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QTL mapping for genetic determinants of lipoprotein cholesterol levels in combined crosses of inbred mouse strains[1,S]

Henning Wittenburg,[2,*,†,§] Malcolm A. Lyons,[3,*] Renhua Li,[*] Ulrike Kurtz,[§] Xiaosong Wang,[*] Joachim Mössner,[§] Gary A. Churchill,[*] Martin C. Carey,[†] and Beverly Paigen[*]

The Jackson Laboratory,* Bar Harbor, ME; Brigham and Women's Hospital and Harvard Medical School,† Boston, MA; and Department of Medicine II,§ University of Leipzig, Leipzig, Germany

Abstract To identify additional loci that influence lipoprotein cholesterol levels, we performed quantitative trait locus (QTL) mapping in offspring of PERA/EiJ×I/LnJ and PERA/EiJ×DBA/2J intercrosses and in a combined data set from both crosses after 8 weeks of consumption of a high fat-diet. Most QTLs identified were concordant with homologous chromosomal regions that were associated with lipoprotein levels in human studies. We detected significant new loci for HDL cholesterol levels on chromosome (Chr) 5 (*Hdlq34*) and for non-HDL cholesterol levels on Chrs 15 (*Nhdlq9*) and 16 (*Nhdlq10*). In addition, the analysis of combined data sets identified a QTL for HDL cholesterol on Chr 17 that was shared between both crosses; lower HDL cholesterol levels were conferred by strain PERA. This QTL colocalized with a shared QTL for cholesterol gallstone formation detected in the same crosses. Haplotype analysis narrowed this QTL, and sequencing of the candidate genes *Abcg5* and *Abcg8* confirmed shared alleles in strains I/LnJ and DBA/2J that differed from the alleles in strain PERA/EiJ. In conclusion, our analysis furthers the knowledge of genetic determinants of lipoprotein cholesterol levels in inbred mice and substantiates the hypothesis that polymorphisms of *Abcg5/Abcg8* contribute to individual variation in both plasma HDL cholesterol levels and susceptibility to cholesterol gallstone formation.—Wittenburg, H., M. A. Lyons, R. Li, U. Kurtz, X. Wang, J. Mössner, G. A. Churchill, M. C. Carey, and B. Paigen. **QTL mapping for genetic determinants of lipoprotein cholesterol levels in combined crosses of inbred mouse strains. *J. Lipid Res.* 2006.** 47: **1780–1790.**

Supplementary key words quantitative trait locus • high density lipoprotein • low density lipoprotein • *Abca1* • *Abcg5* • *Abcg8*

Increased serum LDL cholesterol and decreased HDL cholesterol levels are established risk factors for cardiovascular disease (1). In addition, data from large epidemiological studies support an independent association of low HDL cholesterol levels and gallstone prevalence rates (2). With the exception of rare monogenic disorders of LDL and HDL cholesterol metabolism (3, 4), individual lipoprotein cholesterol levels are determined by a combination of genetic and environmental factors, such as diet and body weight (5). Knowledge of the genes involved would lead to a better understanding of the physiology and pathophysiology of lipoprotein metabolism, and such insight will possibly enable targeted interventions to reduce the risk of cardiovascular disease and gallstone formation.

In different human populations, a number of genome-wide linkage studies localized several genomic regions associated with variation in HDL and LDL cholesterol levels (6). However, this approach has not yet led to the identification of an underlying gene with different alleles influencing lipoprotein levels in humans. Because of the challenges frequently encountered in the identification of genes that modify a quantitative phenotype in humans, we and others propose to ascertain the genetic determinants of lipoprotein levels in mice and to translate these results into directed studies in human populations. To localize genomic regions harboring genes that carry polymorphisms and account for variation of lipoprotein levels in offspring of inbred mouse crosses, quantitative trait locus (QTL) mapping is used (6). To date, using QTL map-

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Abbreviations: Chr, chromosome; cM, centimorgan; D, inbred mouse strain DBA/2J; I, inbred mouse strain I/LnJ; IBS, identical by state; LOD, logarithm of the odds; P, inbred mouse strain PERA/EiJ; QTL, quantitative trait locus; SNP, single nucleotide polymorphism; SSLP, simple sequence length polymorphism.

