were consuming the standard chow diet, a result that also fails to support the role of *Srb1* as a candidate gene. After 18 wk on the atherogenic diet, SR-BI protein concentrations were significantly ($P < 0.01$) decreased in both strains but to a greater degree in SM than in NZB, resulting in a significantly ($P < 0.03$) lower concentration of liver SR-BI protein in SM than in NZB. These data indicate that diet regulates the concentrations of liver *Srb1* mRNA only in SM and regulates the concentrations of liver SR-BI protein in both SM and NZB.

Although strains SM and NZB do not differ in *Srb1* mRNA or protein concentrations in chow-fed mice, *Srb1* could differ in function between the two strains. Since such a functional difference would require a change in amino acid sequence of the protein, we isolated mRNA from both NZB and SM livers, synthesized and amplified the cDNA in two overlapping fragments, and determined their sequences. The NZB and SM sequences were aligned with the complete cDNA sequence obtained from the 3T3-L1 adipocyte cell line. A single transition from a T to C was found in strain NZB at bp 1535. Although this change does not change the amino acid sequence, it does remove an *Alu*I site.

DISCUSSION

The SM and NZB strains of mice exhibit several interesting differences in HDL-C concentrations, as previously reported (36, 38, 40). NZB mice exhibit higher HDL-C concentrations and larger HDL particle sizes than SM mice, regardless of whether mice are fed a chow or an atherogenic diet. To identify the genetic factors responsible for these differences in the concentrations and particle size of HDL-C, we performed a QTL analysis in an (SM × NZB) × NZB backcross. We identified two loci that significantly affect HDL-C concentrations in chow-fed mice (near *D5Mit370* and *D18Mit34*), one locus that significantly affects HDL-C concentrations in mice fed the atherogenic diet for 18 wk (near *D5Mit239*), three loci that affect HDL-C induction of which one (*D15Mit39*) is highly significant, and suggestive loci associated with non-HDL-C concentrations at 0 and 18 wk (Table 4). Investigation of possible interaction effects among the loci identified in this study did not yield any significant results. In light of the small sample size, we cannot rule out the possibility of gene-by-gene interactions as significant contributors to the genetic variation in these traits.

Some of the loci identified in this study confirm loci identified by QTL analysis in previous work, whereas others are new loci. Previous QTL analyses using an NZB × SM F_2 cross (40), a B6 × C3H F_2 cross (25), and a Cast × B6 F_2 intercross (26) identified QTL on chromosome 5 for HDL-C concentrations. Purcell-Huynh and coworkers (40) identified a coincident QTL for HDL-C concentrations in mice fed the chow diet and in those fed the atherogenic diet within an ~20-cM long region on the distal end of chromosome 5. Machleder and coworkers (25) identified an overlapping QTL for HDL-C concentrations in mice fed an atherogenic diet that spanned a portion of the central region of chromosome 5. None of these analyses detected two distinct loci, one for chow-fed and one for atherogenic diet-fed mice, as we did in the present study. This may be the result of our use of a different method of QTL analysis and our use of a larger number of markers in the analysis. All of the previous studies used methods that are based on single QTL models whereas, we utilized both multitrait and multiple QTL models in our analysis. Our analysis suggested that two distinct QTL might exist. Although not unequivocal, this kind of information can be of value in follow up studies that attempt to confirm the QTL or identify the gene(s) responsible. Interestingly, a QTL for HDL-C

Table 6. *Srb1 mRNA and protein concentrations in liver of NZB and SM mice*

	NZB	SM
Srb1 mRNA		
Chow diet	2,810 ± 587	2,930 ± 566
High-fat diet	3,080 ± 686	6,860 ± 1046*†
Membrane SR-B1 protein		
Chow diet	860 ± 173	740 ± 109
High-fat diet	490 ± 94*	250 ± 75*†

Values are means ± SE, of integrated volume, in optical density units. Female mice were fed either a standard chow diet or an atherogenic diet for 18 wk. Values for mRNA were normalized to 18S ribosomal RNA. For SR-BI protein, 50 μg of protein was loaded per lane. *Significantly different from same strain fed chow diet. †Significantly different from NZB fed atherogenic diet.

has been mapped on human chromosome 4 (q13) (6), which is homologous to the region where we mapped *Hdlq2*.

The QTL for HDL-C at 0 wk on chromosome 18 (near *D18Mit34*) might be the same QTL found previously in a cross between C3H/HeJ and C57BL/6J (*D18Mit124–D18Mit142* at cM 22–32) (25), and may be within the confidence interval of a chromosome 18 QTL found previously in a cross between SM and NZB (*D18Mit7* at cM 50) (40). However, we included marker *D18Mit9* at cM 42, which falls between *D18Mit24* and *D18Mit7*, and did not detect significance there. We also found a new QTL not previously reported at *D19Mit71* for HDL-C at 18 wk. Our QTL for non-HDL-C near *D11Mit44* was previously identified as having an effect on cholesterol metabolism (25), although for different traits, plasma HDL-C, and mRNA levels of cholesterol-7α hydroxylase. The QTL we present on chromosome 6 for inducible HDL-C (*D6Mit44*) is also novel.

Several genes that encode proteins known to regulate HDL-C concentrations map to locations different from the QTL identified in this report; these include: *Lcat* on chromosome 8; hepatic lipase (*Lipc*) on chromosome 9; *Apoa1* on chromosome 9; *Apoa2* on chromosome 1; or *Pltp* on chromosome 2. Therefore, none of these genes can account for the observed differences in HDL-C concentrations between these two strains. In addition, the gene for cholesteryl ester transfer protein (*Cetp*) cannot be considered as a candidate gene for these QTL, as it is present in humans but not in mice.

