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Genetic analysis of cholesterol accumulation in inbred mice

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Abstract Genetic linkage analysis in the laboratory mouse identified chromosomal regions containing genes that contribute to cholesterol accumulation in the liver and plasma. Comparisons between five inbred strains of mice obtained from the Jackson Laboratory (DBA/2, AKR, C57BL/6, SJL, and 129P3) revealed a direct correlation between intestinal cholesterol absorption and susceptibility to diet-induced hypercholesterolemia. This correlation was lost in the F_1 generation arising from crosses between high- and low-absorbing strains. Linkage analyses in AKxD recombinant inbred strains and 129xSJL129F_1 N_2 backcross mice identified four quantitative trait loci (QTL) that influenced *L*iver *cho*lesterol accumulation (*Lcho1–4*) and one locus that affected *P*lasma *cho*lesterol accumulation (*Pcho1*). These loci map to five chromosomes and, with one exception, are different from the seven QTL identified previously that influence intestinal cholesterol absorption. We conclude that a large number of genes affects the amount of cholesterol absorbed in the small intestine and its accumulation in the liver and plasma of inbred mice.—Schwarz, M., D. L. Davis, B. R. Vick, and D. W. Russell. **Genetic analysis of cholesterol accumulation in inbred mice. *J. Lipid Res.* 2001.** 42: **1812–1819.**

Supplementary key words quantitative trait mapping • lipid metabolism • liver cholesterol • plasma cholesterol • bile acids • cholesterol absorption

The utilization of cholesterol absorbed by the small intestine takes place via a series of consecutive steps that constitute a metabolic supply pathway (1). Genes and encoded proteins that mediate individual steps in this pathway have been identified, including enzymes that synthesize and transport bile acids (2, 3), proteins that secrete phospholipids and cholesterol into the bile (4, 5), ACAT enzymes involved in the formation of cholesteryl esters (6, 7), and proteins involved in the assembly and metabolism of intestinal chylomicrons (8). Several studies show that variations in the expression of these genes affect the amount of sterol taken up from the intestine. For example, mutations that compromise the synthesis of bile acids decrease intestinal cholesterol absorption (9–11), and the relative levels of the ACAT2 enzyme dictate the quantity of cholesterol absorbed by the intestinal mucosa (12). The assembly of chylomicron particles within the enterocyte is dependent similarly on the expression of genes encoding apolipoprotein B and the microsomal triglyceride transport protein (13). Two cell surface receptors, including the LDL receptor and the LDL receptor-related protein, which bind and internalize chylomicron particles, have also been identified (14). Other catalysts that accelerate steps in the utilization of intestinal cholesterol, such as proteins involved in the transport of cholesterol or its accumulation in the liver, have not been identified.

We are taking a genetic approach to identify genes that participate in the metabolism of intestinal cholesterol and predispose to diet-induced hypercholesterolemia [see the companion article in this issue (15)]. Our initial studies used a dual-isotope fecal absorption assay to classify inbred strains of mice with respect to absorption of intestinal cholesterol. By tracing the inheritance of this phenotype through several generations and performing linkage analyses, we found seven quantitative trait loci (QTL) that influenced the amount of cholesterol absorbed. Here, we show that hepatic cholesterol accumulation is directly correlated with cholesterol absorption in five strains of mice; animals that efficiently absorb cholesterol also accumulate large amounts of cholesterol in their livers when fed a high cholesterol diet. In contrast, plasma cholesterol accumulation does not correlate with the efficiency of intestinal cholesterol absorption in these strains. The correlation between hepatic accumulation and absorption of cholesterol is lost in the F_1 offspring of crosses between high- and low-absorbing strains. Genetic analyses of N_2 backcross animals and recombinant inbred (RI) strains reveal four QTL that influence the accumulation of cholesterol in the liver and one QTL that affects plasma cholesterol ac-

Abbreviations: ABC, ATP binding cassette; cM, centimorgan; Lchol, Liver cholesterol; LOD, logarithm of the odds; QTL, quantitative trait loci; RI, recombinant inbred.

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cumulation. With one exception, these five QTL are different from those that influence cholesterol absorption (15).

MATERIALS AND METHODS

Animals and diets

Male inbred mice (129P3/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, DBA/2J, and SJL/J, n = 8–10 per strain), F_1 hybrids (AKD2F_1/J, D2AKF_1/J, SJL129F_1/J, 129SJLF_1/J, B6129F_1/J, B6D2F_1/J, and B6SJLF_1/J, n = 30 per strain), and the RI set AKxD (n = 8 for 21 available strains) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained as described previously (15) and were fed a cereal-based, pelleted rodent diet ad libitum (Teklad No. 7001, Harlan Teklad, Madison, WI). This diet consisted of crude protein 24% (minimum), crude fat 4% (minimum), and crude fiber 5% (maximum). The basal cholesterol content was 0.016%, and the fatty acid composition, expressed as a percentage of total fat, was oleic acid (31.0%), linoleic acid (30.2%), palmitic acid (19.4%), stearic acid (8.6%), linolenic acid (2.9%), palmitoleic acid (1.7%), myristic acid (0.9%), other (8.2%). The 7001 diet was used as a base to prepare a pelleted high cholesterol (1% w/w) diet (custom research diet No. TD 92181, Harlan Teklad, Madison, WI).