[1] This paper was presented in part at the annual meeting of the American Gastroenterology Association, 2004, in New Orleans, and published as an abstract (*Gastroenterology* 2004; 126: A748). Data from the current study were used in part to exemplify novel statistical means to improve QTL mapping strategies in inbred mice and published in a statistical methods paper (*Genetics* 2005; 169: 1699–1709).

[2] To whom correspondence should be addressed.
e-mail: henning.wittenburg@medizin.uni-leipzig.de

[3] Present address of M. A. Lyons: Centre for Medical Research, University of Western Australia, and Western Australian Institute for Medical Research, Perth WA, Australia.

[S] The online version of this article (available at http://www.jlr.org) contains an additional three tables and two figures.

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ping for lipoprotein levels in mice, 37 genomic regions associated with HDL and 25 genomic regions associated with non-HDL cholesterol levels were identified (6). Importantly, the majority of QTLs for lipoprotein levels in mice were concordant with homologous regions of loci that displayed associations with variation in lipoprotein levels in genome-wide human linkage studies (6). Concordance of genomic loci between mouse and human is not unique to lipoprotein metabolism. Other examples include atherosclerosis (7), hypertension (8), and kidney diseases (9). Recently, a number of studies supported the concept that polymorphisms in orthologous genes underlie concordant loci in murine models and in humans. For example, polymorphisms in *Tnfsf4* (encoding OX40 ligand) were shown to underlie susceptibility to atherosclerosis (10), and polymorphisms in *Hc* (encoding complement factor 5) influence liver fibrogenesis (11) in both mice and humans. Compared with studies in humans, identification of quantitative trait genes in mice is facilitated by the well-known advantages of inbred mice for genetic studies (12) and by novel resources such as the availability of the mouse genome sequence and knowledge of the haplotype structure based on available single nucleotide polymorphism (SNP) databases (13).

In this study, we aimed to differentiate whether the same or distinct genetic loci determine lipoprotein levels and gallstone formation and to identify additional loci that influence the metabolism of HDL and LDL cholesterol in inbred mice. Accordingly, we performed QTL mapping for lipoprotein levels in two inbred mouse intercrosses that were analyzed previously for genetic contributions to cholesterol gallstone susceptibility (14, 15). To increase the power and resolution of our QTL analysis, a recently developed method to combine data from two or more inbred line crosses was used (16). Our analysis furthers the knowledge of genetic determinants of lipoprotein levels in inbred mice both by the identification of novel loci that were associated with HDL and non-HDL cholesterol levels and by the confirmation of a number of QTLs that were detected in previous crosses. Furthermore, a detailed analysis of a locus on distal chromosome (Chr) 17 substantiates the hypothesis that polymorphisms of the *Abcg5* and *Abcg8* genes that encode a heterodimer functioning as a biliary and intestinal cholesterol transporter contribute to individual variation in both plasma HDL cholesterol levels and susceptibility to cholesterol gallstone formation.

MATERIALS AND METHODS

Mice and diets

Mice of parental strains were obtained from The Jackson Laboratory. Both intercrosses were bred and analyzed simultaneously according to the same protocols; breeding protocols, housing, and diets for the PERA/EiJ (P) × I/LnJ (I) cross (14) and the P × DBA/2J (D) cross (15) were described in detail previously. All animals were bred in our colony at The Jackson Laboratory and fed a low-cholesterol (<0.02%) chow (Purina 5001; PMI Feeds, Richmond, IL) until 6–8 weeks of age. Subsequently, experimental animals were transferred to our standard lithogenic/atherogenic diet (containing 1% cholesterol, 15% butter fat, and 0.5% cholic acid) and moved to Brigham and Women's Hospital. To adapt to the different environment, all animals were maintained in a temperature-controlled room for at least 14 days before any experiment was performed. Animals were fed ad libitum and had free access to water. The Institutional Animal Care and Use Committees of The Jackson Laboratory and Harvard Medical School approved the protocols.

