Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7729–7733, August 1995
Genetics

Lith1, a major gene affecting cholesterol gallstone formation among inbred strains of mice

(cholelithiasis/3-hydroxy-3-methylglutaryl-CoA reductase/recombinant inbred strains/lipids/atherosclerosis)

BHUPINDER KHANUJA*, YIN-CHAI CHEAH*, MARK HUNT*, PATSY M. NISHINA*, DAVID Q.-H. WANG†, HARRY W. CHEN‡, JEFFREY T. BILLHEIMER‡, MARTIN C. CAREY†, AND BEVERLY PAIGEN*§

*The Jackson Laboratory, Bar Harbor, ME 04609; ‡DuPont Merck Pharmaceutical Co., Wilmington, DE 19880; and †Brigham and Women's Hospital, Harvard Medical School and Harvard Digestive Diseases Center, Boston, MA 02115

Communicated by Elizabeth S. Russell, The Jackson Laboratory, Bar Harbor, ME, April 21, 1995

ABSTRACT **The prevalence of cholesterol gallstones differs among inbred strains of mice fed a diet containing 15% (wt/wt) dairy fat, 1% (wt/wt) cholesterol, and 0.5% (wt/wt) cholic acid. Strains C57L, SWR, and A were notable for a high prevalence of cholelithiasis; strains C57BL/6, C3H, and SJL had an intermediate prevalence; and strains SM, AKR, and DBA/2 exhibited no cholelithiasis after consuming the diet for 18 weeks. Genetic analysis of the difference in gallstone prevalence rates between strains AKR and C57L was carried out by using the AKXL recombinant inbred strain set and (AKR × C57L)F_1 × AKR backcross mice. Susceptibility to gallstone formation was found to be a dominant trait determined by at least two genes. A major gene, named *Lith1*, mapped to mouse chromosome 2. When examined after 6 weeks on the lithogenic diet, the activity of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.88) was downregulated as expected in the gallstone-resistant strains, AKR and SJL, but this enzyme failed to downregulate in C57L and SWR, the gallstone-susceptible strains. This suggests that regulation of the rate-limiting enzyme in cholesterol biosynthesis may be pivotal in determining the occurrence and severity of cholesterol hypersecretion and hence lithogenicity of gallbladder bile. These studies indicate that genetic factors are critical in determining gallstone formation and that the genetic resources of the mouse model may permit these factors to be identified.**

Both atherosclerosis and cholelithiasis result from excess cholesterol; in the one case cholesterol is deposited in arterial walls, and in the other case cholesterol precipitates in the gallbladder. Both diseases are prevalent in cultures consuming a Western diet, and both can be induced in animal models by a diet high in cholesterol (1, 2). In Western cultures, heart disease is the major cause of death, and gallstone disease is present in 10–40% of individuals over the age of 60 (3).

Genetic factors apparently play an important role in the development of cholesterol gallstone disease. Among studies of gallstone formation in animals, Alexander and Portman (4) demonstrated that C57BL/6 mice are susceptible to cholelithiasis, but CBA mice are resistant. In both strains bile was supersaturated with cholesterol but not to the same degree (4). Fujihara *et al.* (5) reported that the prevalence of gallstones varied from 0% to 100% among six strains of laboratory mice. Evidence for the importance of genetic factors in human cholelithiasis is limited. Gallstone disease can be familial (6–11), and the bile from healthy sisters of female gallstone patients is more lithogenic than controls (11, 12). In certain native populations of North and South America, a high percentage of adults develop cholesterol gallstones, suggesting common genetic factors (13, 14).

In previous studies, high fat plus high cholesterol diets produced atherosclerosis and gallstones in some strains of mice (15). In this report, we survey common inbred strains of mice for susceptibility to cholelithiasis and demonstrate that at least two genes determine the difference in gallstone susceptibility between strains AKR and C57L.

MATERIALS AND METHODS

Animals and Diets. Inbred strains of mice were obtained from The Jackson Laboratory, (AKR × C57L)F_1 × AKR backcross mice were bred in our colony, and AKXL recombinant inbred (RI) strains were a kind gift from Benjamin Taylor (The Jackson Laboratory). Mice were fed low cholesterol laboratory chow (Old Guilford animal diet no. 234; Emory Morse, Guilford, CT) or a semisynthetic lithogenic diet described previously (15) containing 15% (wt/wt) butter fat, 1% (wt/wt) cholesterol, 0.5% (wt/wt) cholic acid, 2% (wt/wt) corn oil, 50% (wt/wt) sucrose, 20% (wt/wt) casein, and essential vitamins and minerals. For euthanasia, 500 mg of 2,2,2-tribromoethanol (Sigma) per kg of body weight was used. Animals were housed in a temperature controlled room (22–23°C) with alternating 12-hr light/12-hr dark cycles.

