Influence of sex and diet on quantitative trait loci for HDL cholesterol levels in an SM/J by NZB/BlNJ intercross population

Ron Korstanje,[1,*] Renhua Li,* Timothy Howard,[†] Peter Kelmenson,* Jan Marshall,* Beverly Paigen,* and Gary Churchill*

The Jackson Laboratory,* 600 Main St., Bar Harbor, ME 04609; and Wake Forest University,[†] Winston-Salem, NC

Abstract **To investigate the dependence of HDL quantitative trait loci (QTL) on sex and diet, we generated a large intercross population of mice from parental strains SM/J and NZB/BlNJ. We measured HDL levels in progeny fed a chow diet and measured them again after 6, 12, and 16 weeks of feeding a high-fat, high-cholesterol diet. QTL analysis was performed on the 260 female and 253 male F_2 progeny. A total of 13 significant QTL were found. Four QTL were specific to female mice: *Hdlq23* (Chr 6, 26 cM), *Hdlq26* (Chr 10, 70 cM), *Hdlq27* (Chr 15, 48 cM), and *Hdlq32* (Chr 19, 40 cM). One significant QTL was specific to male mice: *Hdlq29* (Chr 17, 36 cM). In addition, several QTL were found to have effects that were dependent on diet. Sex- and diet-dependent effects were characterized using a linear model-based genome scan method that avoids the potential pitfalls of subdivided data analysis. The dependence of QTL effects on sex suggests an important role for the sex hormones in HDL regulation. We recommend that sex should be explicitly accounted for in future studies in the genetics of HDL regulation in both mice and humans.**—Korstanje, R., R. Li, T. Howard, P. Kelmenson, J. Marshall, B. Paigen, and G. Churchill. **Influence of sex and diet on quantitative trait loci for HDL cholesterol levels in an SM/J by NZB/BlNJ intercross population.** ***J. Lipid Res.*** **2004.** 45: **881–888.**

Supplementary key words diet • high density lipoprotein • mice • sex

In the past decades, much emphasis has been put on elevated LDL levels as a risk factor for atherosclerosis. Drugs that control LDL levels effectively reduce disease risk (1). More recently, researchers are investigating the possibility of reducing atherosclerosis risk by controlling HDL cholesterol levels (2, 3). HDL has a protective effect, and a growing body of evidence suggests that increasing its levels through therapeutic intervention can reduce atherosclerosis risk (4). In order to enable the development of drugs that increase HDL levels, a better understanding is needed of the mechanism of HDL regulation and the genes that are involved. Several genes involved in HDL metabolism have been identified (e.g., *Apoa2*, *Abca1*) and have been shown to influence HDL levels when mutated. However, the regulation of HDL levels is complex and additional (unknown) genes are involved.

One approach to identify genes involved in HDL regulation is using quantitative trait locus (QTL) analysis in animal models. By crossing inbred strains that significantly differ in HDL levels, and subsequently testing for association between HDL levels and genetic markers in the progeny, many QTL involved in HDL levels have been identified in human, mouse, rat, and rabbit (5–13). A recent review summarized the QTL for HDL that have been found in mouse and human (5). At least 12 murine QTL are located in regions homologous to human QTL, suggesting that the underlying candidate genes may be the same. Several QTL found in mice are found repeatedly in different crosses, suggesting that a limited set of important genes control HDL levels in the mouse.

Despite the many studies involving HDL QTL in mice, not much attention has been paid to the differences that we observe between males and females and between different diets. Mouse inbred strains NZB/BlNJ (NZB) and SM/J (SM) differ in their HDL levels [3.63 and 1.42 mmol/l in females and 4.33 and 1.42 mmol/l in males, respectively, according to the Mouse Phenome Database (14)]. Previously, an intercross between these two strains identified a QTL on Chr 1 (92.3 cM) and two QTL, one each on Chr 5 at 62 cM and 72 cM (11). Another study using the same two strains, but in a backcross to NZB, con-

Manuscript received 3 November 2003 and in revised form 23 December 2003.

Published, JLR Papers in Press, March 1, 2004.
DOI 10.1194/jlr.M300460-JLR200

Abbreviations: apoB, apolipoprotein B; ER, estrogen receptors; QTL, quantitative trait locus; LDLR, LDL receptor; LOD, logarithm of the odds; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor class B type I; VLDLR, VLDL receptor.

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

firmed the loci on Chr 5 (15). The current study, which also uses the NZB and SM inbred strains, differs from previous QTL studies in several ways. First, the cross is considerably larger than in previous studies. Second, HDL was measured prior to and at different time points following the introduction of an atherogenic diet. Lastly, we included both sexes in this study and explicitly tested for dependence of QTL effects on sex. The study confirms the QTL on Chr 1 and Chr 5 found previously between the two inbred strains and reports new QTL on chromosomes 3, 6, 8, 10, 15, 16, 17, 18, and 19. More importantly, this study explicitly characterizes the interaction of QTL with sex.

MATERIALS AND METHODS

Mice and diets

SM/J and NZB/BlNJ inbred strains were obtained from The Jackson Laboratory (Bar Harbor, ME). SM females were mated to NZB males to produce the F_1 progeny, which were then intercrossed to produce 513 F_2 progeny (260 females and 253 males). Mice were housed in a climate-controlled facility with a 14 h:10 h light-dark cycle. After weaning, mice were maintained on a chow diet (Old Guilford 234A, Guilford, CT) and offered free access to food and water throughout the experiment. Mice were fed the chow diet until 8 weeks of age and then fed an atherogenic diet for 16 weeks containing (w/w) 15% dairy fat, 50% sucrose, 20% casein, 0.5% cholic acid, and 1.0% cholesterol, as well as cellulose, vitamins, and minerals. The source of chemicals and the diet have been described previously (16, 17). The Jackson Laboratory's Animal Care and Use Committee approved all experiments.

Lipid measurements

At 0, 6, 12, and 16 weeks of diet consumption, mice were fasted for 4 h, blood was collected by retroorbital bleeding into EDTA-coated tubes, and plasma was separated by centrifugation at 1,500 rpm for 5 min at 4°C. Plasma total cholesterol concentrations were measured by commercial colorimetric enzymatic assay as described previously (18). HDL was measured after selective precipitation of apolipoprotein B (apoB)-containing lipoproteins with polyethylene glycol (19). The results are expressed as mean ± SE in mmol/l.

