The Diet1 Locus Confers Protection against Hypercholesterolemia through Enhanced Bile Acid Metabolism*

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The C57BL/6ByJ (B6By) mouse strain is resistant to diet-induced hypercholesterolemia and atherosclerosis, despite its near genetic identity with the atherosclerosis-susceptible C57BL/6J (B6J) strain. We previously identified a genetic locus, Diet1, which is responsible for the resistant phenotype in B6By mice. To investigate the function of Diet1, we compared mRNA expression profiles in the liver of B6By and B6J mice fed an atherogenic diet using a DNA microarray. These studies revealed elevated expression levels in B6By liver for key bile acid synthesis proteins, including cholesterol 7α-hydroxylase and sterol-27-hydroxylase, and the oxysterol nuclear receptor liver X receptor α. Expression levels for several other genes involved in bile acid metabolism were subsequently found to differ between B6By and B6J mice, including the bile acid receptor farnesoid X receptor, oxysterol 7α-hydroxylase, sterol-12α-hydroxylase, and hepatic bile acid transporters on both sinusoidal and canalicular membranes. The overall expression profile of the B6By strain suggests a higher rate of bile acid synthesis and transport in these mice. Consistent with this interpretation, fecal bile acid excretion is increased 2-fold in B6By mice, and bile acid levels in blood and urine are elevated 3- and 18-fold, respectively. Genetic analysis of serum bile acid levels revealed co-segregation with Diet1, indicating that this locus is likely responsible for both increased bile acid excretion and resistance to hypercholesterolemia in B6By mice.

Hypercholesterolemia is a major risk factor for coronary heart disease. Variations in human plasma cholesterol levels result from both genetic and environmental factors. Although behavioral changes such as lower dietary intake of cholesterol and saturated fatty acids or exercise may alleviate many environmental causes of hypercholesterolemia, genetic factors still account for over 50% of the variation in plasma lipid levels in the human population (1–4). Identifying the genetic components involved in the determination of plasma lipid levels is relevant in understanding the underlying mechanisms involved in complex human diseases such as atherosclerosis, obesity, gall stone formation, and stroke. Because of the relative ease of environmental manipulation and abundance of genetic information, inbred mouse strains provide a useful tool to study these genetic factors.

We previously identified a locus on mouse chromosome 2, Diet1, which is associated with variation in cholesterol levels and susceptibility to atherosclerosis in closely related mouse strains (5). Different alleles at the Diet1 locus that occur in two C57BL/6 mouse strains, C57BL/6J (B6J) and C57BL/6ByJ (B6By), confer strikingly different responses to an atherogenic (Ath) diet. B6J mice fed an Ath diet develop plasma cholesterol levels of ~200 mg/dl and aortic lesions that are among the largest found in standard inbred mouse strains (6). In contrast, B6By mice are largely resistant to diet-induced hypercholesterolemia and aortic lesions. This is associated with lower LDL and VLDL cholesterol levels on the Ath diet, which reach only 40 mg/dl in B6By mice compared with 120 mg/dl in B6J mice (5). The difference in cholesterol response between B6J and B6By mice is particularly striking given the near genetic identity between the two strains; B6J and B6By were isolated as separate breeding stocks of C57BL/6 in the 1960s (~100 generations ago (7)). In fact, in screening 100 microsatellite markers spanning the genome and 20 markers concentrated within the Diet1 locus, no polymorphism has been detected between B6By and B6J mice (5). Thus, variation at the Diet1 locus in the two strains appears to have resulted from de novo mutation rather than genetic contamination from another mouse strain. Scrutiny of known genes mapping to the Diet1 locus, and to the homologous region in the human genome, has revealed no obvious candidate genes, suggesting that Diet1 may represent a novel gene(s) involved in lipid metabolism.