Experimental Protocol. Eight-week-old mice were fed the lithogenic diet for various periods of time, and body weight was monitored every 2 weeks. After sacrifice, the wet weights of livers and gallbladders were determined. To remove the gallbladder, the cystic duct was clamped gently with forceps. Gallbladder volume was determined by measuring water displacement in a partly filled 1- or 3-ml syringe. Gallbladder weight including bile and stones was highly correlated with volume ($r^2 = 0.99$; $n = 94$). To collect bile for analysis, the cystic duct was double-ligated and divided followed by puncturing of the gallbladder fundus with a no. 22 hypodermic needle. Gallstones were usually visible through the gallbladder wall. To collect gallstones, the gallbladder was cut at the tip, gently squeezed with forceps, and washed out with 1 ml of cold 95% ethanol. Stones were dried overnight at 40°C and weighed. Gallstones were light yellow in color and not digitally compressible. Fifteen gallstones were pulverized individually in an agate mortar and dissolved in 2-propanol, and the cholesterol content was determined by HPLC (16).

Assays. Blood for lipid assays was obtained from mice fasted overnight after 4 weeks of lithogenic diet consumption. Measurement of total cholesterol and high density lipoprotein (HDL)-cholesterol levels have been described (17). Non-HDL-cholesterol levels were done by arithmetic difference.

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Abbreviations: HDL, high density lipoprotein; RI, recombinant inbred; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; ACAT, acyl-CoA:cholesterol acyltransferase; C7H, cholesterol 7α-hydroxylase; NS, not significantly different; CSI, cholesterol saturation index (indices).

§To whom reprint requests should be addressed.

Table 1. Gallstone formation, aortic lesions, and plasma lipids in mice fed a lithogenic diet

Mouse strains	Gallstone formation*	Fatty streak size, μm^2	Plasma cholesterol, mg/dl		
			Total	HDL	Non-HDL
AKR	0/3	150 ± 90[a]	110 ± 6[a]	63 ± 3[c,d]	47 ± 4[a]
DBA/2	0/6	230 ± 90[a]	98 ± 9[a]	45 ± 5[b]	53 ± 7[a]
SM	0/5	3100 ± 740[c]	116 ± 23[a]	49 ± 5[b,c]	67 ± 19[a,b]
C57BL/6	1/6	5030 ± 1370[c]	98 ± 7[a]	41 ± 6[b]	57 ± 4[a,b]
SJL	1/5	450 ± 260[a,b]	118 ± 6[a]	51 ± 3[b,c]	67 ± 19[a,b]
C3H	2/5	200 ± 130[a]	202 ± 10[b]	72 ± 4[d]	130 ± 9[c,d]
SWR	4/6	3800 ± 740[c]	174 ± 9[b]	39 ± 3[b]	135 ± 10[d]
C57L	6/6	3700 ± 670[c]	114 ± 11[a]	20 ± 1[a]	94 ± 10[b,c]
A	6/6	670 ± 150[b]	167 ± 6[b]	63 ± 3[c,d]	104 ± 7[c,d]

Values represent the mean ± SEM. Values within columns that do not share superscripts (a–d) are significantly different ($P < 0.01$) from each other as determined by one-way analysis of variance followed by Fisher's least-significant difference test.

*Number of mice with gallstones/total number of animals surviving the 18-week dietary treatment.

Biliary lipid compositions were measured as described (18) on pooled bile from 17 C57L or 27 AKR male mice fed either diet for 2 weeks. To remove microscopic stones and cholesterol crystals, pooled bile was filtered through a 0.22-μm Millipore filter prewarmed to 37°C and vortex mixed prior to sampling. Cholesterol saturation indices (CSI) were calculated from the critical tables of Carey (19). 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR; EC 1.1.1.88), acyl-CoA:cholesterol acyltransferase (ACAT; sterol *O*-acyltransferase, EC 2.3.1.26), and cholesterol 7α-hydroxylase (C7H; cholesterol 7α-monooxygenase, EC 1.14.13.17) were assayed in livers from nonfasted animals fed the diets for 6 weeks and sacrificed between 7 and 9 a.m. Microsomes were prepared from frozen livers after thawing, mincing, and suspending in 0.1 M phosphate buffer (pH 7.4) containing 0.25 M sucrose, 0.5 mM EDTA, 1 mM glutathione, and 20 μM leupeptin, a serine protease inhibitor, at 3 ml/g of tissue. Tissue was homogenized, and microsomes obtained by differential centrifugation were resuspended in 0.1 M phosphate buffer containing 1 mM glutathione to about 20 mg of protein per ml and frozen at −70°C until assayed. Methods for determining enzyme activities of ACAT (20), HMGR (21), and C7H (22) have been described. The same microsomal preparation was utilized for all three enzymes, so no phosphatase inhibitor was added, but recently it was demonstrated that phosphorylation and dephosphorylation do not play a role in feedback regulation of HMGR (23).