DNA isolation and genotyping

DNA was isolated from a centimeter of tail digested overnight in 500 μl of 1× digestion buffer (50 mM Tris-Cl pH 8.0, 100 mM EDTA pH 8.0, 100 mM NaCl, 1% SDS, 1 mg/ml proteinase K) in a 55°C waterbath. Digests were mixed with 1 vol 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 vol of 100% ethanol to the isolated aqueous phase. 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. A list of markers used for this cross is available as an online supplement. Polymorphisms were detected either by capillary electrophoresis or by standard electrophoresis using 4% agarose (Nusieve) gels.

Statistical analyses

Genome scans. We performed genomewide scans and multiple QTL modeling using the method of Sen and Churchill (20). For simple genome scans, this approach is similar to the interval mapping procedure of Lander and Botstein (21) but is based on an imputation algorithm. An advantage of the imputation approach is that it can be generalized to incorporate covariates, such as sex, as well as include multiple QTL in genome scan analyses. Logarithm of the odds (LOD) ratio scores for genome scans were computed at 2 cM intervals across the genome, and significance was determined by permutation testing (22). Significant and suggestive QTL meet or exceed the 95% and 90% genomewide thresholds, respectively. We ran multiple scans on each phenotype, and the usual suggestive threshold [37%, as reviewed in ref. (23)] was deemed too liberal. The software package used in this study was PSEUDOMARKER release version 1.0 (24).

We carried out single-locus genome scans to detect QTL with main effects. These scans included sex as an additive covariate to account for overall differences in HDL levels between the sexes. We then carried out a second set of single-locus scans that included an interaction between sex and the putative QTL at each locus. These scans allow the QTL effect to differ between the two sexes and can be used to identify sex-specific QTL. The difference in LOD scores between these two scans constitutes a test for sex by QTL interaction. This secondary test is carried out with no adjustment for multiple testing, and the threshold, based on the usual chi-square distribution of the likelihood ratio, is 2.0 on the LOD scale. Lastly, we subdivided the population by sex and scanned male and female data separately for the main effect QTL.

We carried out simultaneous genome scans of all pairs of markers in the search for epistatic interactions. The search strategy is described by Sen and Churchill (20) and Sugiyama et al. (25). We failed to detect any significant QTL by QTL interactions using stringent genomewide adjusted criteria.

For some of the QTL detected in the single-locus genome scans, the shape of the LOD curve suggested that there might be two or more QTL present. In order to resolve multiple-linked QTL, we carried out multi-locus scans on a per chromosome basis. The change in the LOD score between one QTL and two QTL models was used as a test statistic. In one case, we also tested two QTL versus three QTL models. Thresholds for these chromosome-wide searches were established by running simulations.

TABLE 1. Comparison of plasma HDL cholesterol concentrations in NZB, SM, reciprocal F_1, and F_2 progeny

	0 Weeks		6 Weeks		12 Weeks		16 Weeks	
	F	M	F	M	F	M	F	M
NZB (n = 20)	2.47 ± 0.06	2.81 ± 0.09	3.96 ± 0.17	3.97 ± 0.16	4.46 ± 0.19	4.64 ± 0.11	3.47 ± 0.34	3.39 ± 0.30
SM (n = 20)	1.28 ± 0.05	1.73 ± 0.07	1.68 ± 0.15	1.62 ± 0.14	1.49 ± 0.07	1.73 ± 0.08	1.75 ± 0.14	1.80 ± 0.10
SM×NZB (n = 15)	1.47 ± 0.14	1.85 ± 0.16	1.51 ± 0.11	2.06 ± 0.16	1.39 ± 0.13	2.62 ± 0.28	1.65 ± 0.15	2.24 ± 0.30
NZB×SM (n = 15)	1.93 ± 0.07	2.70 ± 0.13	2.58 ± 0.12	3.16 ± 0.18	2.66 ± 0.07	4.51 ± 0.31	1.86 ± 0.15	2.82 ± 0.42
(SM×NZB) F_2 F (n = 260); M (n = 253)	1.87 ± 0.04	2.59 ± 0.04	2.73 ± 0.06	3.53 ± 0.07	2.73 ± 0.07	3.46 ± 0.07	2.74 ± 0.07	3.43 ± 0.08

HDL levels (mmol/l) were measured when fed chow (0 weeks) and atherogenic diet at different time points (6, 12, and 16 weeks).

Multiple regression. We selected all significant and suggestive loci and interactions with sex for incorporation into multiple regression analysis and followed a backward elimination strategy to retain only individually significant terms ($P < 0.01$). The percent of variance explained for each QTL is based on the adjusted (type III) sums of squares.

RESULTS

HDL levels in the parental, F_1, and F_2 mice

HDL levels for the parental, reciprocal F_1, and F_2 mice are summarized in **Table 1**. HDL levels in males were consistently higher than in females. Also, HDL levels were significantly different ($P < 0.05$) between the two reciprocal F_1 populations: the HDL levels of SM×NZB F_1 progeny were comparable to those of SM mice at every time point. In contrast, HDL levels of the reciprocal NZB×SM F_1 progeny were significantly higher ($P = 0.003$) than in SM×NZB F_1 progeny (**Fig. 1A** and 1B). In NZB×SM F_1 female mice, HDL levels were intermediate between the two parental strains; in male mice they were similar to NZB. Similar to the NZB×SM F_1 mice, the F_2 population (derived from SM×NZB F_1 mice) had high HDL levels after consuming an atherogenic diet. The distributions of the HDL levels in the F_2 population are shown in **Fig. 2A** and 2B.

The F_2 population was derived from one direction of crossing SM×NZB (except for 8 males and 22 females that were obtained from one breeding pair of NZB×SM F_1 mice by mistake). Thus, it is not possible to investigate genetic effects that may be associated with the different F_1 populations. In all the populations examined, the HDL levels fell at 16 weeks; this was probably due to the occurrence of fat accumulation in the liver in response to the diet (17), leading to liver damage. These observations made us decide to perform QTL analysis for only 0, 6, and 12 weeks.