Phenotyping

Parental animals, F_1 progeny, and F_2 offspring of both sexes were fed the high-cholesterol, cholic acid-enriched diet for 8 weeks. Before surgery, animals fasted overnight. All mice were anesthetized by intraperitoneal injections of a mixture of ketamine (35 mg/kg; Bedford Laboratories, Bedford, OH), xylazine (5 mg/kg; Phoenix Pharmaceutical, Inc., St. Joseph, MO), and atropine (0.05 mg/kg; American Reagent, Inc., Shirley, NY) and euthanized by cervical dislocation. Blood was collected after laparotomy but before cholecystectomy via direct cardiac puncture with an 18 gauge needle using disodium EDTA as anticoagulant. Plasma was isolated from whole blood by centrifugation in a microcentrifuge at 12,000 rpm for 15 min. HDL and total cholesterol concentrations were measured as described previously (17) using an automated analyzer and the manufacturer's reagents (Synchron CX-5 Delta; Beckman, Palo Alto, CA). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol concentrations. Livers and tail tips of all experimental animals were frozen in liquid nitrogen and stored at −80°C until further analysis.

Genotyping

DNA was prepared from tails of mice by phenol-chloroform extraction (14) after digestion with proteinase K (Fisher Scientific, Medford, MA). Individual F_2 progeny were genotyped by PCR of simple sequence length polymorphisms (SSLPs) that discriminate between P and D alleles and between P and I alleles (MapPairs™ primers; Invitrogen, Carlsbad, CA). The markers from both crosses that were used and their map positions are listed in supplementary Table I. For the QTL analysis in the F_2 progeny of the P×I cross, 105 SSLP markers were genotyped, the average distance was 16.0 ± 5.8 centimorgan (cM), and the maximal interval length between markers was 32.0 cM. For the P×D intercross, we genotyped 98 markers with average distance of 17.1 ± 5.5 cM and maximal interval length of 28.5 cM. Polymorphisms were detected by capillary electrophoresis (ABI Prism Sequencer; Applied Biosystems, Foster City, CA) or gel electrophoresis using 4% agarose gels (NuSieve 3:1; FMC BioProducts, Rockland, ME). Map positions were retrieved from the Mouse Genome Database (http://www.informatics.jax.org).

Haplotype analysis and human-mouse orthology

Mouse SNP data were retrieved from a mouse inbred line genotyping database (www.jax.org/phenome). Physical map positions for markers and positional candidate genes are from the Ensembl Genome Browser (http://www.ensembl.org). In an experimental breeding cross, QTLs can only be identified in genomic regions that differ between parental strains. Because the majority of genetic variation between inbred mouse strains is ancestral and the genome of inbred mouse strains consists of haplotype blocks from *Mus musculus* and *Mus domesticus* (and to a lesser extent *Mus castaneus*) species (18), regions that are identical by state (IBS) between the parental strains of a breeding cross can be identified using SNP genotype data, and these regions are unlikely to contain quantitative trait genes. Therefore, it is possi-

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ble to narrow the region of the experimental QTL by excluding genetic segments that are IBS between parental strains of the crosses. As a conservative approach for the region-specific haplotype analysis, we only took SNPs into consideration that were polymorphic in more than five of the standard inbred mouse strains. Because the true boundaries of haplotype blocks are not known, to be deemed IBS we required regions to demonstrate three or more consecutive SNPs to be identical (for details on region-specific haplotype analysis, see 13). Homologous chromosomal regions between mouse and human were found at http://www.informatics.jax.org/reports/homologymap/mouse_ human.shtml.

Sequencing

Overlapping fragments of *Abcg5* (accession number NM_031884) and *Abcg8* (accession number NM_026180) were sequenced in inbred mouse strain D from genomic DNA or cDNA generated from reverse transcription of mRNA (15). Sequencing was performed using an ABI 3700 Capillary Sequencer and the BigDye sequencing kit (Applied Biosystems, Darmstadt, Germany). Primer pairs used for standard PCR are listed in supplementary Table II. The sequence was analyzed using Sequencher software and compared with sequences that we generated previously for strains P and I (14).