RESULTS

Differences in Gallstone Formation Among Inbred Strains. After 18 weeks on the lithogenic diet, female mice of nine inbred strains were tested for the presence of gallstones and the size of fatty streak lesions in the aorta. The strains differed considerably in the development of gallstones (Table 1): strains C57L, SWR, and A had the highest prevalence of gallstones; strains C57BL/6, C3H, and SJL produced an intermediate prevalence; and strains SM, AKR, and DBA/2 formed no gallstones. The 15 stones analyzed from strains C57L, SWR, and C57BL/6 contained 71–99% cholesterol. Susceptibility to gallstones did not correlate with the size of atherosclerotic lesions [$r = 0.27$; P, not significantly different (NS)], total cholesterol ($r = 0.38$; P, NS), or HDL-cholesterol ($r = 0.35$; P, NS). Prevalence of gallstones showed some correlation with non-HDL (i.e., principally low density lipoprotein and very low density lipoprotein) cholesterol, but this relationship did not reach statistical significance among the strains ($r = 0.60$; $P = 0.09$).

Lipid Regulatory Enzyme Activities in Mouse Livers. Three key rate-limiting enzymes affect cholesterol flux from liver to bile: HMGR, the rate-limiting enzyme in *de novo* cholesterol synthesis; C7H, the rate-limiting enzyme in the conversion of cholesterol to bile acids; and ACAT, which converts cholesterol into cholesterol ester for storage and new lipoproteins (24). These enzyme activities were examined in the progenitors of two RI strain sets, chosen because one progenitor was susceptible to gallstones and the other progenitor was relatively resistant. Enzyme activities (Table 2) in the susceptible C57L and SWR strains showed major differences in HMGR compared with the resistant AKR and SJL strains. This enzyme activity was 50% lower in the livers of susceptible compared with resistant strains fed chow ($P < 0.05$, unpaired *t* test), suggesting that HMGR may not upregulate appropriately in gallstone-susceptible mice on a low cholesterol diet. Moreover, when mice were fed the lithogenic diet, HMGR was downregulated in resistant mice but failed to downregulate in susceptible mice ($P < 0.001$). Consequently, the HMGR activities in livers of susceptible mice resulted in the continued synthesis of cholesterol, even in the presence of large amounts of dietary cholesterol, and if other homeostatic controls are equal, this should cause hypersecretion of cholesterol into bile. ACAT activities were upregulated 3-fold by the lithogenic diet ($P < 0.001$) as expected but did not differ significantly between

Table 2. Hepatic lipid regulatory enzyme activities in inbred strains of mice

Cholesterol gallstones	Mouse strain	HMGR,* pmol/min/mg of protein			ACAT,* nmol/min/mg of protein			C7H,* pmol/min/mg of protein		
		Chow	Lith	P†	Chow	Lith	P†	Chow	Lith‡	P†
Susceptible	C57L	28 ± 7[a]	22 ± 3[a]	NS	0.31 ± 0.02[b]	1.24 ± 0.06[b]	<0.001	11 ± 1[a]	6 ± 1[a]	0.03
Susceptible	SWR	24 ± 6[a]	30 ± 3[a]	NS	0.27 ± 0.04[a,b]	1.02 ± 0.03[a]	<0.001	11 ± 1[a]	9 ± 2[a]	NS
Resistant	SJL	51 ± 5[b]	14 ± 3[b]	<0.001	0.30 ± 0.05[b]	1.19 ± 0.05[b]	<0.001	14 ± 1[b]	8 ± 2[a]	0.02
Resistant	AKR	46 ± 13[b]	10 ± 2[b]	<0.001	0.22 ± 0.02[a]	1.00 ± 0.04[a]	<0.001	13 ± 1[a,b]	9 ± 1[a]	0.02

Values represent the mean ± SEM. Values within columns that do not share superscripts (a and b) are significantly different ($P < 0.01$) from each other as determined by one-way analysis of variance followed by Fischer's least-significant difference test.

*Activities based on female mice fed the chow or lithogenic (Lith) diet for 6 weeks. HMGR and C7H, nine mice per group; ACAT, five mice per group.