Many metabolic pathways influence plasma cholesterol homoeostasis, including cholesterol biosynthesis, dietary absorption, uptake by liver and peripheral cells, and clearance from the liver through bile acid metabolism (8–12). We previously determined that food consumption and cholesterol absorption are similar in B6J and B6By mice, suggesting that differences in these factors are not responsible for the lower plasma cholesterol levels in B6By animals (5). In the current study, we have sought to gain insight into the genetic variation between B6By and B6J mice by examining the expression of more than 18,000 genes. We detected elevated expression of genes for

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1 The abbreviations used are: B6J, C57BL/6J; B6By, C57BL/6ByJ; C7AH, cholesterol 7α-hydroxylase; Ath diet, atherogenic diet; LXRα, liver X receptor α; FXR, farnesoid X receptor; SREBP, small heterodimer partner; LRH-1, liver receptor homologue-1; S27H, sterol-27-hydroxylase; OATP, organic anion transporting polypeptide; OAT, organic anion transporting polypeptide; NTCP, Na+-taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; mEH, microsomal epoxide hydrolase; mdr2, multidrug-resistant-2; BSEP, bile salt export pump; 3α-HSD, 3α-hydroxysteroid dehydrogenase; BCB, [B6By × CAST/× B6By] cross; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; RT, reverse transcription; nt, nucleotide(s); cM, centimorgan(s).

2 J. Phan and K. Reue, unpublished data.
of seven proteins involved in bile acid synthesis, including cholesterol-7α-hydroxylase (C7AH) and sterol-27-hydroxylase, the rate-limiting enzymes in the classic and alternative bile acid synthetic pathways (13), and LXRα, which regulates gene expression in response to oxysterols (14). B6By mice also exhibited altered expression levels for additional bile acid synthetic enzymes, bile acid transporters from both the sinusoidal and canalicular membranes, and the bile acid-responsive nuclear receptor, farnesoid X receptor (FXR). These results suggest that more efficient conversion of cholesterol to bile acids and increased bile secretion may contribute to the lower plasma cholesterol levels in B6By mice. In support of this interpretation, B6By mice exhibited increased fecal bile acid excretion and elevated bile acid levels in plasma and urine. Using a genetic cross, we determined that elevated bile acid levels segregate with Diet1, indicating that this locus determines both the resistance to hypercholesterolemia and increased bile acid excretion in B6By mice.

**Experimental Procedures**

**Mice and Diets—**C57BL/6J, C57BL/6ByJ, and CAST/EiJ mice were obtained from the Jackson Laboratory, ME. B6By mice were maintained in a 14-h light/10-h dark cycle and fed ad libitum Purina Mouse Chow 5001 (chow) or an atherogenic diet containing 75% chow, 7.5% cocoa butter, 1.25% cholesterol, and 0.5% sodium cholate (T90221, Teklad Research Diets, Madison, WI). All animals received human care under an institutionally approved experimental animal protocol as outlined in the Guide for the Care and Use of Laboratory Animals.

**Gene Expression Array Hybridization—**Mouse Gene Discovery Array I filters containing 18,378 mouse cDNA clones chosen from the I.M.A.G.E. collection were obtained from Genome Systems Inc. (St. Louis, MO). These arrays contain each mouse cDNA in a double-spotted pattern, as well as 30 double-spotted hybridization controls and 24 double-spotted orientation markers. Hybridization probes were prepared from poly(A)+ RNA isolated from liver of male mice fed the Ath diet for 3 weeks (Poly(A)Tract mRNA isolation system; Promega, Madison, WI). An equal amount of RNA from three mice of each strain was pooled to reduce spurious results because of individual variation. RNA (2 μg) was labeled with [32P]dATP to a specific activity of 7 x 106 cpmp/μg by incubation with 200 units of Moloney murine leukemia virus reverse transcriptase and random hexamer primers (Invitrogen). Unincorporated nucleotides were removed by passing through Sephadex G-50 spin columns (Bio-Rad). Labeled probes (106 cpmp/μl) were combined with 1.5 mg of sheared salmon testes DNA (Sigma) and 5 μg of C5,t-1 DNA (Invitrogen), and a labeled orientation marker (RNA corresponding to Arabidopsis and Drosophila internal control clones spotted on the filters; Genome Systems). DNA was denatured at 95 °C and incubated in roller bottles for 30 min. Filters were then hybridized to labeled laboratory workstation (Beckman Instruments, Inc., Fullerton, CA) (18). (LDL + VLDL) cholesterol levels were determined as the difference between total cholesterol and HDL cholesterol. Bile acids were quantitated using an enzymatic assay (Sigma Diagnostics, St. Louis, MO) using 50 μl of plasma or 25 μl of urine. Fecal bile acid determinations were made after extraction of bile acids using a radioactivity and random primer method (19).