For cholesterol accumulation studies, adult male mice were fed the high cholesterol diet for 21 days. Thereafter animals were killed, exsanguinated via the ascending aorta, and the livers were dissected. Plasma and liver tissue aliquots were used to determine cholesterol content as described below.

Intestinal cholesterol absorption

Intestinal cholesterol absorption was measured in vivo by a fecal dual-isotope ratio method, as described previously (15).

Plasma and tissue cholesterol levels

Lipoproteins in the plasma were separated by fast protein liquid chromatography on Superose 6 resin, and the cholesterol content of individual column fractions was determined using a kit (No. 1127771; Boehringer Mannheim, Indianapolis, IN). Aliquots of liver tissue were saponified and extracted, and their cholesterol concentrations were measured by gas chromatography using stigmastanol (Sigma) as an internal standard (16).

DNA isolation and genotype analysis

Genomic DNA was isolated from mouse tails by phenol/chloroform extraction (17). Genotype analyses using informative microsatellite markers obtained from Research Genetics (Huntsville, AL) were performed as described (15).

Linkage analysis and QTL mapping

The methods of statistical analyses and QTL mapping used in this study were identical to those described in detail in the companion article (15).

RESULTS

Cholesterol responsiveness in inbred strains of mice

To determine whether cholesterol absorption correlates with hepatic and plasma accumulation, we fed five inbred strains of mice with different absorption phenotypes a chow diet supplemented with 1% (w/w) cholesterol. After 21 days, the animals were killed, the amount of total cholesterol in plasma and liver was determined, and lipoprotein cholesterol profiles were generated after separation of plasma lipoprotein fractions by fast protein liquid chromatography. The results from these experiments are summarized in **Table 1**. The baseline level of total hepatic cholesterol was essentially identical among all strains (~2.3 mg/g liver); however, their responsiveness to the high cholesterol diet varied and was reflected in marked differences in hepatic cholesterol accumulation. As shown in **Fig. 1A**, there was a good correlation between rates of intestinal cholesterol absorption on a baseline diet and hepatic cholesterol content on a high cholesterol diet (r = 0.784). High-absorbing strains (129, AKR) accumulated larger amounts of cholesterol in their livers than did low-absorbing strains (DBA/2, SJL), and the C57BL/6 strain showed intermediate values for both traits. This correlation was maintained when a separate experiment was carried out in which mice were fed chow diet supplemented with 2% (w/w) cholesterol, 0.5% (w/w) cholic acid, and 15% (w/w) coconut oil (an atherogenic diet) for 21 days. Hepatic cholesterol levels reached 43.8 and 47.2 mg/g in 129 and AKR strains, respectively, whereas SJL mice accumulated 24.7 mg/g, and DBA/2 mice were unresponsive, accumulating only 6.8 mg cholesterol/g liver. There was no significant change in body weight during the 21-day treatment; however, all mice developed marked hepatomegaly, which was most pronounced in the DBA/2 strain.

The five inbred strains exhibited baseline differences with respect to plasma total cholesterol levels (Table 1),

TABLE 1. Body, organ weights, and cholesterol levels in inbred strains of mice

	129		AKR		DBA/2		SJL		B57BL/6	
	Control	1% Cholesterol	Control	1% Cholesterol	Control	1% Cholesterol	Control	1% Cholesterol	Control	1% Cholesterol
Body weight (g)	24.6 ± 1.2	25.9 ± 0.8	35.1 ± 1.5	35.4 ± 1.5	27.2 ± 1.2	28.6 ± 0.7	29.3 ± 0.6	29.2 ± 0.7	30.8 ± 1.3	31.6 ± 0.9
Liver/body weight (g/100 g body weight)	4.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	6.2 ± 0.1	4.8 ± 0.1	6.1 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	5.0 ± 0.1	5.5 ± 0.2
Hepatic cholesterol (mg/g)	2.3 ± 0.1	8.4 ± 1.1	2.4 ± 0.2	7.4 ± 0.4	2.5 ± 0.1	3.5 ± 0.1	2.1 ± 0.1	4.1 ± 0.1	2.5 ± 0.1	5.7 ± 0.5
Plasma cholesterol (mg/dl)	120.5 ± 6.2	128.5 ± 7.5	78.8 ± 2.7	109 ± 3.9	93.5 ± 1.8	106 ± 7.5	98.2 ± 3.9	121.8 ± 11.7	84 ± 2.2	88.8 ± 2.8
Absorption (%)[a]	67 ± 2	ND	63 ± 1.8	ND	32 ± 4.1	ND	28 ± 3.5	ND	42 ± 4.0	ND

Abbreviation: ND, Not determined.