Statistical analysis

Lipoprotein cholesterol levels in parental strains are expressed as means ± SD. Lipoprotein cholesterol levels among mouse strains and correlations of phenotypes in F_2 progeny were analyzed using one-way ANOVA. In F_2 progeny, the correlations were conditioned on the effect of sex, which was found to be a major covariate. Pairwise comparisons were performed using Student's *t*-test. $P < 0.05$ was considered statistically significant. Single and interacting QTLs associated with lipoprotein levels in the individual crosses were identified using the analysis of Sen and Churchill (19). Significance thresholds were determined by permutation testing (20), and the strength of linkage of a locus to the phenotype was expressed as logarithm of the odds (LOD) score (21). Because the distribution of lipoprotein values was slightly skewed and higher values in the data sets were more variable, a logarithmic transformation of individual lipoprotein cholesterol values was performed to correct for the skewed data and to achieve data on an additive scale, which is needed for statistical modeling. However, the analysis was repeated using the nontransformed data for comparison. To account for differences of lipoprotein levels between male and female mice, analyses included sex as an additive covariate. In addition, we performed individual analyses for males and females and repeated the analyses for combined sexes using sex as an interactive covariate, which is considered the appropriate method to detect sex-specific QTLs (22, 23). The difference in LOD scores between the scans using sex as an additive or interactive covariate indicates sex-specific QTLs if it exceeds a simulation-derived threshold of 2.0 on the LOD scale (24). The posterior probability density statistic was used to calculate 95% confidence intervals of QTLs (19).

After the determination of QTLs in individual crosses, we analyzed the combined data from the P×D and the P×I intercrosses. The purpose of this conjoint QTL analysis was to distinguish those QTLs that were specific to one of the two crosses from QTLs that were shared between both crosses (for details, see 16). The logarithmic transformation of the individual HDL and non-HDL cholesterol values equalized the variances of cholesterol levels between the two intercrosses and enabled us to combine the data in a conjoint analysis. The alleles of F_2 progeny from both crosses were recoded based on lipoprotein cholesterol levels in parental strains (HDL or non-HDL cholesterol) and assigned the genotype H/H if alleles were inherited from a parental strain with high lipoprotein cholesterol levels and L/L if alleles were inherited from a parental strain with low lipoprotein levels. Accordingly, heterozygous alleles in F_2 progeny were assigned the genotype H/L. Genome-wide scans for the joint data sets were performed as described above. Including cross as an additive covariate in the combined genome scans accounted for the difference in mean trait values between crosses; cross-specific QTLs were detected through a cross by QTL interaction term in these scans. The differences between LOD scores derived from both scans (ΔLOD) were calculated, and if ΔLOD exceeded a simulation-derived threshold, the QTL was deemed cross-specific (16). To establish this threshold, we used 1,000 simulated data sets with a common QTL and ran the test for cross-specific effect; we found the threshold of significance (at the 0.05 level) to be ~2.0 in terms of different LOD scores. The contribution of each single locus to the variance of the phenotypes in the combined crosses was identified using the methods of Sen and Churchill (19). The software used in this study, Pseudomarker, is available at http://www.jax.org/research/churchill.

RESULTS

Plasma lipoprotein levels in parental strains of mice and in F_2 progeny

The lipoprotein cholesterol levels in parental strains of mice after consumption of the high-fat diet are displayed in **Fig. 1**. The analysis revealed differences in lipoprotein