**Generation of (B6By × CAST)/B6By Cross—**We crossed male C57BL/6By and female CAST/EiJ mice to produce F1 animals, which were subsequently crossed to C57BL/6ByJ mice. At 2 months of age, mice were fed the Ath diet for 3 weeks. Blood samples obtained before and after the diet were used to determine VLDL/LDL cholesterol levels and bile acid concentrations as described above. Genomic DNA was isolated from tails of mice by phenol/chloroform extraction. Genotyping was performed by PCR using Mouse MapPairs primer sets (Research Genetics) listed in Table I.

**Genetic Mapping Studies—**Chromosomal localization of candidate genes was determined using a mouse-hamster radiation hybrid panel (Research Genetics) and screening with gene-specific primers, usually taken from the 3′-untranslated region of the corresponding gene. Primers were as follows: Cyp8b1, AGCAGAACATGAATCTCCAC (forward primer) and TGAGTGCCACATGAGTCC (reverse primer); Cyp27, GAAGCTGACATGAGTCC (forward primer) and AGTGGTGGCTGGCAGT (reverse primer); Oatp-1 (Slc21a10), ATGATATGACAGGAGAGGAGAGAGG (forward primer) and CCACTCAGCTGTGGT (reverse primer); Lxrα, ATGGCAAGGGGTGGAGAGATG (forward primer) and GTCTGCTCTCAAGAC (reverse primer). Primers were designed for genotyping studies. Genotyping was performed by PCR using Mouse MapPairs primer sets (Research Genetics) listed in Table I.

**RESULTS**

**Elevated Expression of Bile Acid Metabolism Genes in B6By Mice—**Our previous work established that resistance to diet-induced hypercholesterolemia and atherosclerosis is not a result of reduced food consumption or reduced cholesterol...
terol absorption compared with B6J mice (5). To identify differences in gene expression that may reflect metabolic differences between the two strains, we performed gene expression profiling using a DNA array. Hepatic mRNA isolated from B6J and B6By mice fed the Ath diet for 3 weeks was hybridized to arrays containing more than 18,000 mouse cDNA sequences, approximately one-third of which represent known genes and the remainder derived from expressed sequence tags (ESTs). Of the 18,000 genes represented on the array, only 35 (0.19%) differed in expression levels by more than 10-fold between B6J and B6By mice; 80% of the genes in this category were expressed at higher levels in B6J than in B6By. 622 mRNA species (3.5%) exhibited expression levels that differed more than 3-fold between the two strains, with 1.2% having higher expression in B6By and 2.3% having higher expression in B6J.

Based on fold differences in expression levels, we chose the 100 mRNAs having higher expression levels in B6J and the 100 having higher expression levels in B6By for further analysis. Comparison to data base sequences revealed that 29 of the 200 correspond to known mouse genes, 65 are ESTs that appear to be homologous of genes identified in other species, and 106 are ESTs of unknown function. Among the known mouse genes, we recognized three with prominent roles in bile acid synthesis, Cyp7a1, Cyp27, and LXRα. Cyp7a1 encodes C7AH, and Cyp27 encodes sterol-27-hydroxylase, enzymes that catalyze the rate-limiting steps in conversion of cholesterol to bile acids via the classic and alternative pathways, respectively (reviewed in Refs. 20 and 13). LXRα encodes liver X receptor α (LXRα), a nuclear receptor that is activated by oxysterols to stimulate Cyp7a1 transcription and, thus, bile acid synthesis (reviewed in Refs. 9 and 21).