[a] Intestinal cholesterol absorption values for individual strains are reproduced from (15).

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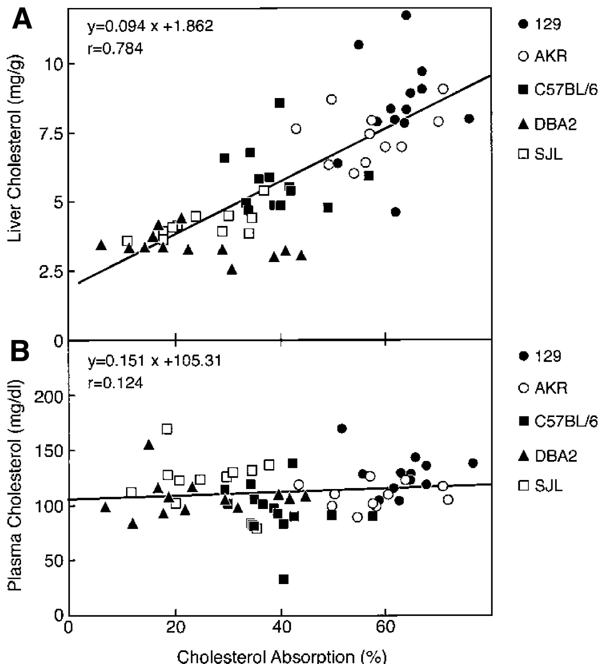

Fig. 1. Levels of liver cholesterol (A) and plasma cholesterol (B) as functions of intestinal cholesterol absorption in five inbred strains of mice. Absorption was measured using a dual-isotope fecal ratio method in 8–15 3-month old male mice fed a normal chow diet (15). Thereafter, the mice were fed a chow diet supplemented with 1% (w/w) cholesterol for 21 days, and cholesterol levels in the plasma and liver were determined enzymatically (plasma) or by gas chromatography (liver). Intestinal cholesterol absorption and liver cholesterol accumulation were correlated strongly (A), but absorption and plasma cholesterol level were not (B).

but in contrast to the data shown in Fig. 1A, there was no correlation between intestinal cholesterol absorption on a basal diet and plasma cholesterol levels of mice maintained on a diet containing 1% (w/w) cholesterol for 21 days (Fig. 1B, r = 0.124). The high-absorbing 129 mice, which had the highest baseline value (120.5 ± 6.2 mg/dl), and the intermediate-absorbing C57BL/6 mice showed no increases in plasma cholesterol concentrations, whereas the other three strains exhibited a significant increase ($P <$ 0.05) in plasma cholesterol irrespective of their intestinal absorption phenotype (Table 1). This increase occurred exclusively in HDL (**Fig. 2**). When mice were fed the atherogenic diet specified above, the low-absorbing SJL strain accumulated the highest amount of plasma cholesterol (230 mg/dl), whereas 129, AKR, and DBA/2 mice accumulated intermediate levels of 182, 161, and 138 mg/dl, respectively.

These results demonstrated that of the two quantitative traits characterizing responsiveness of inbred mice to high cholesterol diets, the accumulation of hepatic cholesterol correlated well with intestinal absorption rates, whereas accumulation of plasma cholesterol did not. The measured differences in hepatic cholesterol accumulation between pairs of high- and low-absorbing strains were statistically significant and varied over 3 to 5 standard deviations. These statistical data suggested that genetic analyses could be used to further dissect this phenotypic trait.

Cholesterol responsiveness in F_1 mice

Crosses were performed between high- and low-absorbing mice to produce F_1 generations of different genetic