QTL analysis

All 513 SM×NZB F_2 progeny were genotyped for 157 markers covering the whole mouse genome, and HDL levels were measured at 0, 6, and 12 weeks feeding of the atherogenic diet. We did not observe a significant difference between the results from analyses of the data at 6 and 12 weeks and, therefore, only show the genomewide scans for 0 and 6 weeks analyses in **Fig. 3**. The significant QTL are summarized in **Table 2**. We have named any QTL that were significant in our cross; some of them were found previously but not named. If a QTL has been found previously and named, the Mouse Nomenclature Committee has asked us to give it a new name if the strains are dif-

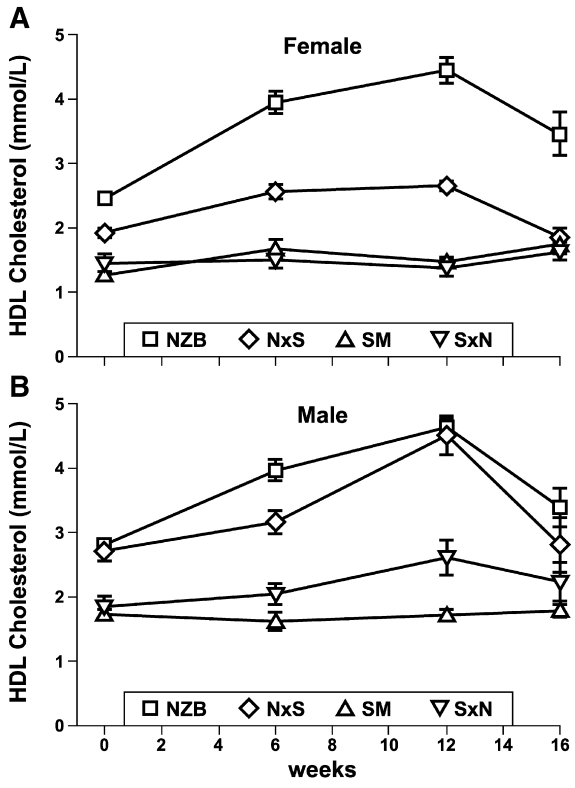

Fig. 1. HDL levels of the parental and reciprocal F_1 animals. Both in females (A) and males (B), SM and SM×NZB F_1 mice did not show a significant increase in HDL levels during the atherogenic diet. In contrast, NZB, NZB×SM F_1 animals showed an increase in the first 6 weeks. At 16 weeks, some groups showed a decrease in HDL levels. This is probably caused by liver damage due to the atherogenic diet.

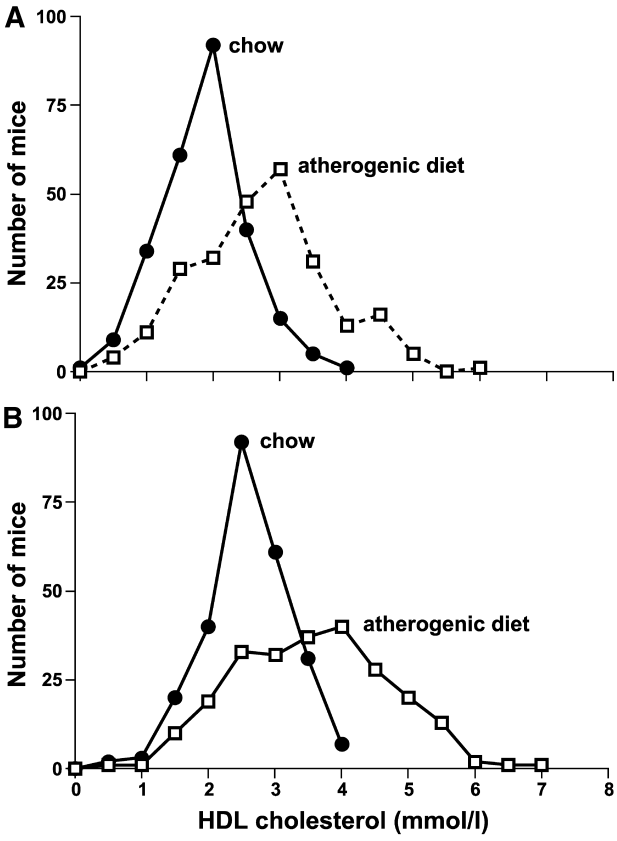

Fig. 2. Frequency distributions of the F_2 animals. The distribution of the levels of F_2 females (A) and F_2 males (B) on chow diet has a narrower range (solid line) than the distribution of the levels after 6 weeks on the atherogenic diet (dotted line). There was no significant difference among the distributions of HDL at 6 weeks and 12 weeks of the atherogenic diet (data not shown). HDL levels are expressed as mean ± SE in mmol/l.