†Probability that enzyme activities in chow-fed compared to lithogenic diet-fed animals is significantly different; as evaluated by unpaired *t*-tests.

‡Activity of C7H for mice fed the lithogenic diet was reported incorrectly in an abstract (47); C7H values in that abstract should be divided by 10.

susceptible and resistant strains on either diet. C7H activities did not differ between susceptible or resistant strains on either diet, but activity decreased 20–50% in mice fed the lithogenic diet (Table 2). Mean C7H activities did not differ between susceptible and resistant strains on the lithogenic diet (7.5 vs. 8.5 pmol/min/mg of protein, respectively).

Genetic Analysis of Gallstone Formation. The strains in Table 1 are progenitors of RI strain sets, a genetic tool in the mouse useful for analyzing complex traits (25). The greatest differences in gallstone susceptibility between progenitors of RI sets are shown by the pairs of strains AKR and C57L (the AKXL RI strain set), by C57BL/6 and A (the AXB, BXA RI strain set), and by SJL and SWR (the SWXJ RI strain set). The choice of which RI set to study further was guided by a previous study that examined these strains for liver weight, liver lipids, and plasma alanine aminotransferase activity, an indicator of liver injury (17). Of the gallstone susceptible strains, only C57L mice showed little evidence of liver injury, so we chose the AKXL RI strain set to avoid any chance of liver injury obscuring the genetic analysis.

Strain C57L males developed gallstones earlier than females (data not shown), so the remaining experiments employed male mice. With respect to CSI at 2 weeks, bile from AKR mice was not saturated on either diet (CSI = 0.4 on chow and 0.8 on lithogenic diet), but bile from C57L mice was supersaturated (CSI = 1.2) in animals fed the lithogenic diet compared with chow (CSI = 0.6). Next, we fed the lithogenic diet to male mice of strains AKR, C57L, and F_1 (AKR × C57L) progeny, sacrificing groups of mice at 0, 4, 6, and 8 weeks (Table 3). Two-thirds of the C57L mice had gallstones at 4 and 6 weeks, and all C57L mice formed gallstones by 8 weeks. Gallbladder volume and gallstone weight increased in C57L mice over the 8 weeks. Gallstone formation is clearly a dominant trait because both prevalence and weight of gallstones in F_1 mice were similar to the C57L parent. However, gallbladder volume in F_1 mice was intermediate in size compared to the parental strains.

Groups of eight male mice from each AKXL RI strain were fed the lithogenic diet and examined for gallstones after 8 weeks. In general, the RI strains segregated into two groups; those with no gallstones like the AKR progenitor and those with gallstones like the C57L progenitor. Only six of eight mice from strains AKXL-29 and AKXL-38 had gallstones, but the remaining mice in these strains exhibited abnormal gallbladders with a thickened white fundus and colorless bile, consistent with chronic cystic duct obstruction, and these gallbladders had accumulated viscous biliary sludge (26). The distribution of RI strains was 5 resistant and 10 susceptible (Fig. 1). Average gallstone weight among the RI strains varied from 0.04 to 2.0 mg per mouse (Table 4). No correlation between gallstone susceptibility and gallbladder volume was observed among the RI strains ($r = 0.4$, NS), indicating that the putative genetic factors affecting gallbladder size could differ from the genetic factors affecting gallstone formation. Variations in gallbladder size could also be influenced by the extent of

Table 3. Formation of gallstones in male mice

Weeks on diet	Strain	n/N*	Gallbladder vol, μl	Gallstone wt, mg
0	AKR	0/5	6 ± 1	0
0	C57L	0/5	8 ± 1	0
4	C57L	5/7	39 ± 7	0.1 ± 0.1
6	C57L	5/8	56 ± 16	0.2 ± 0.1
8	C57L	10/10	86 ± 12	1.0 ± 0.3
8	AKR	0/6	9 ± 1	0
8	F_1	10/10	24 ± 5	1.2 ± 0.1

All measurements are given as the mean ± SEM.
*n/N = number of mice that formed gallstones/total number of mice surviving dietary treatment.