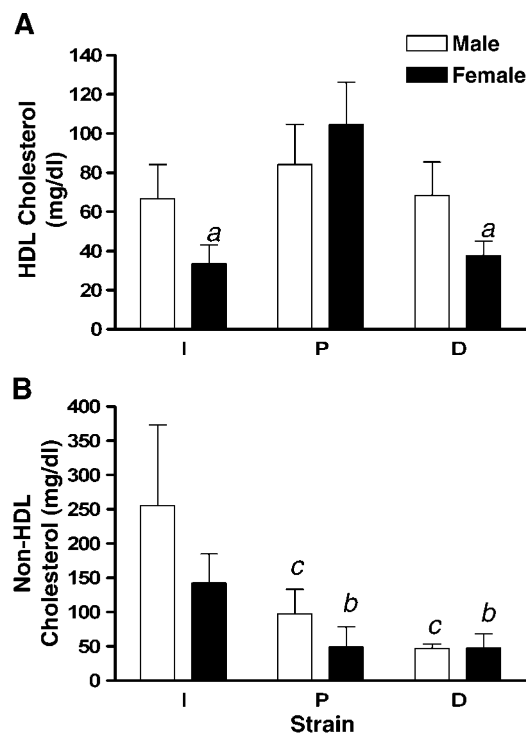

Fig. 1. Lipoprotein cholesterol levels in male (open bars) and female (closed bars) parental strains of mice after consumption of the high-fat diet for 8 weeks. All groups consisted of 8–10 animals with the exception of female I mice (n = 4). Values shown are means ± SD. D, inbred mouse strain DBA/2J; I, inbred mouse strain I/LnJ; P, inbred mouse strain PERA/EiJ. [a] $P < 0.001$ compared with female P mice; [b] $P < 0.05$ compared with female I mice; [c] $P < 0.001$ compared with male I mice.

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cholesterol levels between the three parental strains. HDL cholesterol levels were higher in strain P compared with strains I and D. However, only the differences in female mice were significant (Fig. 1A). In strains I and D, males displayed higher HDL cholesterol levels than females ($P < 0.05$ and $P < 0.005$, respectively). In strain P, the difference of HDL cholesterol levels between males and females was not significant. Non-HDL cholesterol levels were higher in strain I compared with strains P and D in both sexes (Fig. 1B). The non-HDL cholesterol levels in strain P were higher in males than in females ($P < 0.05$), whereas non-HDL cholesterol levels in strains I and D did not differ significantly between sexes.

HDL and non-HDL cholesterol levels in the 305 F_2 progeny from the P×I intercross and in the 324 F_2 progeny from the P×D intercross are shown in **Fig. 2**. Each individual F_2 progeny was analyzed previously for the occurrence of cholesterol gallstones (15). In F_2 progeny of the P×D cross, we observed a positive association between cholesterol gallstone formation [using a "gallstone score" that was derived from the absence or presence of cholesterol monohydrate crystals and the detection of gallstones (14)] and higher non-HDL cholesterol levels ($P < 0.001$, $r^2 = 0.20$). In contrast, no significant association was detected between cholesterol gallstone formation and higher HDL cholesterol levels in the P×D cross ($P = 0.1$) or between cholesterol gallstone formation and non-HDL lipoprotein cholesterol levels or HDL lipoprotein cholesterol levels in the P×I cross ($P = 0.63$ and $P = 0.18$, respectively). The observed correlations of non-HDL lipoprotein levels and gallstone formation in P×D F_2 progeny suggest the possibility that polymorphisms in the same genes may affect both traits in the P×D cross. In F_2 progeny of both crosses, HDL cholesterol levels and non-HDL cholesterol levels were higher in males compared with female offspring ($P < 0.0001$ for all comparisons). Therefore, sex was included as an additive covariate in all QTL analyses performed.