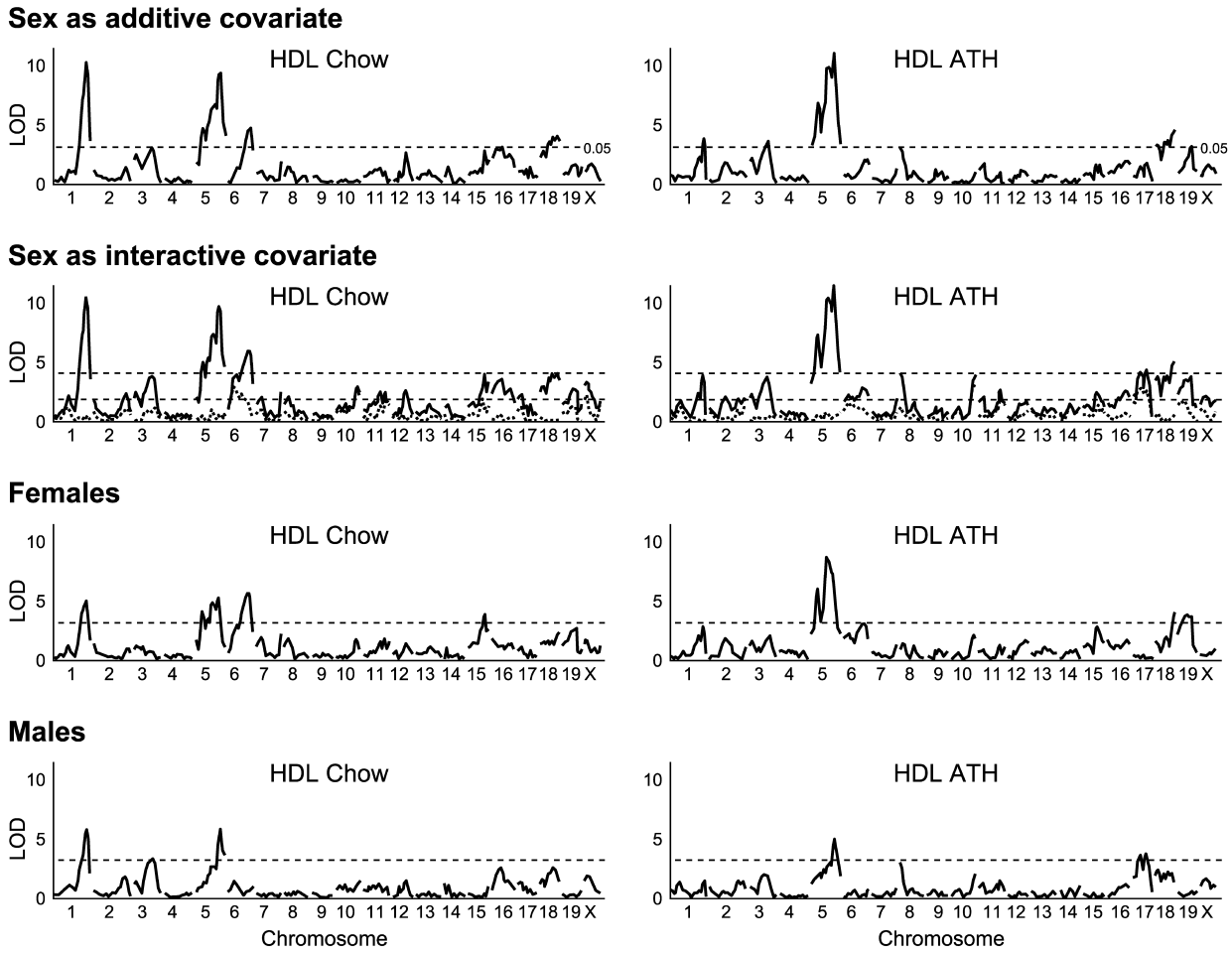

Fig. 3. Genomewide scans for HDL levels on chow and 6 weeks of atherogenic (ATH) diet. Analyses were performed with sex as an additive covariate, sex as an interactive covariate, and with females and males analyzed separately. The line represents the significant ($P = 0.05$) level as determined by permutation tests using 1,000 permutations.

ferent. Thus, we named the QTL on Chr 1 *Hdlq20*, even though it appears to be at the same location as *Hdlq5* found in a different cross (26).

QTL on Chr 1, 6, 15, and 16 were reduced on the atherogenic diet while QTL on Chr 8, 10, and 17 were specific to the atherogenic diet. In all cases, the NZB allele was responsible for the higher HDL levels. Most loci showed additive inheritance except for *Hdlq20*, *Hdlq25*, and *Hdlq28*, where the NZB allele was dominant, and *Hdlq26*, *Hdlq27*, and *Hdlq32*, where the NZB allele was recessive. The allele effects of the sex-specific QTL are shown in **Fig. 4**.

We compared models with one, two, and three QTL on chromosome 5. Maximum LOD scores were established by simultaneous search. **Table 3** shows there is evidence of at least two QTL, both on chow and on the atherogenic diet. We did not find statistical evidence for three QTL. However, in light of low power used to detect linked QTL effects in coupling, we cannot rule out the possibility of three distinct loci on this chromosome. Testing for multiple QTL on Chr 6, we compared models with one versus two QTL. In this case, we allowed the QTL to have sex interaction. We found evidence (ΔLOD = 2.54) for two QTL on the chow diet (Table 3), with one QTL interacting with sex (**Table 4**). Evidence was also found for two QTL (both interacting with sex) on Chr 17 on the atherogenic diet. Furthermore, the tests suggest two QTL each for Chr 16 and Chr 19 on chow and for Chr 18 on the atherogenic diet.

In the multiple regression models for the chow and atherogenic diet, we used a different criterion for the significance of QTL. In a genome scan, the evidence for a QTL is based on its *marginal* effect, taking no account of other QTL. Multiple regression models allow us to assess a QTL effect while accounting for all the other QTL. We constructed a large model with all suggestive QTL (and interactions with sex), then eliminated those that did not achieve a $P < 0.01$ level of significance based on the multiple regression F test. Table 4 shows both models. The two models only shared *Hdlq20* on chromosome 1 and the QTL cluster on chromosome 5. In addition to these QTL, the model for the chow diet contained QTL found on chow only, and the model for the atherogenic diet contained QTL found on the atherogenic diet only. Interestingly, a significant component in the model for the chow diet was the *Hdlq2*×sex interaction, which was not detected as significant in the

TABLE 2. Significant QTL for HDL cholesterol levels identified in the SM×NZB intercross

QTL[a]	Chr	LOD[b] Chow	LOD[b] Ath	Peak (cM) (95% CI)	Sex Specificity (Δ LOD)[c]	Nearest Marker	High Allele	Mode of Inheritance[d]	Coincident QTL	
									Name	Reference
Hdlq20	1	11.0	4.1	96 (94–103)		*D1Mit291*	NZB	Dominant	*Hdlq5*	(26)
Hdlq21	3	4.0	3.8	56 (34–60)		*D3Mit11*	NZB	Additive	Not assigned	(9, 13)
Hdlq22	5	5.2	7.6	18		*D5Mit228*	NZB	Additive		
Hdlq2	5	7.6	10.8	50		*D5Mit205*	NZB	Additive	*Hdlq2*	(9, 15)
Hdlq1	5	10.1	12.1	66 (12–85)[e]		*D5Mit161*	NZB	Additive	*Hdlq1*	(10, 11, 15)
Hdlq23	6	4.2	—	26 (12–32)	Female (3.1)	*D6Mit74*	NZB	Additive		
Hdlq24	6	6.3	—	66 (54–70)		*D6Mit259*	NZB	Additive	*Hdlq11,Hdlq12*	(42)
Hdlq25	8	—	4.2	0 (0–12)		*D8Mit58*	NZB	Dominant		
Hdlq26	10	—	4.1	70 (60–70)	Female (3.2)	*D10Mit271*	NZB	Recessive		
Hdlq27	15	4.2	—	48 (44–60)	Female (2.0)	*D15Mit70*	NZB	Recessive	Not assigned	(10)
Hdlq28	16	3.7	—	26 (0–60)		*D16Mit57*	NZB	Dominant	Not assigned	(10)
Hdlq29	17	—	4.5	36 (30–42)	Male (2.9)	*D17Mit20*	NZB	Additive	Not assigned	(10)
Hdlq30	18	4.2	—	48		*D18Mit9*	NZB	Additive		
Hdlq31	18	4.1	5.2	56 (42–60)[e]		*D18Mit4*	NZB	Additive	Not assigned	(10)
Hdlq32	19	—	4.0	26 (10–70)	Female (2.0)	*D19Mit11*	NZB	Recessive	Not assigned	(8)

Ath, atherogenic diet; LOD, logarithm of the odds; QTL, quantitative trait locus.