It has been shown that C7AH activity levels change in parallel with C7AH mRNA levels (22–24). To confirm the results of the DNA array experiment and to obtain absolute quantitation of C7AH mRNA levels, we performed competitive RT-PCR (15). Consistent with the array data, the C7AH mRNA levels were 3-fold higher in B6By than B6J mice fed the Ath diet (218 versus 61 pM, Fig. 1). Furthermore, B6By mRNA levels were higher in mice fed a chow diet (615 versus 227 pM), indicating that the difference between the strains is evident even under basal conditions and is not diet-dependent. In agreement with previous findings, the Ath diet repressed C7AH mRNA levels by 70–80% in both strains (13, 25, 26). The expression difference between the two strains for sterol-27-hydroxylase (S27H) and LXRα mRNAs seen on the array were confirmed by Northern blot analysis (Fig. 2). As with C7AH, mRNA levels for these genes were higher in B6By mice on both chow and Ath diets, with differences of 1.5–2.5-fold.

The detection of higher C7AH and S27H mRNA levels in B6By versus B6J mice prompted us to examine expression of additional genes involved in bile acid synthesis. Oxysterol-7α-hydroxylase (O7AH) is the mitochondrial counterpart to C7AH and functions to catalyze the C-7 hydroxylation of 27-hydroxylated bile acids (25). We observed elevated expression of O7AH in B6By, with 70% higher levels on the chow diet, and 50% higher levels on the Ath diet (Fig. 2). We also examined mRNA levels for sterol-12α-hydroxylase, the enzyme responsible for the C-12 hydroxylation of chenodeoxycholic acid to form cholic acid.

![Fig. 1. Elevated C7AH mRNA levels in B6By liver. A competitive RT-PCR assay was used to determine absolute amounts of C7AH mRNA in liver of B6J and B6By mice fed the chow and Ath diets. Liver RNA was converted to cDNA in the presence of serial dilutions of a synthetic competitor RNA, and the resulting cDNA samples were PCR-amplified using a single primer pair that amplifies both the liver C7AH mRNA and competitor samples. RT-PCR products were quantitated, and the ratio of product produced from sample target to competitor was plotted against the concentration of competitor RNA (pM). The concentration of C7AH in liver samples was determined as the point at which the ratio of sample RNA to competitor was equal to 1 (represented by dashed lines in graphs). Panels a–d show a representative determination from one of five animals of each strain and diet treatment. a and b, B6J (a) and B6By (b) mice on chow diet. c and d, B6J (c) and B6By (d) mice on Ath diet.](http://www.jbc.org/thesis.Sleep/471/Fig.1)
mRNA levels for this enzyme were slightly higher in B6By mice on a chow diet, but slightly lower in B6By on the Ath diet (Fig. 2). Thus, the genes for four enzymes involved in bile acid synthesis had a higher basal expression level in chow-fed B6By compared with B6J mice. Although the Ath diet repressed expression in both strains, mRNA levels remained higher in B6By liver for three of the enzymes, C7AH, C27H, and O7AH.

As described above, we observed a modest increase in mRNA levels for the nuclear receptor LXRα in the liver of B6By mice. We subsequently examined expression levels for three additional nuclear receptors that, together with LXRα, serve as sensors for the regulation of cholesterol and bile acid metabolism in the cell: the FXR, small heterodimer partner (SHP), and LRH-1 (27). FXR acts as a bile acid receptor, maintaining homeostasis in response to increased bile acid levels by repressing genes involved in bile acid synthesis. In contrast to LXRα, FXR mRNA levels were reduced ~50% in B6By compared with B6J on both chow and Ath diets (Fig. 2). SHP, which is a key target gene of FXR, showed 6-fold lower mRNA levels in liver of B6By mice on the chow diet, but levels increased on the Ath diet to nearly the same as B6J mice, in which SHP levels did not change substantially in response to diet (Fig. 2). LRH-1 acts as a competence factor to promote C7AH expression in the liver, but becomes inactivated upon formation of a heterodimeric complex with SHP. LRH-1 mRNA expression in B6By compared with B6J liver was reduced slightly on a chow diet, and by about 50% on the Ath diet (Fig. 2). The lower levels of FXR and SHP expression in B6By liver are consistent with less robust repression of C7AH expression in B6By mice. In light of the higher C7AH mRNA levels in B6By mice, the somewhat lower LRH-1 expression levels are unexpected, but suggest that complex regulatory interactions may occur in the liver under the conditions produced by feeding the atherogenic diet.

