Genetic contributors to lipoprotein cholesterol levels in an intercross of 129S1/SvImJ and RIIIS/J inbred mice

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Lyons, Malcolm A., Ron Korstanje, Renhua Li, Kenneth A. Walsh, Gary A. Churchill, Martin C. Carey, and Beverly Paigen. Genetic contributors to lipoprotein cholesterol levels in an intercross of 129S1/SvImJ and RIIIS/J inbred mice. Physiol Genomics 17: 114–121, 2004. First published February 10, 2004; 10.1152/physiolgenomics.00168.2003.—To determine the genetic contribution to variation among lipoprotein cholesterol levels, we performed quantitative trait locus (QTL) analyses on an intercross between mouse strains RIIIS/J and 129S1/SvImJ. Male mice of the parental strains and the reciprocal F1 and F2 populations were fed a high-cholesterol, cholic acid-containing diet for 8–12 wk. At the end of the feeding period, plasma total, high-density lipoprotein (HDL), and non-HDL cholesterol were determined. For HDL cholesterol, we identified three significant QTLs on chromosomes (Chrs) 1 (D1Mit507, 88 cM, 72–105 cM, 4.8 LOD), 9 (D11Mit149, 14 cM, 10–25 cM, 9.4 LOD), and 12 (D12Mit60, 20 cM, 0–50 cM, 5.0 LOD). These QTLs were considered identical to QTLs previously named Hdlq5, Hdlq17, and Hdlq18, respectively, in crosses sharing strain 129. For total cholesterol, we identified two suggestive QTLs on Chrs 12 (D12Mit60, 88 cM, 10–105 cM, 3.9 LOD) and Chr 11 (D11Mit149, 14 cM, 0–30 cM, 4.4 LOD), respectively. In addition, for total cholesterol, we identified two suggestive QTLs on Chrs 12 (distal and 17, which remain unnamed. For non-HDL cholesterol, we identified and named one new QTL on Chr 17, Nhdlq3 (D17Mit221, 58 cM, 45–60 cM, 3.4 LOD). Nhdlq3 colocalized with orthologous human QTLs for lipoprotein phenotypes, and with Abcg5 and Abcg8. Overall, we detected eight QTLs for lipoprotein cholesterol concentrations on Chrs 1, 9, 12, and 17 (each two per chromosome), including a new QTL for non-HDL cholesterol, Nhdlq3, on Chr 17. These QTLs are in agreement with human QTLs for non-HDL cholesterol, which colocalize with LDL receptor (LDLR), APOB, LCAT, and LPL (4) are known to cause HDL deficiency, whereas mutations in LDLR, APOB, ARH, ABCG5, and/or ABCG8 (8) and CYP7A1 (26) are known to cause increases in LDL concentrations. One may then postulate that polymorphisms in the genes that cause the monogenic lipoprotein disorders, but distinct from those mutations, also may contribute to quantitative variations in lipoprotein levels in general.

We document here our investigation of the genetic loci determining lipoprotein cholesterol concentrations among intercross (F2) progeny generated from strains RIIIS/J (RIIIS) and 129S1/SvImJ (129). Four QTLs were detected for total cholesterol, three for HDL cholesterol, and one for non-HDL cholesterol. Since a number of QTLs apparently were detected for more than one phenotype, we conclude that five distinct QTLs were detected. Nhdlq3, a new QTL for non-HDL cholesterol concentrations, colocalized with human QTLs for lipoprotein traits, and with Abcg5/Abcg8. Interestingly, nonfunctional mutations in either of the human homologues of these genes cause a rare monogenic disorder, which primarily affects plant sterol absorption and biliary secretion, i.e., sitosterolemia, but in some cases also affects LDL concentrations.