[a] If a QTL has been found previously and named, the Mouse Nomenclature Committee has asked us to give it a new name if the strains are different. Thus, we named the QTL on Chr 1 *Hdlq18*, even though it appears to be at the same location as *Hdlq5* found in a different cross.

[b] LOD scores of the analysis with sex as an interactive covariate are given.

[c] The difference in the LOD score when adding sex×QTL as an interaction term.

[d] Mode of inheritance of the high allele.

[e] Overlapping QTL did not allow the authors to determine the interval for the individual QTL. The interval for the overlapping QTL is shown.

single-locus scans. The allele effect plot for *Hdlq21* (Fig. 4A) shows a stronger effect in males than in females, but it is not as profound as in the other sex-specific QTL.

DISCUSSION

The results of our study and of previous ones (9, 10, 27) lead to several conclusions. First, an atherogenic diet does not have the same effect on HDL levels in all mouse strains. There appear to be three different groups of strains: *1)* those whose HDL levels decrease in response to an atherogenic diet (e.g., C57BL/6J, CAST/Ei), *2)* those whose HDL levels do not change in response to an atherogenic diet (e.g., SM/J, BALB/cJ, C3H/HeJ), and *3)* those whose HDL levels increase in response to an atherogenic diet (e.g., PERA/Ei, AKR/J, NZB/BlNJ). This suggests that many polymorphic genes regulate HDL levels, and that unique combinations of these polymorphisms in the various inbred mouse strains determine HDL concentrations.

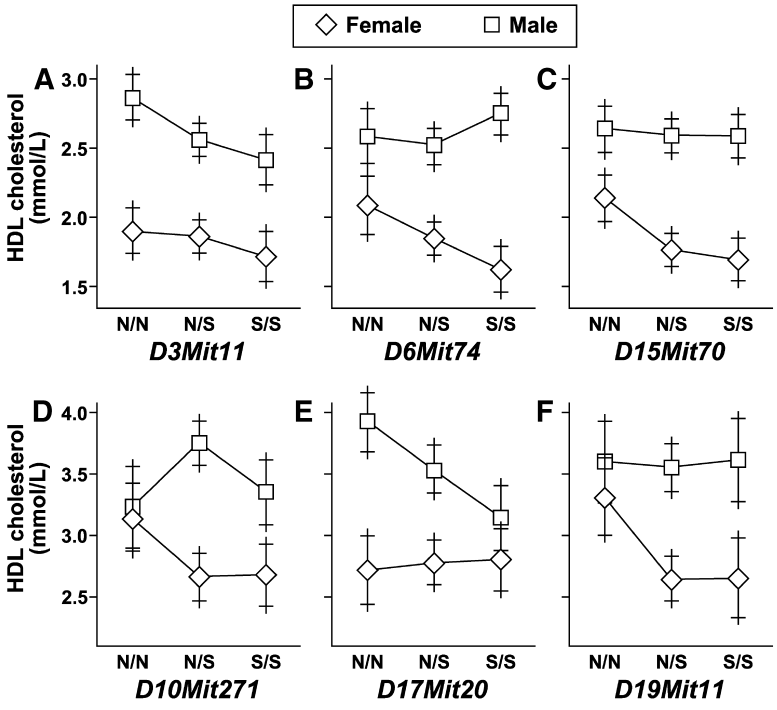

Fig. 4. Allele effects for the sex-specific quantitative trait loci *Hdlq21* (A), *Hdlq23* (B), and *Hdlq27* (C) found on the chow diet, and *Hdlq26* (D), *Hdlq29* (E), and *Hdlq32* (F) found on the atherogenic diet. Homozygous NZB alleles are represented by N/N, homozygous SM alleles by S/S, and heterozygous alleles by N/S. HDL levels are expressed as mean ± SE in mmol/l.

TABLE 3. Evidence for multiple-linked QTL

Chr	Δ LOD (Chow)	Δ LOD (Atherogenic)
5 (1 vs. 2 QTL)	2.59	4.13
5 (2 vs. 3 QTL)	0.89 (ns)	0.79 (ns)
6[a]	2.54	1.66 (ns)
16	2.19	0.64 (ns)
17[b]	1.28 (ns)	3.68
18	1.98	2.91
19	2.29	1.71 (ns)

ns, not significant. Multi-locus scans were carried out on a per chromosome basis. The change in the LOD score between one QTL and two QTL models was used as a test statistic. Thresholds for these tests (ΔLOD = 2.0) were established by running simulations.

[a] One QTL interacting with sex (threshold = 2.0).

[b] Both QTL interacting with sex (threshold = 3.2).

TABLE 4. Multiple regression models for loci involved in HDL cholesterol levels on chow and atherogenic diet

	DF	% Variance	Female	*P*
Sex	5	24.5	48.07	0
Hdlq20	2	5.3	26.29	1.45×10^{-11}
Hdlq27	2	1.6	7.84	0.000444
Hdlq28	2	1.2	5.88	0.003011
Hdlq30	2	1.6	7.78	0.000475
Hdlq21×sex	4	1.6	4.03	0.003160
Hdlq1, 2, and *22*	6	5.5	9.02	2.30×10^{-9}
Hdlq23×sex	4	1.7	4.23	0.002245
Hdlq24	2	2.2	10.74	2.74×10^{-5}
Total		43.6		
Sex	3	11.4	27.35	2.22×10^{-16}
Hdlq20	2	2.1	7.62	0.000556
Hdlq29×sex	4	3.6	6.49	4.35×10^{-5}
Hdlq31	2	2.1	7.39	0.000690
Hdlq32	2	1.3	4.78	0.008790
Hdlq1, 2, and 22	6	8.9	10.63	4.40×10^{-11}
Hdlq25	2	2.4	8.80	0.000178
Total		31.8		

DF, degrees of freedom. The percent of variance explained for each QTL is based on adjusted (type III) sums of squares. The chromosome 5 QTL (*Hdlq1*, *2*, and *22*) were retained as a block and reported as a unit because of positive correlation.