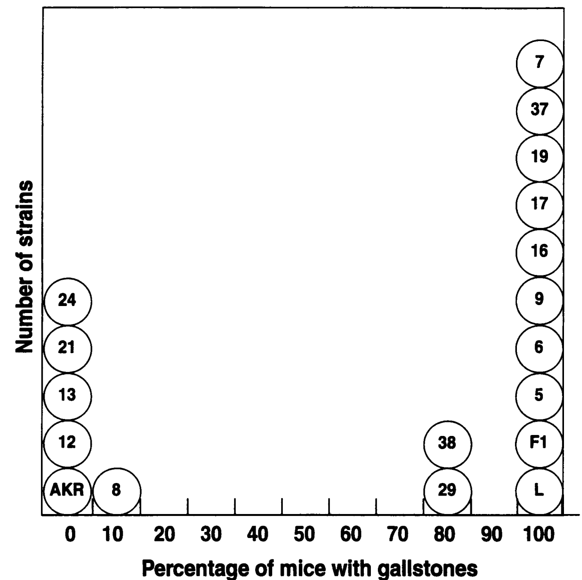

FIG. 1. Percentage of mice with cholesterol gallstones in AKXL RI strains. Each circle represents a group of eight male mice from a given strain. The strain abbreviations are L for C57L and F_1 for the progeny of a cross between AKR and C57L. The numbers in the remaining circles are abbreviations for RI strains; i.e., 24 is AKXL-24, 21 is AKXL-21, etc.

mucosal absorption of cholesterol and toxic injury to smooth muscle cell function (26).

The distribution of AKXL RI strains into two groups resembling one or the other progenitor strains (Fig. 1) was consistent with the hypothesis that the difference in gallstone susceptibility between the progenitor strains is determined by a single gene. If so, gallstone susceptibility among backcross animals should segregate into two equal groups for the presence or absence of gallstones. To test this, F_1 mice were backcrossed to the resistant parent AKR, and male progeny were examined for cholelithiasis after 8 weeks of lithogenic diet consumption. Among 135 backcross progeny, 102 had gallstones and 33 did not, a ratio of 3:1, which is significantly different ($P < 0.0001$) from the equal numbers expected if a single gene determined the trait. The weight of the gallstones among backcross mice varied from 0.1 to 5.8 mg of gallstones

Table 4. Gallbladder volume and gallstone weight among AKXL RI strains

Mouse strain	Gallbladder vol, μl	Gallstone wt, mg
AKXL-5	7 ± 2	0.5 ± 0.2
AKXL-6	18 ± 4	1.0 ± 0.2
AKXL-7	74 ± 13	1.8 ± 0.4
AKXL-8	11 ± 2	<0.01*
AKXL-9	18 ± 3	0.8 ± 0.4
AKXL-12	5 ± 1	0
AKXL-13	7 ± 1	0
AKXL-16	83 ± 6	1.1 ± 0.4
AKXL-17	14 ± 3	0.4 ± 0.1
AKXL-19	65 ± 20	0.04 ± 0.02
AKXL-21	36 ± 23	0
AKXL-24	14 ± 2	0
AKXL-29	28 ± 6	2.0 ± 0.6
AKXL-37	18 ± 3	0.1 ± 0.03
AKXL-38	25 ± 7	0.7 ± 0.3

All measurements are given as the mean ± SEM.
*Cholesterol crystals from this mouse were too small to weigh precisely.

per mouse, which is similar to the weights in the RI strains (Table 4).

We tested 33 backcross mice without gallstones and 22 mice with the largest gallstones (>2 mg per mouse) for *D13Mit53* and *D13Mit78*, two polymorphisms flanking the HMGR structural gene on chromosome 13 (27), but no linkage was found, indicating that a gene affecting gallstone formation does not map at HMGR (Table 5). However, a genome-wide search of polymorphic markers did reveal a highly significant association ($P < 0.0005$) between gallstone formation and a region on chromosome 2 defined by the polymorphic markers *D2Mit11* and *D2Mit66*, which are 4 centimorgans apart (Table 5). We have chosen the name *Lith1* for this major lithogenic gene.

DISCUSSION

This report demonstrates that gallstone formation is genetically determined and that susceptibility to gallstones is a dominant trait. A backcross to the resistant parent gave a 3:1 distribution of susceptible to resistant mice among backcross progeny. The most parsimonious interpretation of this distribution is that two unlinked genes determine the difference in susceptibility to gallstones between strains AKR and C57L, but other interpretations with more complex genetics and more genes are also possible. Genotyping the entire backcross as well as examining additional crosses between other susceptible and resistant strains should provide information as to the number and location of genes affecting gallstones. The better our understanding of the genetics of gallstone formation in mice, the easier it will be to elucidate the genetic determinants of this disease in humans (7, 10, 11).