MATERIALS AND METHODS

Animals and Diet

Animals were obtained from and maintained at the Jackson Laboratory (Bar Harbor, ME). Breeding facilities and protocols were identical to those described in detail previously (16). First filial generation (F1) progeny were produced from both breeding directions (i.e., 129 × RIIIS and RIIIS × 129). The F2 progeny were generated from both lineages by intercrossing either (i.e., 129 × RIIIS)F1 or (RIIIS × 129)F1 animals. Experimental animals were housed at a density of five mice per cage. At 6–8 wk of age, mice of the parental strains (n = 5 per strain) and F2 progeny (n = 9–10 per direction of cross) were transferred from standard diet to the atherogenic diet for 8 wk. The atherogenic diet is identical to that of the “lithogenic,” or gallstone-promoting diet, reported previously (13) and comprised, by

ATHEROSCLEROSIS is correlated inversely with high-density lipoprotein (HDL) concentrations (9), but correlated directly with low-density lipoprotein (LDL) concentrations (12). HDL performs key roles in the removal of cholesterol from extrahepatic tissues and its transport to the liver for elimination (34), perturbation of which can result in cholesterol gallstone formation. Hence, a negative association exists between HDL levels and cholesterol gallstones (22).

Rare mutations in crucial lipoprotein metabolic genes cause premature atherosclerosis in a small group of patients (4, 8, 26). In the general population, however, lipoprotein concentrations are regulated by multiple genes and their interactions with the environment. Therefore, elucidation of the pathophysiological and genetic underpinnings of variation in individual lipoprotein levels should afford novel molecular targets for risk assessment and new or enhanced drug therapies.

When exposed to an identical environment, diverse inbred mouse strains exhibit great variation in plasma lipoprotein concentrations (21). Therefore, the genetic background of the strains must determine these differences. Consequently, quantitative trait locus (QTL) analysis may be employed to map the genes that affect lipoprotein concentrations (20, 38). Furthermore, QTLs determining HDL cholesterol levels in mice and humans are localized in orthologous chromosomal (Chr) regions (38), thereby generating the hypothesis that polymorphisms within the orthologous genes underlie the QTL in each species. To date, rare mutations in ABCA1 (3, 27), APOA1, LCAT, and LPL (4) are known to cause HDL deficiency, whereas mutations in LDLR, APOB, ARH, ABCG5, and/or ABCG8 (8) and CYP7A1 (26) are known to cause increases in LDL concentrations. One may then postulate that polymorphisms in the genes that cause the monogenic lipoprotein disorders, but distinct from those mutations, also may contribute to quantitative variations in lipoprotein levels in general.

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weight, butter fat (15%), cholesterol (1%), cholic acid (0.5%), corn oil (2%), sucrose (50%), casein (20%), and essential minerals and vitamins. To enhance the sensitivity of cholesterol gallstone evaluation (M. A. Lyons, R. Korstanje, M. C. Carey, and B. Paigen, unpublished observations), the feeding duration was prolonged to 12 wk (n = 180) from 8 wk (n = 150) for the second half of the F2 population. However, so few F2 mice developed cholesterol gallstones, the gallstones were not amenable to QTL analysis. Hence, we report our QTLs for lipoprotein cholesterol levels only. Male mice only were studied and had free access to food and water. All animals fasted 4 h prior to euthanasia. The Institutional Animal Care and Use Committees of The Jackson Laboratory and Harvard University approved all experimental protocols.

Phenotyping

Phenotyping was performed as described (16). Briefly, blood was collected via the retro-orbital sinus with EDTA as anticoagulant. Plasma was isolated by centrifugation. HDL and total cholesterol concentrations were measured using an automated chemistry analyzer (Synchrom CX5 Delta; Beckman, Palo Alto, CA) and the manufacturer’s reagents (HDLD and total cholesterol reagents, respectively; Beckman). Because mice were fasted before blood was collected and because chylochromes display very short half-lives (7), non-HDL was presumed to comprise predominantly VLDL and LDL. Non-HDL was calculated as the difference between total and HDL cholesterol concentrations.