Second, the results from this study imply that it is important to differentiate between the two sexes in QTL analysis because four QTL were found to have effects only in female mice, and one has effects only in male mice. The phenomenon of differences between sexes is not restricted to HDL (e.g., atherosclerosis, gallstones, albuminuria), and it is not restricted to phenotypes that show an overall difference between the two sexes. To identify sex-specific QTL, it is not sufficient to carry out separate analyses of males and females. This approach can be misleading, as the subdivided populations will be much smaller than the combined population, and this increases the likelihood that real QTL effects would fail to be detected in one or both subsets of the data. Analysis of the entire population with and without a sex-by-QTL interaction provides a more appropriate basis for interpretation. Failure to detect a significant interaction suggests a common QTL, the null hypothesis, and a sex-specific QTL is declared only when there is substantial evidence to support the claim. The shortcoming of the subdivided analysis approach is highlighted by our analysis of *Hdlq26* on chromosome 10, where the individual scans do not detect a QTL, but the analysis with sex as an interactive covariate does. This strategy also applies for humans, where QTL analysis is commonly carried out on mixed-sex populations.

It would be interesting to examine the QTL in female mice that do not have estradiol. Will the QTL that are present only in females disappear? Will QTL that are normally present only in males now appear in females as well? A recently developed method for chemically-induced menopause in mice by follicle depletion using vinylcyclohexene diepoxide (28) might be a good way to address these questions.

A strong gender effect can be seen not only in the F_2 population, but also in the analysis of the reciprocal F_1 populations. In females, the SM×NZB F_1 animals resemble the SM parents, while the reciprocal NZB×SM F_1 animals are intermediate between SM and NZB. In males, the SM×NZB F_1 animals also resemble the SM parents (although HDL levels are significantly higher), and the reciprocal NZB×SM F_1 animals resemble the NZB parents. These observations do seem to indicate that an interaction between the X-chromosome (animals with the X-chromosome from SM have lower HDL levels) and the mother (animals with an SM mother have lower HDL levels) may be acting in crosses between the strains SM and NZB. This nurture effect has been observed in NZO mice, which are closely related to NZB (29).

Third, the most important changes happen in the first weeks on the high fat diet. We observed different QTL between chow and 6 weeks of high-fat diet, which suggests that different genes are regulating HDL, but after 6 weeks, the HDL levels remain relatively stable.

Fourth, male mice have significantly higher HDL levels than female mice. In contrast, premenopausal human females have higher HDL levels and reduced cardiovascular disease compared with men (30). Several studies have shown that the relatively higher estrogen levels in women cause this difference. Estrogen may protect women against atherosclerosis by increasing HDL levels and stimulating reverse cholesterol transport. One way is through the binding of the estrogen receptors (ER), as homo- or heterodimers, to an estrogen response element found in the promoter of estrogen sensitive genes. Another way is through the binding of the ER to other transcription factors, resulting in enhancement or repression of transcription (31). In the human male population, higher levels of testosterone are associated with higher HDL levels (32), and it has been shown that testosterone treatment causes female mice to have HDL levels that resemble that of the male (27).

Most QTL that we describe have been reported previously; only five out of the 15 QTL found in this study are novel, while two of the novel QTL (*Hdlq26* and *Hdlq28*) show overlap with the QTL recently found for phospholipid transfer protein (PLTP) activity in the same cross (33). PLTP is known to be involved in HDL metabolism, and it is possible that genes involved in PLTP activity are

indirectly involved in HDL levels. These findings confirm our earlier statement that a limited set of genes control HDL levels in the mouse (5). Of special interest are the QTL found on Chr 1, 5, 15, and 16, which are located in regions homologous to human QTL for HDL on chromosomes 1q21-25, 13q12, 8q24, and 3q26, respectively (5). The identification of the underlying genes may provide therapeutic targets to increase HDL levels in humans. The gene underlying the Chr 1 QTL (*Hdlq20*) is most likely *Apoa2* [Wang and Paigen (5), submitted] and is caused by an amino acid difference at position 61 in the protein (valine in NZB, alanine in SM). The QTL on Chr 3 (*Hdlq21*) overlaps with QTL found in previous crosses (10, 13) and a QTL for PLTP activity in this cross (33). We have tested the gene encoding the apoA-I binding protein (*Apoa1bp*) as a candidate for the PLTP-activity QTL by sequence comparison and expression analysis in the liver but found no evidence to support the candidacy of the gene (33). The gene encoding the LDL receptor (LDLR)-related protein associated protein 1 (*Lrpap1*) is a good candidate for *Hdlq22*, the most proximal QTL (at 18 cM) on Chr 5. An overexpression of this gene in the liver is associated with decreased apoA-I and HDL levels (34). The gene encoding the HDL receptor (HDL-R) scavenger receptor class B type I (SR-BI) has been suggested as a candidate gene for *Hdlq1*, the distal QTL (at 70 cM) on Chr 5 (10, 15). However, both studies found no significant differences in *Scarb1* (formerly *Srb1*) expression, in sequence variation between CAST and B6, or between SM and NZB mice. *Tcf1*, the gene encoding the transcription factor HNF1A, also maps within the confidence interval of the QTL, and it has been demonstrated that HNF1A is an essential transcriptional regulator of HDL metabolism (35). *Hdlq27* on Chr 15 overlaps with *Pltpq4*, a QTL for PLTP activity. *Ppara* expression in the liver was shown to be significantly higher (~4-fold) in male SM mice compared with male NZB mice fed a chow diet (33). The apoD gene (*Apod*) is located close to the peak of *Hdlq28* on Chr 16. Polymorphism in this gene has been associated with HDL levels in the African population (36). A good candidate gene for *Hdlq31* is the gene encoding endothelial lipase (*Lipg*). Overexpression of endothelial lipase in mice reduces the plasma concentration of HDL (37). Of more interest in this study are the sex-specific QTL. In considering candidate genes for these QTL, we have to keep in mind that these are probably genes that are either regulated at the hormone level or act in combination with sex-specific factors. The gene for apoF (*Apof*) is located near the peak of *Hdlq26* on Chr 10. This gene has also been suggested as a candidate for *Pltpq2*, a QTL for PLTP activity. Interestingly, *Hdlq26* has only been found in females, while *Pltpq2* has been observed in males. Sequence comparison of *Apof* between SM and NZB shows a basepair change leading to an amino acid difference in the protein (33). However, it is difficult to find a sex-specific link with this amino acid difference. *Hdlq23*, which was specific for females on the chow diet, was mapped to Chr 6. Searching the MGI and Ensembl databases did not lead to any obvious candidate genes.