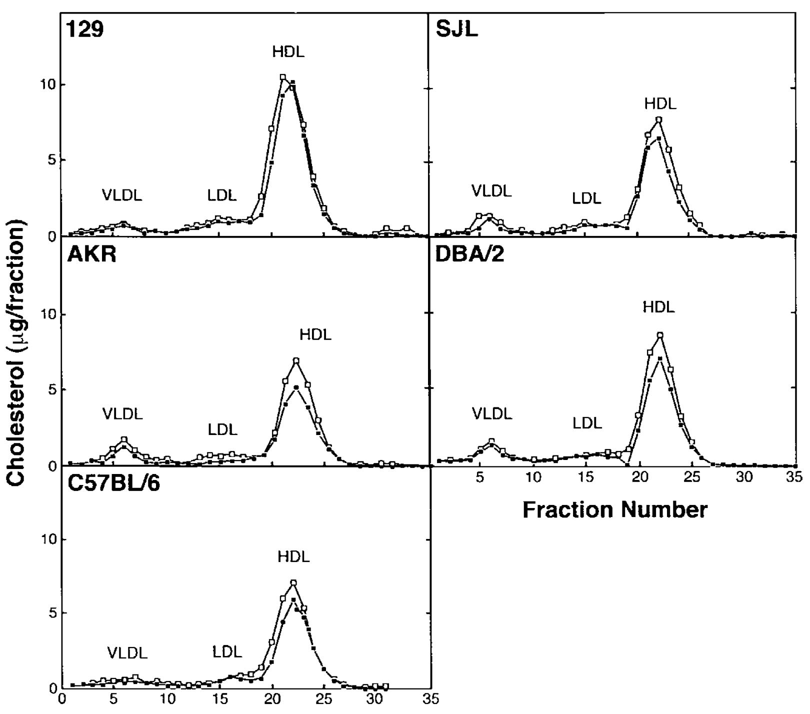

Fig. 2. Distribution of plasma lipoprotein cholesterol by particle size in five inbred strains of mice fed either a chow diet (filled squares) or a chow diet supplemented with 1% (w/w) cholesterol (open squares). After 21 days on the indicated diet, the mice were killed, and equal volumes of plasma from five animals per group were pooled. Lipoproteins were separated by fast protein liquid chromatography on a Superose 6 column, and 1-ml fractions from the column were assayed for cholesterol content.

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backgrounds. The crosses were between high-absorbing female AKR mice and low-absorbing DBA/2 males to produce an AKD2F$_1$ generation, low-absorbing SJL females and high-absorbing 129 males to produce SJL129F$_1$ mice, and intermediate-absorbing female C57BL/6 mice and male high-absorbing 129 mice to produce B6129F$_1$ mice. Cholesterol absorption was measured in 10 adult male offspring from each cross. These animals were then placed on the 1% (w/w) cholesterol diet, and accumulation of sterol in the liver and plasma was determined after 21 days (**Fig. 3**).

Whereas parental AKR mice accumulated hepatic cholesterol to a mean value of 7.4 mg/g tissue and DBA/2 mice to a mean value of 3.5 mg/g, their offspring exhibited an intermediate accumulation of 5.5 mg/g (Fig. 3A). In contrast, cholesterol absorption in the F$_1$ animals of this genotype was almost identical to that of the high-absorbing AKR parent (Fig. 3A). Similarly, the mean hepatic sterol accumulation in SJL129F$_1$ mice was intermediate (5.5 mg/g tissue) to those of the parental 129 (8.4 mg/g) and SJL (4.1 mg/g) strains (Fig. 3B). The inheritance of the accumulation phenotype in the F$_1$ generation was therefore different from that of the absorption phenotype, which segregated as a dominant low-absorption trait. Finally, B6129F$_1$ mice accumulated hepatic cholesterol to a mean value of 4.8 mg/g, which most closely resembled the value in the parental C57BL/6 strain (5.7 mg/g). This inheritance pattern was opposite that observed with the absorption trait in this cross; B6129F$_1$ mice showed a mean absorption value (61%) similar to that of the high-absorbing 129 parental strain (63%) (Fig. 3C). Taken together, these results indicated that whereas cholesterol absorption was inherited as either a dominant low- or high-absorption trait in crosses of different inbred strains, the inheritance of hepatic cholesterol accumulation was characterized by varying degrees of dominance and the apparent contributions of multiple genes.

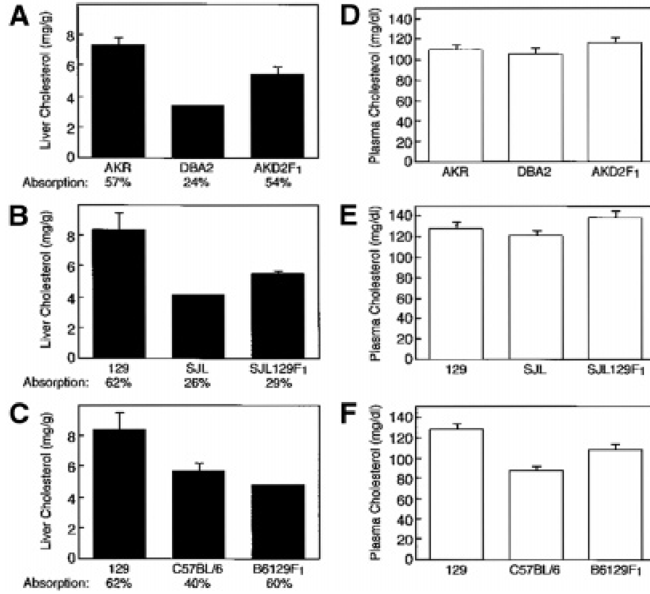

Fig. 3. Liver and plasma cholesterol accumulation in parental and F$_1$ mice. Hepatic cholesterol accumulation and absorption were measured in 8–30 males of AKR, DBA/2, and AKD2F$_1$ mice (A and D), 129, SJL, and SJL129F$_1$ mice (B and E), and 129, C57BL/6, and B6129F$_1$ mice (C and F), as described in Materials and Methods. Cholesterol contents are expressed as means of individual measurements (y-axes) with SEMs (vertical lines above histogram bars). For comparison purposes, the intestinal cholesterol absorption values determined previously (15) are indicated below the hepatic accumulation data in A, B, and C. A strong correlation was found between cholesterol absorption and hepatic accumulation in parental but not F$_1$ mice.