QTL Analyses

Genotyping. DNA was prepared from tail samples, and genotyping was performed as described (16). Initially, to perform the genome-wide QTL analyses, we genotyped 92 F2 mice randomly from each of the two feeding periods (i.e., n = 184). Subsequently, we genotyped the remaining 146 mice (total n = 330) at simple sequence length polymorphism (SSLP) markers in the regions with QTLs. Simple sequence length polymorphisms (MapPrimers, Research Genetics, Huntsville, AL) that discriminated between RIIS and 129 alleles were employed for QTL analyses (n = 87, interval range 4.7 to 36.0 cm; interval mean ± SD 18.7 ± 5.8 cm). Report genetic map positions were retrieved from the Mouse Genome Database (http://www.informatics.jax.org) and checked against the physical position in the sequence database (ENSEMBL) (i.e., D1Mit49 map to Chr 9). These data are available from the authors and presented in Supplemental Table S1, available at the Physiological Genomics web site.1

QTL analyses. To identify QTLs associated with lipoprotein cholesterol concentrations, a three-stage analysis was employed as detailed previously (30, 41). This approach uses single QTL scans to identify main effects, all-pairs genome scans to identify epistatic QTLs, and multiple QTL modeling to confirm significance and estimate the QTL main and interaction effects. Analyses were performed using Pseudomarker version 0.9 (available at http://www.jax.org/staff/churchill/labsite/software/index.html). This software employs a multiple imputation algorithm for interval mapping (n = 64 imputations) (30). Each trait was analyzed separately. In the first stage, single loci associated with the traits were detected by a genome-wide scan using 5-cM intervals. Fine mapping of those loci was performed using 2-cM intervals. Significance thresholds were determined by permutation testing (n = 1,000 permutations) (6). We defined significant loci as those that exceeded the 95th percentile (i.e., P < 0.05) of the permutation distribution, whereas the suggestive loci exceeded the 90th percentile (P < 0.10). The 95% confidence intervals (CI) were calculated as described (30). Results are expressed as logarithm of the odds ratio (LOD) scores. In the second stage, QTLs that affected the trait by interacting with one another (epistasis) were sought using a genome-wide simultaneous search for marker pairs employing a two-way ANOVA model, with an interaction term. We required overall significance of the locus pair at the genome-wide P < 0.05 level as determined by permutation analysis (6). Furthermore, we required the interaction component of the two-way ANOVA model to be significant at the (unadjusted) P < 0.01 level. The third stage comprises a multiple regression analysis in which the single and interacting loci identified in the first two stages are integrated to determine the contribution of each QTL in combination with all other QTLs. However, no epistasis was detected in this study, so only single QTLs and their contributions are reported.

In this study, two QTLs were detected on Chr 12, one for total cholesterol (46 cM) and one for HDL cholesterol (20 cM). To determine whether these represented one QTL with pleotropic effects or whether these represented two distinct QTLs, we applied two multivariate models to the phenotype data. The first model fitted one common QTL (pleiotropy model) to explain variation in both traits, and the second fitted two QTLs (heterogeneity model). A large change in the likelihood indicates heterogeneity (5, 25). Previous simulation studies (R. Li and G. A. Churchill, unpublished data) indicated that the 0.05 threshold for this test corresponds to a LOD change (ΔLOD) > 2.1, whereas the 0.10 threshold corresponds to ΔLOD > 1.9. Therefore, if the LOD increases by greater than 2.1 when the heterogeneity model is applied, then we conclude that two distinct QTLs exist. However, failure to achieve significance should not be interpreted as evidence for a single QTL.