Since HMGR activity appeared to be important in gallstone formation, we examined whether any genetic determinant of gallstones was located in the region of the structural gene for HMGR. Regulatory elements controlling the transcriptional regulation of HMGR are located in the proximal 5′ flanking region of this gene, in particular the sequence known as SRE-1, which determines the responsiveness to sterols (28). We found no evidence for a gene affecting gallstones near HMGR on chromosome 13. This is consistent with a previous report that HMGR activity is controlled by a genetic locus not linked to the structural gene (27). Since HMGR is regulated at multiple levels including transcription, translation, and turnover, there are several opportunities for mutations that alter the concentration of a trans-acting regulatory factor. The failure of gallstone-susceptible mice to downregulate HMGR activity in the presence of dietary cholesterol (Table 2) is reminiscent of the mutant Chinese hamster ovary cell lines that failed to downregulate HMGR in the presence of 25-hydroxycholesterol (29, 30).

The major gene affecting gallstone formation, *Lith1*, maps to chromosome 2, as demonstrated by a distribution of AKR and C57L alleles that differs significantly from that expected using the contingency table χ^2 analysis. We did not use the

Table 5. Single sequence-length polymorphisms in backcross mouse progeny

	Resistant		Susceptible	
Marker	AKR/AKR	AKR/L	AKR/AKR	AKR/L
D13Mit53	17	16	12	10
D13Mit78	16	17	13	9
D2Mit64	9	9	5	17*
D2Mit11	25	8*	5	17*
D2Mit66	25	8*	5	17*
D2Mit109	8	10	10	12

*The distribution of homozygous or heterozygous AKR alleles among mice resistant or susceptible to gallstones is significantly different from 1:1 as determined by contingency table χ^2 analysis ($P < 0.01$).

quantitative trait loci mapping statistics (31) contained in the computer program MAPMAKER QTL (32), which was designed for the analysis of quantitative rather than qualitative phenotypes such as gallstones. Further analysis using interval mapping techniques adopted for use with qualitative traits (N. J. Schork, J. A. Lonmgate, T. Morel, and E. K. Wakeland, personal communication) suggests that a logarithm of odds score of 4.1 could be assigned to the putative gallstone susceptibility locus on chromosome 2 (N. J. Schork and B.P., unpublished observations). No known regulator of HMGR maps to this region of chromosome 2.

It is believed that the primary defect in human gallstone formation is hypersecretion of cholesterol into bile (24, 33). The secretion of cholesterol from the liver is affected by several factors, which include (*i*) activity of LDL receptors, which transport cholesterol from plasma lipoproteins into the liver; (*ii*) *de novo* synthesis of cholesterol by the rate-limiting enzyme HMGR, an enzyme activity elevated in most gallstone patients (34–37); (*iii*) conversion of cholesterol to bile salts by the rate-limiting enzyme C7H; (*iv*) conversion of cholesterol into cholesteryl esters controlled by the rate-limiting enzyme ACAT; and (*v*) excess dietary cholesterol. We did not measure the level of low density lipoprotein receptors, but these have been reported to be coordinately regulated with HMGR in mice (38). Mice resistant to gallstone formation downregulated HMGR when consuming a lithogenic diet, but the susceptible strains did not (Table 2). This suggests that HMGR activity may be pivotal in determining whether gallstones are formed in the mouse model. This hypothesis could be tested by using one of the many inhibitors of HMGR activity. Inhibiting cholesterol biosynthesis should reduce the saturation index of bile and prevent or greatly diminish gallstone formation in susceptible mice. Two recent studies are of direct relevance in this connection. Administration of lovastatin, an HMGR inhibitor, markedly decreased the saturation index of bile in human subjects (39). Furthermore, Kern (40) demonstrated that cholesterol synthesis is higher in peripheral monocytes from gallstone patients compared to controls. Both of these observations are consistent with the higher hepatic HMGR activity we observed in gallstone-susceptible compared to gallstone-resistant mice (Table 2). In two human studies (36, 37), the magnitude of the HMGR activity increase in gallstone patients compared to controls was as much as 2-fold, which is similar to the difference we observed in mice (Table 2).

The lithogenic diet decreased C7H activities by a small but significant extent in three of the four strains. Again this is consistent with human data, which shows about a 20% decrease in bile acid synthesis in gallstone patients compared to controls when both were consuming a high cholesterol diet (40). The expression of C7H mRNA and C7H activity are upregulated by cholesterol feeding and downregulated by dietary taurocholate (34, 41) or its 7α dehydroxylation product, deoxycholate. After absorption, cholic acid is conjugated intrahepatically and becomes taurocholate, which replaces muricholates in the mouse bile, thereby substituting a good cholesterol solubilizer for poor ones (19). Nevertheless Dueland *et al.* (41) have suggested that taurocholate does not affect cholesterol absorption but does downregulate C7H activity in mice. The control of C7H activity is highly complex and is controlled by many factors in rodents including hepatic cholesterol flux, which upregulates activity, and enterohepatic bile salt flux, which downregulates activity in proportion to hydrophobicity (42, 43).