We also investigated genes involved in the hepatic transport of bile acids and found increased mRNA levels for several membrane and intracellular hepatic bile acid transporters in B6By mice. Distinct transporters on the sinusoidal membrane mediate hepatic bile acid uptake from the enterohepatic circulation by sodium-dependent and sodium-independent processes. We examined expression levels of three of these transporters: the sodium/taurocholate cotransporting polypeptide (NTCP), the mouse homologue of the rat liver-specific organic anion transporting polypeptide-1 (OATP-1), and microsomal epoxide hydrolase (mEH). Expression levels for NTCP and OATP-1 were higher in the liver of chow-fed B6By than B6J mice by 90 and 40%, respectively (Fig. 2), whereas mEH levels were similar for the two strains (data not shown). On the Ath diet, NTCP expression was diminished in both strains, whereas OATP-1 expression levels increased, with B6By maintaining 2-fold higher mRNA levels. Expression of mEH was unchanged by the Ath diet (not shown). We also measured expression levels of two canalicular membrane proteins involved in hepatobiliary excretion, the bile salt export pump (BSEP; also known as sister of P glycoprotein, Spgp) and the multidrug-resistant 2 (mdr2) phospholipid translocator. BSEP is a member of the ABC transporter family and mediates ATP-dependent bile acid efflux from the canalicular membrane, which is considered the rate-determining step in bile acid secretion (28,

![Fig. 2. Differential expression of bile acid synthesis and transport genes in B6By and B6J liver.](http://www.jbc.org/)

Hepatic mRNA levels for proteins involved in bile acid metabolism were determined by RT-PCR (C7AH) or Northern blot (all other mRNA species). Values shown represent the average of RT-PCR performed on five samples of each strain and diet or Northern blot hybridizations performed on samples pooled from five mice. Northern blots contained 2 µg of poly(A) RNA/lane, and signals were quantitated by integration on a PhosphorImager. For each mRNA species, the levels detected in B6J mice fed the chow diet were set to 1, and values for other samples expressed relative to that value. Note that the ordinate scales in the left and right panels are different. S12AH, sterol-12α-hydroxylase; other abbreviations are defined in Footnote 1.
examined serum bile acid concentrations. B6J and B6By mice results from impaired intestinal uptake and recirculation, we whether the increased fecal bile acid excretion in B6By mice bile acid secretion from the liver or impaired intestinal uptake to levels 2-fold higher than B6J mice (Fig. 4 chow diet, and this difference was accentuated on the Ath diet Fecal bile acid content was higher in B6By mice by 40% on a receptor LXR thesis (C7AH, S27H, O7AH), the nuclear bile acid-binding genes involved in bile acid synthesis. Relative hepatic mRNA levels in B6By mice of each strain fed the Ath diet for 2 weeks. Cholesterol stion. We measured hepatic cholesterol concentration in three differences between B6J and B6By. Excretion in B6By Mice—We previously mapped the Diet1 locus conferring resistance to diet-induced elevations in LDL/VLDL cholesterol in B6By mice to a 20-cM interval on proximal chromosome 2 using a cross between B6By and A/J mice (5). In an effort to narrow this interval further, we have now produced a genetic cross between B6By mice and the CAST/EJ strain, which has diverged widely from common laboratory strains, affording abundant polymorphic markers for use in mapping. We used a [(B6By × CAST) × B6By] backcross strategy (referred to subsequently as BC) to maximize the number of offspring homozygous for B6By alleles. Unexpectedly, LDL/VLDL cholesterol levels in offspring from the BC cross did not segregate with chromosome 2 markers, which were previously shown to be linked to the Diet1 locus (Fig. 5, left panel). This may reflect the influence of additional loci contributed by the CAST strain that affect cholesterol levels and mask the effect of the Diet1 allele. However, serum bile acid levels in BC mice did segregate with the Diet1 locus, with significantly higher bile acid levels observed in mice homozygous for the B6By allele at the D2Mit117 marker that lies in the middle of the Diet1 locus (Fig. 5, right panel). Co-segregation of bile acid levels and the Diet1 locus was confirmed by typing additional chromosome 2 markers (Table 1). These results demonstrate that the Diet1 locus on proximal chromosome 2 segregates with serum bile acid levels in the BC cross, and are consistent with the interpretation that genetic variation at the Diet1 locus influences plasma bile acid levels, with a secondary effect on plasma cholesterol levels
that may be apparent in some genetic backgrounds (i.e. the A/J strain used in the previous study; Ref. 5) and not in others (i.e. the CAST strain).