As was the case in the progenitor strains, plasma cholesterol levels in the various F$_1$ crosses fed the 1% cholesterol diet did not correlate with absorption rates or with hepatic cholesterol accumulation (Fig. 3D–F). There were no statistically significant differences in this parameter among the parental strains and their respective F$_1$ offspring.

Cholesterol responsiveness in N$_2$ mice

N$_2$ backcross generations were constructed by crossing AKDF$_1$ males to DBA/2 females and SJL129F$_1$ males to 129 females. The intestinal cholesterol absorption phenotype of individual N$_2$ mice was determined, and animals classified as low or high absorbers (15) were placed on the high cholesterol diet for 3 weeks. The phenotypes of liver cholesterol accumulation in the two groups of N$_2$ animals were unrelated to their absorption phenotypes (**Fig. 4A** and **B**). The range of hepatic cholesterol accumulation in the low-absorbing group of DBA/2xAKDF$_1$ N$_2$ mice was 3.03–4.78 mg/g tissue, and that for the high-absorbing group was similar (3.01–5.45 mg/g, Fig. 4A). The mean values of low- and high-absorbing groups of DBA/2xAKDF$_1$ N$_2$ mice (4.1 ± 0.1 and 3.9 ± 0.1, respectively) were slightly above the mean for the low-absorbing parental DBA/2 strain (3.5 ± 0.1) and were significantly lower than the 7.4 mg/g value observed in the high-absorbing parental AKR strain.

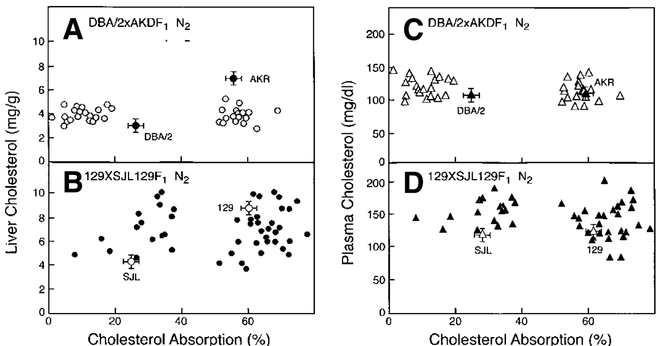

Fig. 4. Hepatic and plasma cholesterol accumulation as a function of intestinal cholesterol absorption in N$_2$ backcross mice. DBA/2xAKDF$_1$ N$_2$ (A and C) and 129xSJL129F$_1$ N$_2$ (B and D) mice were bred and assayed for cholesterol accumulation phenotypes, as described in Materials and Methods. Values for each parameter determined in individual N$_2$ backcross mice were plotted and compared to mean values ± SEM calculated for the parental strains.

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In the 129xSJL129F$_1$ N$_2$ population, means of hepatic cholesterol values were again similar between low- and high-absorbing groups (7.1 ± 0.3, and 7.2 ± 0.4, respectively, Fig. 4B). The standard deviation within each group was significantly larger than that measured in the DBA/2xAKDF$_1$ N$_2$ mice, and the range encompassed the values for the parental strains. This outcome was indicative of the contribution of multiple genes with large effects in the 129xSJL129F$_1$ N$_2$ population and provided a data set suitable for linkage analysis.

With respect to plasma cholesterol accumulation, low- and high-absorbing groups within each N$_2$ population were virtually identical (112 ± 4 vs. 117 ± 3 mg/dl for DBA/2xAKDF$_1$ N$_2$ mice; 142 ± 5 vs. 156 ± 5 mg/dl for 129xSJL129F$_1$ N$_2$ mice) (Figs. 4C and D). However, in the 129xSJL129F$_1$ N$_2$ population, the range of values was larger than in DBA/2xAKDF$_1$ N$_2$ mice, and the group means were significantly higher than those of the parental SJL and 129 strains (Fig. 4D). This distribution suggested the presence of additive gene effects, and again provided a data set amenable to linkage analysis.