Tepperman and coworkers (44, 45), who first reported gallstone formation in mice, found that both cholesterol and cholic acid were essential factors. Although we found cholic acid to be necessary to produce gallstones in the 8- to 18-week feeding time of the present studies, we have not fed C57L mice a high cholesterol diet without cholic acid for more extended times. With atherosclerosis, we found that cholic acid was

required to produce lesions in an experimental time of 4–6 months, but atherosclerotic lesions did form eventually by 18 months when C57BL/6 mice were fed a high cholesterol diet without cholic acid (B.P., unpublished observations). These long experimental times, which extend to half the lifetime of the mouse, are more comparable to the times required for gallstone formation and atherosclerosis development in humans. To determine the relative importance of dietary cholic acid for gallstone formation in the mouse model, it would be interesting to feed C57L mice a lithogenic diet without cholic acid for prolonged periods.

Although the same diet produces atherosclerosis and gallstones in mice and although genetic factors now clearly control susceptibility to both diseases (1), no correlation between susceptibility to gallstones and atherosclerosis was found. Moreover, no correlation between plasma cholesterol and gallstones among mouse strains was observed, confirming the failure to find such a relationship in human populations (46). However, the suggested relationship between the non-HDL-cholesterol fractions and susceptibility to gallstones, which failed to reach statistical significance ($P = 0.09$), is interesting enough to suggest a repeat study with additional strains and a greater number of mice from each strain.

This report identifying a major gene affecting cholesterol gallstone formation in a laboratory animal opens the door to a new experimental approach to gallstone disease. This includes a variety of genetic manipulations that are possible in the mouse, such as the use of transgenic, knockout, and mutant mice to test specific hypotheses concerning cholesterol gallstone formation. Even though results obtained with an animal model must be extrapolated to humans with caution, development of the mouse as a genetic model for cholesterol gallstones should lead to a greater understanding of the critical factors underlying the prevalence and pathophysiology of this common disease in humans.

We thank Debra Cromely and Kathy Kieras for technical assistance and Camille Falkner and Sarah Mukta, students in The Jackson Laboratory Summer Program, for pilot studies on the AKXL RI strains. Support for this work came from National Institutes of Health Grants RR8911, HL32087, DK36588, and DK34854 and Grant BG88-192 from the National Dairy Promotion and Research Board administered by the National Dairy Council.