Allele effects. For each QTL, we determined the allele effect by calculating the phenotype mean for each of the three possible genotypes. We then determined qualitatively which strain contributed the allele that increased lipoprotein cholesterol concentration and inferred whether that allele caused dominant, additive, or recessive inheritance of the lipoprotein cholesterol phenotype. A dominant allele was defined as that of a cholesterol level by the heterozygous genotype F2 population indistinguishable from the high-cholesterol homozygous genotype F2 population, whereas a recessive allele was defined as the heterozygous genotype F2 group being indistinguishable from the low-cholesterol homozygous genotype F2 group. An additive allele was defined as an allele of a genotype that produced a lipoprotein cholesterol phenotype intermediate between each of the homozygous-low and homozygous-high F2 genotype populations.

mRNA Expression Analyses

In the parental strains of mice, RIIS and 129, hepatic levels of mRNA expression were determined for obvious candidate genes that colocalized with QTLs and were known to have a direct or indirect role in lipid metabolism. Assuming a steady state for the expression of genes induced by the diet, hepatic tissue was harvested as described from male parental mice (5 per strain) fed the atherogenic diet for 4 wk (16). mRNA expression levels were determined using quantitative (real-time) PCR and oligonucleotide primers designed as detailed elsewhere (16). Primer sequences were: ApoA2, ggc cat act ctt cca tca tga (forward) and ggt ctt ggc ctt ctc cat ca (reverse); Abcg5, ctt cga can aat tgc cat cc (forward) and gaa agg acc cct gaa gg (reverse); Abcg8, taa act tgg aca tgg tgt gga tag t (forward) and gac aat gga gta gat cgc ata tag t (reverse). Data were expressed per 106 molecules of Gapd. Statistical analyses were performed on the normalized data.

General Statistical Analyses

Plasma lipoprotein cholesterol concentration data are presented as scatter plots. All other data are presented as mean ± SE. Data were analyzed using GraphPad Prism (Windows 3.0; GraphPad Software, San Diego, CA). The lipoprotein cholesterol concentrations and mRNA expression data were analyzed using Student’s t-test with the Bonferroni adjustment for multiple comparisons. Phenotypes were
associated using the Pearson correlation. $P < 0.05$ was considered significant.

**RESULTS**

**Plasma Lipoprotein Cholesterol Concentrations**

The individual lipoprotein cholesterol concentrations are depicted in Fig. 1, whereas the mean cholesterol values are listed in Table 1. Subsequent to atherogenic diet feeding for 8 wk, strain 129 exhibited significantly greater cholesterol concentrations in each lipoprotein class. The reciprocal $F_1$ groups displayed similar cholesterol levels in each lipoprotein class; hence, they were combined for comparison with each of the two parental strains. Although the non-HDL cholesterol level of the combined $F_1$ mice was similar to strain 129, it was not significantly different from strain RIHIS ($P = 0.07$). However, the total and HDL cholesterol concentrations of the combined $F_1$ mice resembled strain 129 and were significantly different from strain RIHIS, indicating that these two phenotypes were inherited dominantly in this intercross.

Since the reciprocal $F_1$ progeny did not differ in this cross, we concluded that lipoprotein cholesterol concentrations were not affected by maternal (e.g., mitochondrial) or imprinted (gene expression predominantly from either a maternal or paternal allele) genetic factors. Therefore, we focused on autosomal regions that may carry genes affecting lipoprotein cholesterol levels using QTL analyses.

To enhance the phenotyping for cholesterol gallstones, which was investigated concurrently with lipoprotein cholesterol levels, the feeding period for the $F_2$ progeny was extended from 8 to 12 wk. HDL cholesterol was not different between the $F_2$ groups fed the lithogenic diet for 8 vs. 12 wk (Table 1). In contrast, there was a small but significant decrease in total cholesterol of the $F_2$ mice fed for 12 wk (Table 1). Similarly, the $F_2$ mice fed for 12 wk displayed decreased non-HDL cholesterol levels compared with the group fed for 8 wk (Table 1). These data indicated that the QTL analyses should be executed using the duration of feeding as a covariate.