To determine whether any of the bile acid metabolism genes identified as having altered expression in B6By mice might represent candidates for Diet1, we determined the map positions of several of these genes using a mouse-hamster radiation hybrid panel. We also typed nearby microsatellite markers for

**Fig. 5. Genetic variation in Diet1 co-segregates with bile acid levels.** Offspring of a backcross between B6By and the genetically diverse CAST/EiJ strain were screened for cholesterol levels before and after feeding on the Ath diet for 3 weeks, and for serum bile acid levels after the Ath diet. ∆(VLDL + LDL) represents the change in cholesterol levels on the Ath compared with chow diet. Animals were genotyped for the D2Mit117 marker within the Diet1 locus and classified as either homozygous for the B6By allele (designated BB) or heterozygous (one allele each from B6By and CAST, BC). Homozygosity for the B6By allele was associated with elevated bile acid levels, although no difference was seen between the two genotypes in ∆(VLDL + LDL) cholesterol levels.
each of these genes for possible segregation with LDL/VLDL cholesterol levels and bile acid levels in BCB mice (Table I). Most candidates were eliminated by their localization to chromosomes other than chromosome 2. Genes for LXR and BSEP both map to mouse chromosome 2, but are located 25 cM distal to the Diet1 locus and failed to show association with elevated serum bile acid levels (Table I). Thus, none of the bile acid metabolism genes we identified as having altered expression levels in B6By compared with B6J mice are likely candidates for the Diet1 gene. Diet1 may represent a novel gene or one with a previously unrecognized role in cholesterol/bile acid metabolism.

**DISCUSSION**

Data from the current and previous study (5) show that B6By mice differ from B6J mice in three parameters that are metabolically linked: lower lipoprotein cholesterol levels, elevated expression levels for the rate-limiting enzymes in both the classic (C7AH) and alternative (sterol-27-hydroxylase) pathways of bile acid synthesis are elevated in B6By mice on both the chow and Ath diets. Both of these enzymes are regulated predominantly at the transcriptional level (22–24, 31, 32), indicating that mRNA levels are a good reflection of protein levels. Elevated C7AH expression in B6By is notable in that several other studies in animal models and man support an association between C7AH expression and plasma cholesterol levels. For example, in a survey of nine inbred mouse strains fed cholesterol and fat-enriched diets, there was an inverse correlation between levels of C7AH mRNA and plasma and hepatic cholesterol levels (33).

Furthermore, overexpression of C7AH via adenovirus infection in liver of mice and hamsters leads to reduction of total and low density lipoprotein cholesterol levels of a magnitude similar to that observed in B6By mice (34, 35). The relationship between C7AH and plasma cholesterol levels is not limited to rodents. In rabbits, a genetic variant that is resistant to diet-induced hypercholesterolemia has increased C7AH expression and fecal bile acid excretion (36), and hepatocytes isolated from out-bred rabbits that are hyporesponsive to dietary cholesterol produce bile acids at a rate 2-fold higher than those from hyper-responsive animals (37). In addition, administration of bile acid sequestrants to humans increases C7AH activity and lowers LDL cholesterol levels (reviewed in Ref. 38).