Linkage analysis of N$_2$ backcross animals

Linkage analysis was performed in the N$_2$ populations to identify loci that affected plasma and hepatic cholesterol accumulation. To this end, whole-genome scans were carried out using cross-specific panels of 100 informative polymorphic microsatellite markers distributed on average every 15 centimorgans (cM) across the chromosomal complement. The resulting genotypic data were analyzed with computer programs (MapMaker/Exp 3.0, MapMaker/QTL 1.16) to identify markers that segregated with accumulation of plasma and/or liver cholesterol.

No loci were identified in the DBA/2xAKDF$_1$ N$_2$ population that exhibited statistically significant linkage to either phenotype. However, analysis of genotype-phenotype correlations in 129xSJL129F$_1$ N$_2$ mice revealed one locus that influenced hepatic sterol accumulation. This locus, which was designated *L*iver *cho*lesterol *1* (*Lcho1*), was linked to the D9Mit279 marker on chromosome 9 with a logarithm of the odds (LOD) score of 2.2 (**Fig. 5**). Additional analysis of the linkage data revealed one locus that influenced plasma cholesterol accumulation, termed *P*lasma *cho*lesterol *1* (*Pcho1*), which was linked to the D1Mit166 marker on chromosome 1 with a LOD score of 4.6 (Fig. 5).

Cholesterol responsiveness in AKxD RI strains

RI strains were used in a further effort to map genes contributing to the relationship between intestinal cholesterol absorption and cholesterol accumulation in the liver and plasma compartments. Each of the strains is fixed to homozygosity at all loci. Thus, based on the parental phenotypes, individual RI strains would be expected to be homozygous for certain AKR loci that specify high cholesterol accumulation, whereas other strains would be homozygous for DBA/2 alleles that specify low accumulation. A multigenic trait would yield a continuous phenotypic pattern rather than a discrete high-accumulating or low-accumulating phenotype in each strain.

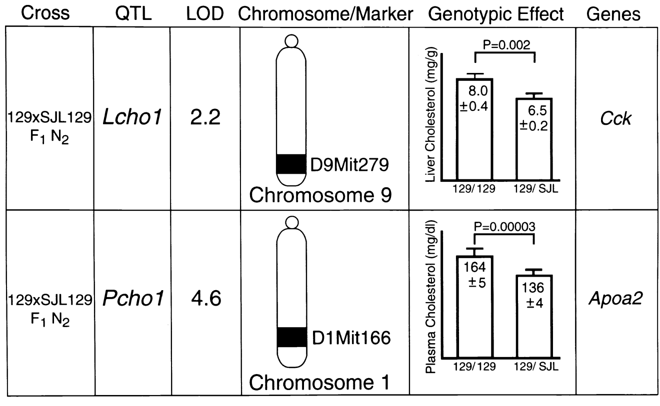

Fig. 5. Chromosomal locations of loci linked to liver cholesterol accumulation (*Lcho1*) and plasma cholesterol accumulation (*Pcho1*) identified in 129xSJL129F$_1$ N$_2$ backcross animals. Idiograms of two chromosomes are shown together with the locations of markers linked to the *Lcho1* and *Pcho1* QTL. The distributions of the 129 and SJL alleles in the N$_2$ backcross population animals in the low and high cholesterol accumulation intervals are indicated by histogram bars. Candidate genes that map in the identified intervals are shown at right.

Ten RI strains with absorption phenotypes ranging from 27% to 66% were analyzed for plasma and hepatic total cholesterol values. When maintained on a regular chow diet, cholesterol levels varied from 2.0 to 2.4 mg/g tissue in the liver and from 80 to 100 mg/dl in the plasma compartment (data not shown). These values did not correlate with the observed absorption phenotype. When a second set of the same RI strains with absorption values ranging from 24% to 56% were fed the high cholesterol diet, hepatic cholesterol levels varied significantly between the strains (**Fig. 6A**), suggesting that homozygosity at certain loci was required for manifestation of this phenotypic trait. In some strains there was concordance between hepatic cholesterol accumulation and absorption, whereas in others there was discordance between these two traits. For example, strain 22 was both a high hepatic accumulator (6.1 mg/g) and a high absorber (55%), and strain 23 was a low absorber (26%) and a low hepatic accumulator (3.8 mg/g). Discordance was illustrated by the results obtained in RI strain 18, which had one of the highest hepatic accumulation values (5.8 mg/g) but one of the lowest absorption values (24%) (Fig. 6A).

As with the results obtained with the DBA/2xAKDF$_1$ N$_2$ population (Fig. 4C), plasma cholesterol values did not vary significantly among the different RI strains tested here (Fig. 6B).