**QTL Analyses**

As indicated, QTL analyses were performed using the duration of feeding as a covariate. The genome-wide QTL anal-
determined by permutation testing (6), suggestive QTL LOD/H11022 5 and 8 (canalicular cholesterol transporter). Given that much greater strength for HDL compared with total cholesterol on Chr 17 was suggestive only, it was not named, although it is likely identical to Nhdlq3. Finally, the QTL for total cholesterol on distal Chr 12 colocalized with a QTL for HDL cholesterol (coef cient 0.78, P < 0.0001) and non-HDL cholesterol (coef cient 0.75, P < 0.0001), but HDL correlated weakly with non-HDL cholesterol (coef cient 0.17, P < 0.005). Therefore, Chol10 and Chol11 likely are identical to Hdlq5 and Hdlq7, respectively. Because the QTL for total cholesterol on Chr 17 was suggestive only, it was not named, although it is likely identical to Nhdlq3. Figure 2. Genome-wide QTL analyses for loci determining plasma lipoprotein cholesterol levels in the F2 population derived from strains RIHIS and 129. Chromosomes (Chrs) 1 through X are represented numerically on the abscissa. The relative width of the space allotted for each chromosome reflects the relative number of SSLP markers for each chromosome. The ordinate represents the LOD score. The significant (P < 0.01, LOD > 2.5) levels of linkage were determined by permutation testing (6). Total cholesterol levels are presented in A; HDL cholesterol in B, and non-HDL cholesterol in C.

yses are depicted in Fig. 2 and summarized in Table 2. For the lipoprotein total cholesterol concentration, significant QTLs were detected on Chrs 1 and 9, whereas suggestive QTLs were detected on Chrs 12 and 17, the latter being the only QTL that were detected with duration of feeding as covariate. Colocalizing significant QTLs for HDL cholesterol levels were detected on Chrs 1 and 9, although the latter QTL was detected with much greater strength for HDL compared with total cholesterol concentrations (9.4 vs. 4.4 LOD, respectively; Table 2). A third significant QTL for HDL cholesterol concentration was detected on Chr 12; however, the peak for this QTL was proximal (20 cM) relative to that for total cholesterol (46 cM). One significant QTL for non-HDL cholesterol concentration was detected on Chr 17, which colocalized with a suggestive QTL for total cholesterol.

When the two multivariate models were applied to the phenotype data giving rise to the QTL on Chr 12, we observed \(\Delta\text{LOD} = 2.8\) between the pleiotropy model and the heterogeneity model. Since this exceeded the \(P < 0.05\) threshold of \(\Delta\text{LOD} > 2.1\), we concluded that the QTLs for total and HDL cholesterol on Chr 12 were not likely to represent the same loci; rather, they represent two distinct QTLs.