Genetic variation in C7AH expression levels in humans and animal models may be associated with polymorphism at the C7AH gene locus itself or at distinct loci. In humans, for example, a polymorphism in the 5′-flanking region of the CYP7A gene is associated with high LDL cholesterol levels (39), whereas genetic studies in the mouse have identified three loci distinct from Cyp7a1, which coordinately regulate C7AH mRNA levels and HDL cholesterol levels (40). In the case of B6By mice, the expression data suggest a potential mechanism for elevated C7AH expression via altered expression levels for nuclear receptors LXRα and FXR, known to regulate Cyp7a1 gene expression in response to oxysterols and bile acids. LXRα mRNA levels were moderately higher and FXR mRNA levels lower in B6By mice on both chow and Ath diets. LXRα is activated by cholesterol metabolites to increase Cyp7a1 gene transcription (14, 41, 42), and LXRα-deficient mice fail to induce Cyp7a1 gene expression in response to dietary cholesterol, leading to massive accumulation of cholesterol the liver (43). In contrast, FXR is involved in the negative regulation of Cyp7a1 gene expression in response to bile acids. Activated FXR subsequently induces expression of SHP, which antagonizes the function of the positive Cyp7a1 transcriptional regulator, LRH-1 (44, 45). The mRNA levels for SHP and LRH-1 also differ between B6J and B6By mice under some dietary condi-

**Table I**

| Candidate gene | Location | Marker | (LDL + VLDL) cholesterol | Serum bile acids |
|----------------|----------|--------|--------------------------|-----------------|
| Diet1 locus    | Chr 2 (2 cM) | D2Mit76 | 225 | 287 | p = NS | 50 | 17 | 0.002 |
| Diet1 locus    | Chr 2 (5 cM) | D2Mit117 | 235 | 224 | p = NS | 49 | 15 | 9e-5 |
| Diet1 locus    | Chr 2 (10 cM) | D2Mit79 | 240 | 224 | p = NS | 54 | 15 | 4e-6 |
| Cyp7a1         | Chr 4d   | D2Mit315 | 204 | 288 | p = NS | 39 | 22 | NS   |
| Cyp27          | Chr 1     | D1Mit46 | 207 | 286 | p = NS | 30 | 30 | NS   |
| Cyp7b1         | Chr 3     | D3Mit60 | 245 | 214 | p = NS | 36 | 25 | NS   |
| Cyp8b1         | Chr 9     | D9Mit18 | 283 | 185 | p = NS | 36 | 26 | NS   |
| LXRA           | Chr 2 (38 cM) | D2Mit327 | 259 | 236 | p = NS | 35 | 28 | NS   |
| FXR            | Chr 10    | D10Mit302 | 255 | 210 | p = NS | 29 | 32 | NS   |
| SHP            | Chr 4     | D4Mit203 | 225 | 229 | p = NS | 37 | 24 | NS   |
| LRH-1          | Chr 1     | D1Mit101 | 251 | 259 | p = NS | 37 | 29 | NS   |
| Ntcp           | Chr 12    | D12Mit49 | 268 | 186 | p = NS | 30 | 31 | NS   |
| Slc21a10       | Chr 6     | D6Mit59 | 241 | 212 | p = NS | 34 | 29 | NS   |
| Bsep           | Chr 2 (39 cM) | D2Mit327 | 259 | 236 | p = NS | 35 | 28 | NS   |
| mdr2           | Chr 5     | D5Mit10 | 274 | 180 | p = NS | 31 | 29 | NS   |
| 3ox-HSD        | Chr 13    | D13Mit300 | 252 | 228 | p = NS | 22 | 37 | NS   |

*Genotype at the locus is BB (both alleles from B6By).

*Genotype at the locus is BC (one allele each from B6By and CAST/Eij).

*p value calculated by Student’s t test.

*Chromosomal map positions determined in this study by mapping in a radiation hybrid panel (see “Experimental Procedures”).

**Diet1 and Bile Acid Metabolism**

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*p value calculated by Student’s t test.

*Chromosomal map positions determined in this study by mapping in a radiation hybrid panel (see “Experimental Procedures”).
tions. On the chow diet, B6By had substantially reduced SHP mRNA levels, which could contribute to the higher basal C7AH levels in this strain. Both strains increased SHP mRNA levels in response to the Ath diet, as has been observed previously for A129 mice on a 0.2% cholesterol/0.1% cholic acid diet (44), and which is associated with reduced C7AH expression on the Ath diet. However, the differences in SHP and LRH-1 levels between B6J and B6By mice cannot account for the observed differences between the strains in C7AHH levels on the Ath diet, indicating that additional factors may play a role under these dietary conditions. Further studies are required to determine whether the altered nuclear receptor mRNA levels translate to altered activity of these proteins. It is clear that genes for LXRα, FXR, SHP, and LRH-1 all map outside of the Diet1 interval on chromosome 2, indicating that the altered expression levels are a secondary effect of Diet1 genetic variation in B6By mice.