Linkage analysis in AKxD RI strains

The continuous phenotypic pattern observed with respect to hepatic cholesterol accumulation in the RI strains suggested that more than one locus influenced this phenotype in AKR and DBA/2 mice. To map these loci, we performed genotype-phenotype correlation using the MapManager computer program. This analysis revealed three loci that segregated in a statistically significant ($P <$

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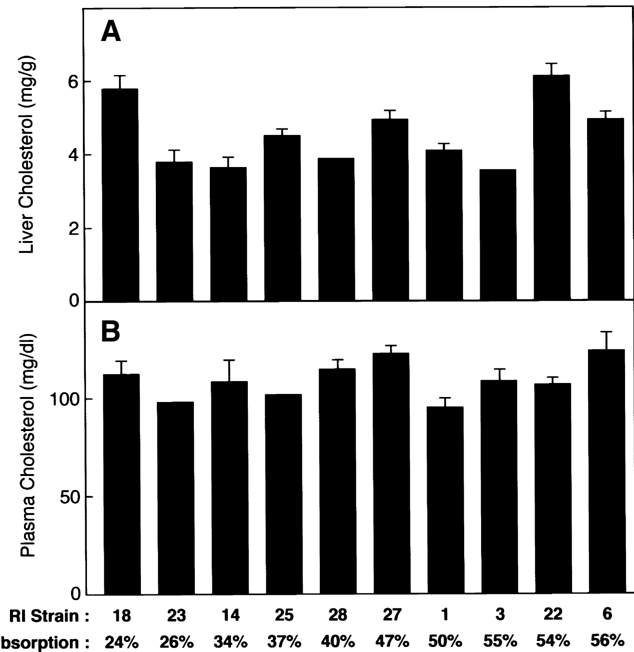

Fig. 6. Cholesterol accumulation and absorption in AKxD recombinant inbred (RI) strains. Five to eight male animals from each of the indicated RI strains were assayed for hepatic (A) and plasma (B) cholesterol accumulation. Measurements from individual animals of each strain were used to calculate a mean cholesterol accumulation value (y-axis) and SEM (vertical lines on histogram bars). For comparison purposes, the amount of cholesterol absorbed by each RI strain is shown below the panels (15).

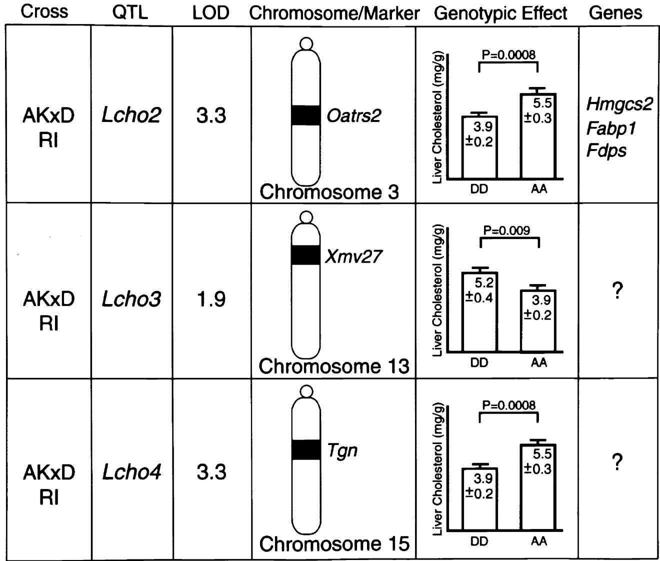

Fig. 7. Chromosomal locations of the liver cholesterol accumulation loci (*Lcho2*, *Lcho3*, and *Lcho4*) identified in AKxD RI strains. Idiograms of three chromosomes are shown together with the locations of markers linked to individual QTL. Histogram bars indicate the distributions of the DBA/2 (D) and AKR (A) alleles in the high- and low-accumulating RI strains. Candidate genes that map in the identified chromosomal regions are shown at right.

0.01) manner with the hepatic cholesterol accumulation trait. The *Lcho2* locus was mapped to chromosome 3 closely linked to the *Oatrs2* gene and exhibited a LOD score of 3.3 (**Fig. 7**). This locus accounted for ~42% of the genetic variance observed in hepatic cholesterol accumulation in the RI mice. The *Lcho3* locus on chromosome 13 was linked (LOD = 1.9) to the X*mv27* insertion and accounted for 15% of the genetic variance, whereas the *Lcho4* locus on chromosome 15 was linked (LOD = 3.3) to the *Tgn* gene and generated 42% of the variance.

DISCUSSION

In this study, we identify regions of five mouse chromosomes that predispose to diet-induced hypercholesterolemia. These QTL, termed *L*iver *cho*lesterol 1–4 (*Lcho1–4*) and *P*lasma *cho*lesterol 1 (*Pcho1*), were identified by phenotype-genotype comparisons in RI strains and N_2 backcross animals. Parental inbred strains exhibited a direct correlation between the amount of cholesterol absorbed in the small intestine and subsequent accumulation in the liver. However, the correlation was lost in F_1 and N_2 backcross generations arising from matings between high- and low-absorber/accumulator strains. This pattern of inheritance was different from that observed for the intestinal cholesterol absorption phenotype (15) and suggested that the genes affecting accumulation are different from those influencing absorption. Thus a high intestinal cholesterol absorption does not necessarily predispose to diet-induced hypercholesterolemia, as has been generally assumed. Consistent with this interpretation, the five accumulation loci identified here in AKR, DBA/2, 129, and SJL strains map to different chromosomes from those that influence absorption (15).