The QTL for inducible HDL-C on chromosomes 6 and 15 do have interesting candidate genes in the regions. On chromosome 6 is the gene for the peroxisome-proliferator-activated receptor-γ (*Pparg*), a transcription factor that is induced in macrophages by oxidized LDL (42). The consequences of induction are, in part, anti-inflammatory. On chromosome 15 is the gene for the α-subunit of the peroxisome-proliferator-activated receptor (*Ppara*), which stimulates the β-oxidative degradation of fatty acids. *Ppara* is expressed in aortic smooth muscle cells; it has recently been shown that activators of PPARA inhibit the inflammatory response of smooth muscle cells (48). Since oxidized HDL is cleared from plasma faster than native HDL, any transcription factor that could reduce the oxidative and inflammatory response in artery walls could lead to increases in plasma HDL-C concentrations. Further experiments are needed to clarify whether *Ppara* and *Pparg* are promising candidates for the QTL on chromosomes 6 and 15.

However, we did identify one gene for which the mapping and functional evidence was sufficiently strong to merit testing as a candidate gene for our QTL. The murine *Srb1* gene encodes the scavenger receptor, class B, type I (SR-BI), maps to the distal end of chromosome 5 (54), and has been identified as the first known cell-surface HDL-C receptor (1). Murine *Srb1* is expressed most abundantly in liver and steroidogenic tissues (1, 24, 28). Hepatic overexpression of *Srb1* reduces plasma HDL-C and increases cholesterol concentrations in bile (21), indicating that SR-BI is a physiologically significant HDL-C receptor. This key role for SR-BI in HDL-C metabolism was confirmed when investigators created a targeted null mutation in the gene for murine SR-BI. *Srb1*-null mice exhibited concentrations of HDL-C that were dramatically increased in the plasma but decreased in the adrenal gland and other steroidogenic tissues (45). Mice with the null mutation exhibited increased concentrations of large HDL-C particles. These changes in HDL-C concentration and particle size are similar to the HDL we observed in strain NZB and suggested to us that *Srb1* is a strong candidate gene for this locus.

To test *Srb1* as a candidate gene, we carried out experiments to determine whether *Srb1* expression or its coding sequence differs between strains SM and NZB. We tested the expression of *Srb1* protein and mRNA by performing Western analyses on liver membranes and Northern blot analyses on total liver mRNA, respectively. We anticipated that if *Srb1* were the gene encoding the QTL for HDL, then strain NZB would exhibit lower concentrations of *Srb1* mRNA and protein than SM on a standard chow diet. This would explain the higher basal concentrations of plasma HDL-C in NZB compared with SM, as *Srb1* expression is inversely correlated with plasma concentrations of HDL-C. We also expected that when challenged with an atherogenic diet, both strains would exhibit decreased concentrations of *Srb1* mRNA and protein, thereby explaining the inducibility of HDL-C in both strains; induction of *Srb1* expression by the atherogenic diet would reduce plasma concentrations of HDL-C. We expected the decrease in *Srb1* expression in response to the atherogenic diet to be more pronounced in strain NZB than in strain SM. Contrary to our expectations, there was not a significant difference between the basal levels of *Srb1* mRNA in the liver between the SM and NZB strains when mice were fed a chow diet (Fig. 5; Table 6). Consistent with our observations of mRNA levels, there also was no significant difference between the basal concentrations of SR-BI protein in the liver between the SM and NZB strains when mice were fed the chow diet (Table 6). However, the effect of the atherogenic diet on *Srb1* mRNA expression did not mirror its effect on *Srb1* protein expression in either strain. When challenged with the atherogenic diet, liver concentrations of SR-BI protein decreased significantly in both strains. This lack of coordinate regulation of *Srb1* mRNA and protein concentrations in mice fed an atherogenic diet has been observed previously (H. Hobbs, personal communication).

Since *Srb1* expression did not differ between strains NZB and SM when mice were fed the chow diet, the HDL-C phenotype could be explained by a difference in *Srb1* protein activity. Such a functional difference in the protein would be accompanied by a difference in the coding region of *Srb1*. However, sequencing the cDNA for *Srb1* shows that the sequence is identical in strains NZB and SM with the exception of a single nucleotide polymorphism that does not result in an amino acid change. The expression and sequencing

data does not provide persuasive evidence that *Srb1* is a candidate gene for our HDL-C locus.

Similar analyses have been used to test *Srb1* as a candidate gene for a QTL for HDL-C in a cross between CAST/Ei and C57BL/6J; these authors also were not able to find any evidence to support the candidacy of *Srb1* (26). However, the possibility remains that some posttranslational difference in SR-BI occurs between strains NZB and SM. We have not attempted to test that possibility. Recent research has identified biochemical pathways involving SR-BI that could account for such a difference: SR-BI protein and mRNA are under hormonal regulation in mouse steroidogenic tissue (43, 44, 52) and levels of *Srb1* mRNA are altered by mutations in apoA-I and LCAT (29, 52).

These QTL for plasma HDL-C and VLDL-C concentrations identify chromosomal regions containing genes that participate in lipoprotein metabolism. Identifying the genes responsible for these QTL offers the possibility of identifying novel genes affecting lipoprotein metabolism.

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