Elevated Serum Bile Acid Levels and Altered Bile Acid Transporter Gene Expression in B6By and Other Mouse Models—B6By mice exhibit elevated serum bile acids in conjunction with increased expression of several major hepatic bile acid transporters, including both a sodium-sensitive bile salt transporter (NTCP) and sodium-insensitive transporter (liver-specific OATP) located on the basolateral hepatocyte membrane (30). This is in contrast to other mouse models, where elevated serum bile acid is associated with a targeted disruption or decreased expression of a particular bile acid transporter that results in impaired bile transport. For example, diminished expression of NTCP in HNF-3β transgenic mice leads to elevated serum bile acids due to impaired hepatocyte uptake of recirculating bile acids (46). Thus, increased NTCP expression in B6By mice may represent a compensatory response aimed at alleviating the elevated serum bile acid levels that occur as a result of a primary defect in some other protein. Like NTCP, members of the OATP family are involved in extracting recirculating bile acids, and increased expression in B6By mice may represent a compensatory response.

Bile acid secretion is driven by bile (BSEP) and phospholipid (mdr2) transporters residing on the canicular membrane. mRNA levels for both transporters are increased in B6By compared with B6J liver on both chow and Ath diets. In other mouse models, expression levels of both BSEP and mdr2 have been shown to directly correlate with bile acid/cholesterol secretion rates. For example, overexpression of BSEP in the C57L mouse strain leads to hypersecretion of bile salts, which in turn promotes hypersecretion of cholesterol (30, 47). Additionally, elevated mdr2 mRNA is associated with increased bile acid pool size, resulting from cholic acid feeding in mice (48), and mdr2 deficiency leads to impaired cholesterol secretion in bile. The chromosomal map positions of BSEP and mdr2 genes are distinct from Diet1, indicating that the increased expression of these genes is not the primary difference between B6By and B6J mice, but the increased expression likely contributes to the enhanced bile acid transport in B6By mice.

Diet1 and Bile Acid Metabolism—Cholesterol excretion rate is influenced by several factors. The rates of bile acid synthesis from cholesterol, secretion of bile from the liver, resorption of bile acids in the intestines, and uptake of bile acids returning to liver via the enterohepatic circulation each contribute to the final bile excretion rate. The rate-determining factors for the processes occurring in liver are C7AH (cholesterol conversion to bile acids), BSEP (bile secretion from liver), and NTCP (uptake of recirculating bile acids). As discussed above, mouse models with alterations in expression levels for any of these key factors have a corresponding change in the excretion rate of bile acids or cholesterol (34, 35, 46, 47, 49, 50). In B6By mice, increased bile excretion is associated with a coordinate increase in mRNA levels for C7AH, BSEP, and NTCP, indicating that all three processes contributing to bile secretion from the liver are enhanced in these mice. The increased bile excretion in B6By mice provides a mechanism for elimination of cholesterol, both in the form of bile acids and as free cholesterol, which are secreted in a coupled manner in bile (51, 52). Together with the demonstration that serum bile acid levels co-segregate with the Diet1 locus, these data provide a plausible mechanism for the lower lipoprotein cholesterol levels that occur in B6By compared with B6J mice.

The genetic mapping data presented here eliminate several known bile acid metabolism genes as candidates for Diet1, suggesting that a gene with an indirect or unrecognized effect on bile acid metabolism is involved. An attractive possibility for Diet1 would be a transcriptional activator or co-activator that influences the expression of several genes involved in bile acid metabolism. An example of such a model exists in mice deficient in the transcription factor HNF-1α (53), which have defective bile acid transport and altered expression of many of the same genes as seen in B6By. Beyond those reported here, there are dozens of additional mRNA species that were detected through our DNA array analysis as having expression differences between B6J and B6By mice. The majority of these are ESTs with unknown function or genomic map position. Our ongoing efforts to identify the Diet1 gene include screening these ESTs for possible localization to the Diet1 locus on chromosome 2, as well as application of a positional cloning strategy.

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