In several cases, candidate genes reside in the regions of the chromosome that contain accumulation loci. For example, the *Lcho1* locus on chromosome 9 maps to a region containing the cholecystokinin gene (*Cck*), which encodes a gut hormone that modulates the enteric nervous system and contraction of the gallbladder. The *Lcho2* locus on chromosome 3 maps to a region containing the 3-hydroxymethyl-3-glutaryl coenzyme A synthase gene (*Hmgcs2*) encoding an enzyme involved in ketone body formation, the fatty acid binding protein 1 gene (*Fabp1*) whose product may be involved in storing or transporting lipids in the hepatocyte, and the farnesylpyrophosphate synthetase gene (*Fdps*) encoding a cholesterol biosynthetic enzyme. The *Pcho1* gene maps to a region of chromosome 1 containing the gene encoding apolipoprotein A-II (*Apoa2*). Polymorphisms in *Apoa2* influence the size and lipid content of circulating HDL particles in mice and thus influence plasma cholesterol levels (18).

The localization of a cholesterol biosynthetic gene in the *Lcho2* region is noteworthy because differences in the regulation of enzymes in this pathway have been shown to underlie the hyperresponder and hyporesponder phenotypes in cynomolgus monkeys (19). Hyperresponder monkeys accumulate large quantities of cholesterol in their liver and plasma LDL fraction when fed a high cholesterol

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diet, whereas hyporesponders accumulate less tissue and serum LDL cholesterol. Detailed cholesterol balance studies in these outbred animals indicate that this phenotypic difference is caused by alterations in the regulation of hepatic sterol synthesis; hyperresponders synthesize 6-fold more sterol in the face of excess dietary cholesterol than do hyporesponders, leading to an expansion of the cholesterol pool in the liver and subsequent accumulation in the plasma (19). The accumulation of hepatic cholesterol associated with different alleles of the *Lcho2* locus in the mouse may reflect a similar alteration in the regulation of one or more cholesterol biosynthetic genes.

Additional genes that affect cholesterol accumulation also may reside within the identified chromosomal regions. These include genes that encode proteins associated with the formation of intracellular lipid droplets, such as the perilipins, a related group of proteins associated with the external face of the lipid droplet whose expression level is increased by lipid accumulation and regulates fat metabolism (20–22). Another class may encode enzymes involved in the synthesis (e.g., ACAT) or breakdown (cholesterol esterases) of cholesterol esters within the liver, although we note that neither of the two known ACAT genes (12, 23) or the single known cholesterol esterase gene (24) map within the regions identified here. How much cholesterol accumulates in a given compartment will depend on its availability for packaging into lipoproteins and the secretion of these particles. This dependence could explain the tight linkage we identified between *Pcho1* (Fig. 7) and the apolipoprotein A2 gene (*Apoa2*) (18).

The rates of catabolism and secretion into the bile will also determine how much cholesterol accumulates in the liver. Catabolism involves the conversion of cholesterol into bile acids via several pathways (25) and in the mouse is chiefly regulated by the level of the enzyme cholesterol 7α-hydroxylase in the hepatocyte (9). The expression of the encoding gene is in turn regulated by the levels of bile acids and cholesterol via several nuclear hormone receptors (26). In the five inbred strains of mice studied here, each responded appropriately to dietary cholesterol by inducing the levels of cholesterol 7α-hydroxylase, and we could detect no differences in the extent of this induction (data not shown). There also were no significant interstrain differences in other parameters of bile acid metabolism such as pool size and composition (15), and no linkage was detected between the *Lcho* and *Pcho* loci and genes encoding bile acid synthetic enzymes or regulatory factors (Figs. 5 and 7). Thus, although some strains of mice (e.g., BALB/c, C57BL/6, C3H/HeJ) differ in their expression and regulation of cholesterol 7α-hydroxylase (27, 28), we have been unable to find an obvious link between cholesterol catabolism and accumulation in the livers of C57BL/6, DBA/2, AKR, SJL, and 129 animals. The gene encoding the ileal lipid binding protein (29), which has been identified as a positional candidate for the regulation of hepatic cholesterol accumulation in QTL analyses using inbred rats (30), also is not contained within the chromosomal regions identified here.

Proteins that mediate cholesterol secretion into the bile include the scavenger receptor B (31) and members of the ATP binding cassette (ABC) transporter family (4, 32, 33). Although none of the known genes in these families map to the QTL identified here, the possibility remains that yet to be identified transporter or receptor genes reside in each of these intervals. These and other candidate gene hypotheses will be tested in future studies.

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