Using our statistical criteria for significance, and after increasing the cohort to 330 individuals, we detected no gene interactions (epistasis) in the second stage of our analysis. Therefore, only single QTLs are presented. Consistent with previous reports, the QTL for non-HDL cholesterol concentration on Chr 17 was named Nhdlq3 ("non-HDL cholesterol QTL 3"). The significant QTLs for total cholesterol on Chrs 1 and 9 were named Chol10 and Chol11, respectively. The QTL for HDL cholesterol on Chr 1 overlapped with previously described QTL for HDL cholesterol (38). Previous crosses that shared strain 129 as one parent reported and named QTLs for HDL cholesterol on Chr 1 (Hdlq5) (37), Chr 9 (Hdlq17) and proximal Chr 12 (Hdlq18) (11), which colocalized with the QTLs for HDL cholesterol from this cross. According to the rules of the International Mouse Nomenclature Committee, QTLs are given an independent name even if they map to the same location as a previously described QTL unless the cross shares a parental strain with the described QTL and the allele effect, peak marker, and confidence intervals are substantially overlapping. Since strain 129 contributed the alleles that determined the high phenotype in all crosses, we retained the previous names for these QTLs; Hdlq5, Hdlq17, and Hdlq18. Total cholesterol correlated strongly with HDL cholesterol (Pearson correlation coef cient 0.75, P < 0.0001), but HDL correlated weakly with non-HDL cholesterol (coef cient 0.17, P < 0.005). Therefore, Chol10 and Chol11 likely are identical to Hdlq5 and Hdlq17, respectively. Because the QTL for total cholesterol on Chr 17 was suggestive only, it was not named, although it is likely identical to Nhdlq3. Finally, the QTL for total cholesterol on distal Chr 12 colocalized with a QTL for HDL cholesterol (coef cient 0.78, P < 0.0001) and non-HDL cholesterol (coef cient 0.75, P < 0.0001), but HDL correlated weakly with non-HDL cholesterol (coef cient 0.17, P < 0.005). Therefore, Chol10 and Chol11 likely are identical to Hdlq5 and Hdlq17, respectively. Because the QTL for total cholesterol on Chr 17 was suggestive only, it was not named, although it is likely identical to Nhdlq3. Finally, the QTL for total cholesterol on distal Chr 12 colocalized with a QTL for HDL cholesterol (coef cient 0.78, P < 0.0001) and non-HDL cholesterol (coef cient 0.75, P < 0.0001), but HDL correlated weakly with non-HDL cholesterol (coef cient 0.17, P < 0.005). Therefore, Chol10 and Chol11 likely are identical to Hdlq5 and Hdlq17, respectively. Because the QTL for total cholesterol on Chr 17 was suggestive only, it was not named, although it is likely identical to Nhdlq3. Finally, the QTL for total cholesterol on distal Chr 12 colocalized with a QTL for HDL cholesterol.
HDL cholesterol identified in a previous cross (38). However, because the QTL detected here was suggestive, but the QTL detected previously was for a different, albeit related, trait and because that cross employed different inbred strains, the locus remains unnamed.

The allelic contribution of each QTL to the respective lipoprotein cholesterol phenotypes is presented in Fig. 3. The alleles determining increased cholesterol concentrations were contributed by strain 129 at all loci except for Nhdlq3, which was contributed by strain RIIIS. The allelic effects for increased lipoprotein cholesterol concentrations were deemed to be additive at all loci except at the QTL for total cholesterol on distal Chr 12 and at Nhdlq3 on Chr 17, which were recessive and dominant, respectively.

![Fig. 3. Allelic contributions to the QTLs detected for total (A), HDL (B), and non-HDL (C) cholesterol concentrations using an intercross between strains RIIIS and 129. Homozygous 129 genotypes are represented by 129/129 (solid bars), homozygous RIIIS genotypes by RIIIS/RIIIS (open bars), and heterozygous genotypes by 129/RIIIS (hatched bars). Data are means + SE (n = 39–145).](image)

**Candidate Gene Expression Analyses**

We evaluated mRNA expression of selected candidate genes for Hdlq5 and Nhdlq3 between strains RIIIS and 129. For Hdlq5, strain 129 exhibited 3.6-fold greater expression of Atpo2 compared with strain RIIIS (P < 0.0005). For Nhdlq3, neither of the candidate genes Abcg5 or Abcg8 exhibited differential mRNA expression between strains RIIIS and 129.

**DISCUSSION**

Employing an intercross between strains RIIIS and 129, we detected eight QTLs for lipoprotein cholesterol concentrations: 1) Chol10, Chr 1; 2) Chol11, Chr 9; 3) Hdlq5, Chr 1; 4) Hdlq17, Chr 9; 5) Hdlq18, proximal Chr 12; 6) Nhdlq3, Chr 17; 7) a suggestive QTL for total cholesterol on distal Chr 12; and 8) a suggestive QTL for non-HDL cholesterol on Chr 17. Each of these confirms or colocalizes with previously identified QTLs, except Nhdlq3, which is a new QTL for non-HDL cholesterol concentrations.