The QTL on Chr 17 (*Hdlq29*) overlaps with an unnamed QTL found between CAST/Ei and C57BL/6J (10). *Abcg5/Abcg8* have been suggested as candidate genes for this QTL (10).

The locus on Chr 19 (*Hdlq32*) has been reported previously in a cross between MRL/*lpr* and BALB/cJ (8). Also, a QTL for cholesterol absorption (*Chab5*) has been mapped to this region (38). The first report (8) suggests that *Fas* underlies this QTL. MRL/*lpr* has a defective *Fas* gene, which leads to a breakdown of the central and/or peripheral tolerance, resulting in the failure to properly clear CD4/CD8 negative cells. The *Fas* gene is identified as the major defect underlying the autoimmune manifestation, especially autoantibody levels, which strongly correlated with HDL cholesterol levels (8). Toda, Wickham, and Sullivan (37) have shown gender differences in *Fas* expression and the alteration of *Fas* expression by testosterone treatment in MRL/*lpr* mice. The gene encoding the VLDL receptor (*Vldlr*) is also located in this region.

In a human population (Alberta Hutteries), genetic variation of *VLDLR* is associated with variation in HDL cholesterol levels (40). However, *Vldlr*$^{-/-}$ mice have normal plasma lipoprotein levels (41).

In summary, we have identified 15 significant QTL involved in HDL cholesterol levels. Most of these loci have been found in crosses using other mouse inbred strains and in homologous human regions. There are currently 64 HDL QTL mapped in the mouse using 16 different crosses. Most QTL have been found multiple times, resulting in 23 confirmed locations and four locations found only once. Recurrence of the same QTL in different crosses suggests a limited number of key genes involved in the phenotype. Furthermore, concordance between species shows that 22 QTL found in the mouse are also found at the homologous regions in human, and one QTL is concordant between mouse, rat, and rabbit (5). The combining of all this data provides an excellent basis for gene identification.

After applying statistical analyses that investigate sex dependence of these QTL, we found five QTL to be sex-specific (four specific for female and one for male). A likely explanation is the influence of sex hormones (estrogen and testosterone) on the regulation of the genes that underlie these QTL. Our findings highlight the importance of taking gender into account in the analysis of HDL QTL in both animal models and human studies.

The authors thank Tina Snow, Colleen Bradstreet, Eric Taylor, Susan Sheehan, and David Higgins for their technical assistance. This work was funded by The Program for Genomic Applications (Grant HLB-66611) from the Heart, Lung, Blood and Sleep Disorders Institute (National Institutes of Health).

REFERENCES

1. Shepard, J., S. M. Cobbe, and I. Ford. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N. Engl. J. Med.* **333:** 1301–1307.