1. Paigen, B., Ishida, B. Y., Verstuyft, J., Winters, R. B. & Albee, D. (1990) *Arteriosclerosis* **10,** 316–323.
2. Liepa, G. U., Gorman, M. A. & Duffy, A. M. (1988) in *Use of Animal Models for Research in Human Nutrition*, eds. Beynen, A. C. & West, C. E. (Karger, Basel), pp. 149–173.
3. Johnston, D. E. & Kaplan, M. M. (1993) *N. Engl. J. Med.* **328,** 412–421.
4. Alexander, M. & Portman, O. W. (1987) *Hepatology* **7,** 257–265.
5. Fujihara, E., Kaneta, S. & Oshima, T. (1978) *Biochem. Med.* **19,** 211–217.
6. Jackson, C. D. & Gay, B. C. (1959) *Surgery* **46,** 853–857.
7. Van der Linden, W. & Lindelof, G. (1965) *Acta Genet. Stat. Med.* **15,** 159–164.
8. Jørgensen, T. (1988) *J. Biosocial Sci.* **20,** 111–120.
9. Gilat, T., Feldman, C., Halpern, Z., Dan, M. & Bar-Meir, S. (1983) *Gastroenterology* **84,** 242–246.
10. Van der Linden, W. (1973) *Clin. Gastroenterol.* **2,** 603–614.
11. Kesäniemi, Y. A., Kosekenvuo, M., Vuoristo, M. & Miettinen, T. A. (1989) *Gut* **30,** 1750–1756.
12. Danzinger, R. G., Gordon, J., Schoenfield, L. J. & Thistle, J. L. (1972) *Mayo Clin. Proc.* **47,** 762–766.
13. Sampliner, R. E., Bennett, P. H., Comess, L. J., Rose, F. A. & Burch, T. A. (1970) *N. Engl. J. Med.* **283,** 1358–1364.
14. Weiss, K. M., Ferrell, R. E., Hanis, C. L. & Styne, P. N. (1984) *Am. J. Hum. Genet.* **36,** 1259–1278.
15. Nishina, P. M., Verstuyft, J. & Paigen, B. (1990) *J. Lipid Res.* **31,** 859–869.
16. Goh, E. H., Colles, S. M. & Otte, K. D. (1989) *Lipids* **24,** 652–655.
17. Nishina, P. M., Wang, J., Toyofuku, W., Kuypers, F. A., Ishida, B. Y. & Paigen, B. (1993) *Lipids* **28,** 599–605.
18. Cohen, D. E., Leighton, L. S. & Carey, M. C. (1992) *Am. J. Physiol.* **263,** G386–G395.
19. Carey, M. C. (1978) *J. Lipid Res.* **19,** 945–955.
20. Billheimer, J. T., Tavani, D. M. & Nes, W. R. (1981) *Anal. Biochem.* **111,** 331–335.
21. Favata, M. F., Trzaskos, J. M., Chen, H. W., Fischer, R. T. & Greenberg, R. S. (1987) *J. Biol. Chem.* **262,** 12254–12260.
22. Einarsson, K., Angelin, B., Ewerth, S., Nielsel, K. & Bjorken, I. (1986) *J. Lipid Res.* **27,** 80–88.
23. Sato, R., Goldstein, J. L. & Brown, M. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9261–9265.
24. Carey, M. C. & LaMont, J. T. (1992) *Prog. Liver Dis.* **10,** 139–163.
25. Taylor, B. (1978) in *Origins of Inbred Mice*, ed. Morse, H. C. I. (Academic, New York), pp. 423–432.
26. LaMont, J. T. & Carey, M. C. (1992) *Prog. Liver Dis.* **10,** 165–191.
27. Hwa, J. J., Zollman, S., Warden, C. H., Taylor, B. A., Edwards, P. A., Fogelman, A. M. & Lusis, A. J. (1992) *J. Lipid Res.* **33,** 711–725.
28. Goldstein, J. L. & Brown, M. S. (1990) *Nature (London)* **343,** 425–430.
29. Leonard, S. & Sinensky, M. (1988) *Biochim. Biophys. Acta* **947,** 101–112.
30. Metherall, J. E., Goldstein, J. L., Luskey, K. L. & Brown, M. S. (1989) *J. Biol. Chem.* **263,** 18480–18487.
31. Lander, E. S. & Botstein, D. (1989) *Genetics* **121,** 185–199.
32. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M., Lincoln, S. & Newburg, L. (1987) *Genomics* **1,** 174–181.
33. Carey, M. C. (1993) *Recent Prog. Med.* **83,** 379–391.
34. Salen, G., Nicolau, G., Shafer, S. & Mosbach, E. H. (1975) *Gastroenterology* **69,** 676–684.
35. Coyne, M. J., Bonorris, G. G., Goldstein, L. I. & Schoenfield, L. J. (1976) *J. Lab. Clin. Med.* **87,** 281–291.
36. Maton, P. N., Ellis, H. J., Higgins, M. J. P. & Dowling, R. H. (1980) *Eur. J. Invest.* **10,** 325–332.
37. Key, P. H., Bonorris, G. G., Marks, J. W., Chung, A. & Schoenfield, L. J. (1980) *J. Lab. Clin. Med.* **95,** 816–826.
38. Rudling, M. (1992) *J. Lipid Res.* **33,** 493–501.
39. Duane, W. C. (1994) *J. Lipid Res.* **35,** 501–509.
40. Kern, F., Jr. (1994) *J. Clin. Invest.* **93,** 1186–1194.
41. Dueland, S., Drisko, J., Graf, L., Machleder, D., Lusis, A. J. & Davis, R. (1993) *J. Lipid Res.* **34,** 923–931.
42. Vlahcevic, Z. R., Pandak, W. M., Heuman, D. M. & Hylemon, P. B. (1992) *Semin. Liver Dis.* **12,** 403–419.
43. Ramirez, M. I., Karaoglu, D., Haro, D., Barillas, C., Bashizadek, R. & Gil, G. (1994) *Mol. Cell. Biol.* **14,** 2809–2821.
44. Tepperman, J., Caldwell, F. T. & Tepperman, H. M. (1964) *Am. J. Physiol.* **206,** 628–634.
45. Pedreira, F. & Tepperman, J. (1964) *Am. J. Physiol.* **206,** 635–640.
46. Thijs, C., Knipschild, P. & Brombacher, P. (1990) *Gastroenterology* **99,** 843–849.
47. Paigen, B., Chen, H. W., Billheimer, J. T. & Carey, M. C. (1994) *Hepatology* **20,** 206 (abstr.).