Cholic acid, a component of our experimental diet, decreased HDL levels in some strains of mice (18, 23, 32) primarily via a putative bile acid response element that mediates transcriptional repression of apolipoprotein A1 (32). Nevertheless, most QTLs for HDL phenotypes tend to colocalize irrespective of the experimental diet (38). The replacement of tauromuricholates (endogenous mouse bile acids) with an increased concentration of taurocholate by feeding cholic acid performs the additional function of increasing the concentration of lower density lipoproteins, a situation that resembles human lipoprotein profiles more closely than mice fed standard diet. It is for these reasons that we consider the effect of cholic acid inclusion negligible with regard to detecting QTLs for lipoprotein cholesterol levels. Moreover, cholic acid is required to promote cholesterol absorption and subsequent cholesterol gallstone formation (36), a major focus for this and other QTL mapping studies of our laboratories (42).

We measured gene expression using mRNA derived from mice fed the atherogenic diet for 4 wk rather than 8 or 12 wk, which marked the end of the study when we evaluated gallstone formation and plasma lipids. The 4 wk time point allows sufficient time for stabilization of altered mRNA expression and is early in the disease process (atherosclerosis, for which HDL levels are a major risk factor). Thus, changes in mRNA expression at 4 wk are likely to be causal because they preclude secondary effects of overt disease manifestation on gene transcription.

The three QTLs for HDL cholesterol, Hdlq5, Hdlq17, and Hdlq18, essentially explained the increase in the HDL cholesterol levels displayed by the F1 mice. Combined, a single copy of the strain 129 alleles at each of the three QTLs caused an increase of 37 mg/dl, whereas the F1 mice exhibited an increase of 42 mg/dl compared with the RIIIS mice. The three QTLs for total cholesterol, Chol10, Chol11, and the suggestive QTL on Chr 17, accounted for an increase of 47 mg/dl, whereas the F1 mice exhibited an increase of 75 mg/dl. The fourth QTL for total cholesterol on distal Chr 12 was recessive and did not contribute to the increase in the F1 mice. Therefore, the total cholesterol QTLs only partially explained the changes observed in the F1 mice. Similarly, since Nhdlq3 caused an increase in non-HDL cholesterol of 15 mg/dl, but the total increase in the F1 population was 35 mg/dl, it only partially
explained the observed increase in non-HDL cholesterol. Hence, other QTLs that were not detected in this study must contribute to non-HDL cholesterol levels.

Hdlq5 (88 cM) colocalized with a variety of QTLs from previous murine crosses (Table 2) and also with QTLs derived from human studies (38). An attractive candidate gene for Hdlq5 is Apoa2 (92.6 cM) encoding apolipoprotein A2. Consistent with the allele effect, which indicated that strain 129 contributed the allele that increased HDL cholesterol levels (Fig. 3B), strain 129 demonstrated increased Apoa2 mRNA expression compared with strain RIIS (Fig. 4). These data are supported by studies of knockout (null expression) (40) and transgenic (overexpression) (39) mouse models, which demonstrated decreased and increased HDL cholesterol concentrations, respectively. A comprehensive analysis of the amino acid sequence of APOA2 among 42 inbred mouse strains indicated that in the 16 crosses that exhibited Hdlq5, including the present investigation, a valine (129) to alanine (RIIIS) amino acid substitution at residue 61 is likely responsible for this QTL (X. Wang, R. Korstanje, and B. Paigen, unpublished observations). It remains to be determined whether Hdlq5 in this cross is due to both the amino acid substitution and promoter polymorphisms or whether the mRNA expression difference is secondary to mRNA stability arising from altered cDNA sequences. Nevertheless, taking all results together, it appears that Apoa2 is very likely to underlie Hdlq5, and Apoa2 is very likely to underlie the orthologous human QTL for HDL levels.

Hdlq17, the QTL for HDL cholesterol on Chr 9, colocalized with two murine QTLs (19, 33) and at least one human QTL (14) for the HDL phenotype. However, no immediately obvious candidate genes were recognized; the APOA1/APOC3/APOA4 gene cluster was distinct from the human QTL (14) and was outside the 95% CI for the Hdlq17 detected in the present study. The colocalization of mouse and human QTLs for HDL phenotypes indicates that this represents an important locus to pursue.