2. Goldburt, U., E. Holtzman, and H. N. Neufel. 1985. Total and high density lipoprotein cholesterol in the serum and risk of mortality: evidence of a threshold effect. *BMJ.* **290:** 1239–1243.
3. Castelli, W. P., R. J. Garrison, and P. W. Wilson. 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels: Framingham Study. *JAMA.* **256:** 2835–2838.
4. Rubins, H. B., S. J. Robins, and D. Collins. 1999. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veteran Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N. Engl. J. Med.* **341:** 410–418.
5. Wang, X., and B. Paigen. 2002. Quantitative trait loci and candidate genes regulating HDL cholesterol: a murine chromosome map. *Arterioscler. Thromb. Vasc. Biol.* **22:** 1390–1401.
6. Bottger, A., H. A. van Lith, V. Kren, D. Krenova, V. Bila, J. Vorlicek, V. Zidek, A. Musilova, M. Zdobinska, J. Wang, L. F. M. van Zutphen, T. W. Kurtz, and M. Pravenec. 1996. Quantitative trait loci influencing cholesterol and phospholipid phenotypes map to chromosomes that contain genes regulating blood pressure in the spontaneously hypertensive rat. *J. Clin. Invest.* **98:** 856–862.
7. van Haeringen, W. A., M. den Bieman, G. F. Gillissen, A. Lankhorst, M. T. R. Kuiper, L. F. M. van Zutphen, and H. A. van Lith. 2001. Mapping of a QTL for serum HDL cholesterol in the rabbit using AFLP technology. *J. Hered.* **92:** 322–326.
8. Gu, L., M. W. Johnson, and A. J. Lusis. 1999. Quantitative trait locus analysis of plasma lipoprotein levels in an autoimmune mouse model. *Arterioscler. Thromb. Vasc. Biol.* **19:** 442–453.
9. Machleder, D., B. Ivandic, C. Welch, L. Castellani, K. Reue, and A. J. Lusis. 1997. Complex genetic control of HDL levels in mice in response to an atherogenic diet. *J. Clin. Invest.* **99:** 1406–1419.
10. Mehrabian, M., L. W. Castellani, P. Z. Wen, J. Wong, T. Rithporn, S. Y. Hama, G. P. Hough, D. Johnson, J. J. Albers, G. A. Mottino, J. S. Frank, M. Navab, A. M. Fogelman, and A. J. Lusis. 2000. Genetic control of HDL levels and composition in an interspecific mouse cross (CAST/Ei x C57BL/6J). *J. Lipid Res.* **41:** 1936–1946.
11. Purcell-Huynh, D. A., A. Weinreb, L. W. Castellani, M. Mehrabian, M. H. Doolittle, and A. J. Lusis. 1995. Genetic factors in lipoprotein metabolism. Analysis of a genetic cross between inbred mouse strains NZB/BINJ and SM/J using a complete linkage map approach. *J. Clin. Invest.* **96:** 1845–1858.
12. Sehayek, E., E. M. Duncan, H. J. Yu, L. Petukhova, and J. L. Breslow. 2003. Loci controlling plasma non-HDL and HDL cholesterol levels in a C57BL/6J × CASA/Rk intercross. *J. Lipid Res.* **44:** 1744–1750.
13. Suto, J., S. Matsuura, H. Yamanaka, and K. Sekikawa. 1999. Quantitative trait loci that regulate plasma lipid concentration in hereditary obese KK and KK-A mice. *Biochim. Biophys. Acta.* **1453:** 385–395.
14. Mouse Phenome Database. (Accessed October 30, 2003, at http://www.jax.org/phenome.)
15. Pitman, W. A., R. Korstanje, G. A. Churchill, E. Nicodeme, J. J. Albers, M. C. Cheung, M. A. Staton, S. S. Sampson, S. Harris, and B. Paigen. 2002. Quantitative trait locus mapping of genes that regulate high density lipoprotein cholesterol in SM/J and NZB/BINJ inbred mice. *Physiol. Genomics.* **9:** 93–102.
16. Nishina, P. M., S. Lowe, J. Verstuyft, J. K. Naggert, F. A. Kuypers, and B. Paigen. 1993. Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J. Lipid Res.* **34:** 1413–1422.
17. Nishina, P. M., J. Verstuyft, and B. Paigen. 1990. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J. Lipid Res.* **31:** 859–869.
18. Allain, C. C., L. S. Poon, C. S. G. Chang, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
19. Izzo, C. G. F., and E. Murador. 1981. Improved method for determination of high density lipoproteins by using polyethylene glycol 6000. *Clin. Chem.* **27:** 371–374.
20. Sen, S., and G. A. Churchill. 2001. A statistical framework for quantitative trait mapping. *Genetics.* **159:** 371–387.
21. Lander, E. S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics.* **121:** 185–199.
22. Churchill, G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics.* **138:** 963–971.
23. Lander, E. S., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11:** 241–247.
24. Hiekkalinna, T., and J. D. Terwilliger. 200X. PSEUDOMARKER Ver. 1.0. (Accessed October 30, 2003, at http://www.jax.org/staff/churchill/labsite).
25. Sugiyama, F., G. A. Churchill, D. C. Higgins, C. Johns, K.P. Makaritsis, H. Gavras, and B. Paigen. 2000. Quantitative trait loci analysis of blood pressure in mice on a high salt regimen: concordance with rat and human loci. *Genomics.* **71**: 70–77
26. Wang, X., I. Le Roy, E. Nicodeme, R. Li, R. Wagner, C. Petros, G. A. Churchill, S. Harris, A. Darvasi, J. Kirilovsky, P. L. Roubertoux, and B. Paigen. 2003. Using advanced intercross lines for high-resolution mapping of HDL cholesterol quantitative trait loci. *Genome Res.* **13:** 1654–1664.
27. Paigen, B., P. A. Holmes, D. Mitchell, and D. Albee. 1987. Comparison of atherosclerotic lesions and HDL-lipid levels in male, female, and testosterone-treated female mice from strains C57BL/6, BALB/c, and C3H. *Atherosclerosis.* **64:** 215–221.
28. Mayer, L. P., R. Easdgard, C. A. Dyer, P. B. Hoyer, and C. L. Banka. 2003. Chemically-induced menopause and atherosclerosis in mice (In 4th Annual Conference on Arteriosclerosis, Thrombosis and Vascular Biology. May 8–10, 2003, Washington, DC, 2003.)
29. Reifsnyder, P. C., G. A. Churchill, and E. H. Leiter. 2000. Maternal environment and genotype interact to establish diabesity in mice. *Genome Res.* **10:** 1568–1578.
30. Lopez, D., M. D. Sanchez, W. Shea-Eaton, and M. P. McLean. 2002. Estrogen activates the high-density lipoprotein receptor gene via binding to estrogen response elements and interaction with sterol regulatory element binding protein-1A. *Endocrinology.* **143:** 2155–2168.
31. Tsai, M., and B. W. O'Malley. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* **63:** 451–486.
32. Dai, W. S., J. P. Gutai, L. H. Kuller, R. E. LaPorte, L. Falvo-Gerard, and A. Caggiula. 1984. Relation between plasma high-density lipoprotein cholesterol and sex hormone concentrations in men. *Am. J. Cardiol.* **53:** 1259–1263.
33. Korstanje, R., J. J. Albers, G. Wolfbauer, R. Li, A. Y. Tu, G. A. Churchill, and B. Paigen. 2004. Quantitative trait locus mapping of genes that regulate phospholipid transfer activity in SM/J and NZB/BlNJ inbred mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 155–160.
34. Willnow, T. E., Z. Sheng, S. Ishibashi, and J. Herz. 1994. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science.* **264:** 1471–1474.
35. Shih, D. Q., M. Bussen, E. Sehayek, M. Ananthanarayanan, B. L. Schneider, F. J. Suchy, S. Shefer, J. S. Biollileni, F. J. Gonzalez, J. L. Breslow, and M. Stoffel. 2001. Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat. Genet.* **27:** 375–382.
36. Desai, P. P., C. H. Bunker, F. A. M. Ukoli, and M. I. Kamboh. 2002. Genetic variation in the apolipoprotein D gene among African blacks and its significance in lipid metabolism. *Atherosclerosis.* **163:** 329–338.
37. Jaye, M., K. J. Lynch, J. Krawiec, D. Marchadier, C. Maugeais, K. Doan, V. South, D. Amin, M. Perrone, and D. J. Rader. 1999. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat. Genet.* **21:** 424–428.
38. Schwarz, M., D. L. Davis, B. R. Vick, and D. W. Russell. 2001. Genetic analysis of intestinal cholesterol absorption in inbred mice. *J. Lipid Res.* **42:** 1801–1811.
39. Toda, I., A. Wickham, and D. A. Sullivan. 1998. Gender and androgen treatment influence the expression of proto-oncogenes and apoptotic factors in lacrimal and salivary tissues of MRL/*lpr* mice. *Clin. Immunol. Immunopathol.* **86:** 59–71.
40. Hegele, R. A., J. H. Brunt, and P. W. Connelly. 1995. Multiple genetic determinants of variation of plasma lipoproteins in Alberta Hutterites. *Arterioscler. Thromb. Vasc. Biol.* **15:** 861–871.
41. Frykman, P. K., M. S. Brown, T. Yamamoto, J. L. Goldstein, and J. Herz. 1995. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **92:** 8453–8457.
42. Lyons, M. A., H. Wittenburg, R. Li, K. A. Walsh, G. A. Churchill, M. C. Carey, and B. Paigen. 2003. Quantitative trait loci that determine lipoprotein cholesterol levels in DBA/2J and CAST/Ei inbred mice. *J. Lipid Res.* **44:** 953–967.