Two genes that underlie Nhdlq3 (58 cM) are Abcg5 and Abcg8, which encode ATP-binding cassette (ABC) half-transporters comprising a canalicular sterol transporter (43, 44). The role of ABCG5/ABCG8 is to limit intestinal sterol absorption and facilitate biliary sterol secretion (43, 44). In some patients (8), but not all patients (2), mutations in ABCG5 and/or ABCG8, both located at 2p21, caused increases in LDL levels, a significant risk factor for atherosclerosis. Therefore, we considered Abcg5 and Abcg8 candidate genes for Nhdlq3. Because QTLs can be caused by promoter polymorphisms that affect gene transcription or polymorphisms that affect mRNA stability, we evaluated the hepatic mRNA expression levels of Abcg5 and Abcg8 as a preliminary test for polymorphisms that affect mRNA levels of these genes. Between strain RIIS and 129, we did not observe differential mRNA expression of either gene. These data suggest that either Nhdlq3 is caused by a different gene or that a coding variant causing an amino acid substitution is responsible for the QTL. Recent DNA sequence analyses demonstrated that RIIS exhibits a T354M polymorphism compared with strain 129/SvEv (15), a 129 substrain closely related to 129S1/SvImJ (31). However, it remains to be determined whether this amino acid substitution exerts any effect upon ABCG5/ABCG8 activity, whether any such effect determines Nhdlq3, or whether another gene underlies Nhdlq3. Additionally, familial combined hyperlipidemia (FCHL), which is characterized by elevated very-low-density lipoprotein (VLDL; triacylglycerol) and LDL (cholesterol), demonstrated linkage to Chr 2p in human studies (1, 24). Another human study demonstrated genetic linkage between triacylglycerol, a large component of VLDL, and Chr 2p (10). It is likely, therefore, that orthologous genes underlie both Nhdlq3 and the corresponding human QTL for lipid protein levels. Similar to Hdlq17, the orthologous genes responsible for these QTLs ought to be pursued.

The study reported here is a single QTL study, but it also comprises part of a larger, ongoing study involving four gallstone-susceptible (RIIS, C58J, CAST/Ei, PERA/Ei) and four gallstone-resistant (129, DBA/2J, 129L/SvJ, LPIJ) inbred mouse strains that also vary in their lipoprotein profiles. In this “daisy chain,” each susceptible strain is crossed to two different resistant strains. These intercrosses form a daisy chain experimental design (17). This design increases the probability of detecting alleles affecting a trait possessed by a single parental strain because it is crossed into two other genetic backgrounds (35). Another advantage of this design is that by combining data from separate crosses, QTLs will be narrowed and closely linked QTLs may be resolved (35). An additional advantage of this daisy chain design is that haplotype analysis can be used to narrow the QTL and that candidate genes can be tested in those crosses that find the QTL and those that fail to find the QTL. Therefore, the goals of this experimental design are to uncover a substantial fraction of alleles that contribute to the complex lipoprotein phenotypes and to narrow their 95% CI.

QTL analysis of this intercross between strains RIIS and 129 confirmed three QTLs for HDL cholesterol (Hdlq5, Hdlq17, and Hdlq18) that were identified previously using crosses that shared strain 129. Additionally, we uncovered a suggestive QTL for total cholesterol on distal Chr 12 that colocalized with a QTL detected in a cross between two separate strains (SM/J and NZB/BINJ). It remains to be determined whether these QTLs are identical and whether the same allele underlies both QTLs. Most importantly, we revealed Nhdlq3, a new QTL for non-HDL cholesterol concentrations,
which colocalized with human QTLs for lipoproteins. Abcg5 and Abcg8 cannot be eliminated as candidate genes for this QTL, but it remains to be determined which gene gives rise to Nhdlq3 and thus contributes to quantitative lipoprotein variation in the mouse and likely in human populations.

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