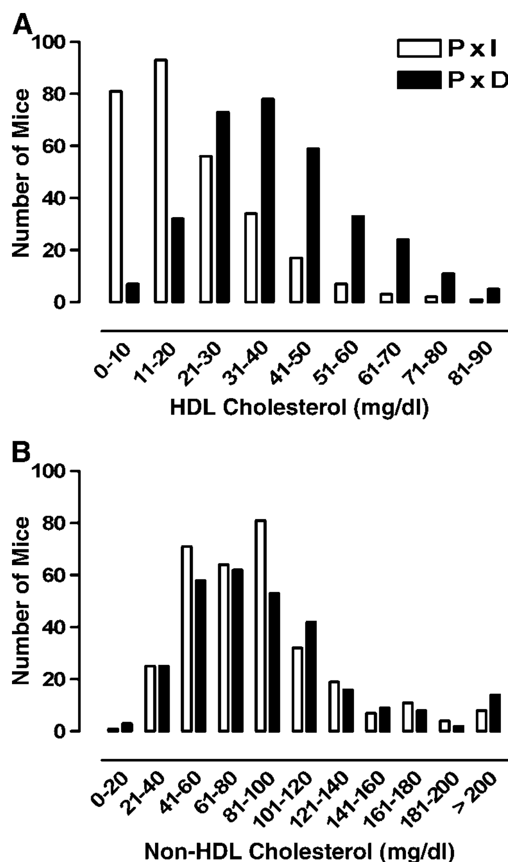

Fig. 2. Distribution of lipoprotein cholesterol levels between the 305 P×I (open bars) and the 324 P×D (closed bars) F_2 intercross progeny after consumption of the high-fat diet for 8 weeks.

QTL mapping for lipoprotein cholesterol levels

Figures 3 and 4 display the results of the genome-wide scans for single loci that were associated with the range of

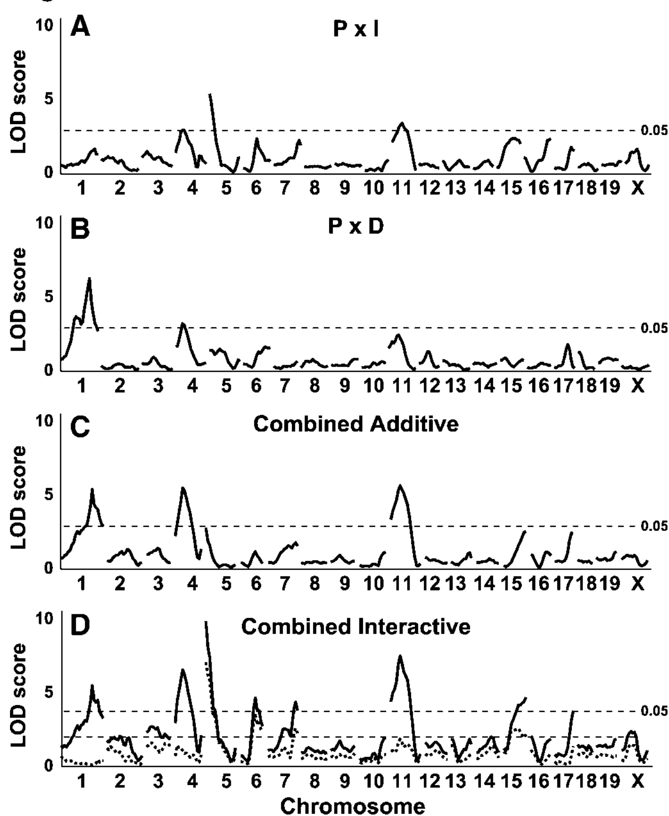

Fig. 3. Genome-wide scan plots for log HDL cholesterol concentrations for the P×I intercross (A), the P×D intercross (B), the combined crosses with cross as an additive covariate (C), and the combined crosses with cross as an interactive covariate (D). All scans (A–D) incorporated sex as an additive covariate. The horizontal broken lines (in A–C and the upper line in D) are thresholds for genome-wide significant ($P < 0.05$) quantitative trait loci (QTLs). Logarithm of the odds (LOD) scores exceeding 2.9 (A), 2.9 (B), 3.0 (C), and 3.7 (D) were determined by permutation testing to represent thresholds for significant QTLs. The broken curves at the bottom of D depict the ΔLOD scores between scans C and D; ΔLOD > 2.0 (denoted by the lower horizontal broken line in D) indicates allele effects that differ significantly between crosses ($P < 0.05$) as for the cross-specific QTLs on chromosomes (Chrs) 5, 6, 7, and 15. Which cross confers the QTL can be determined by comparing A and B.

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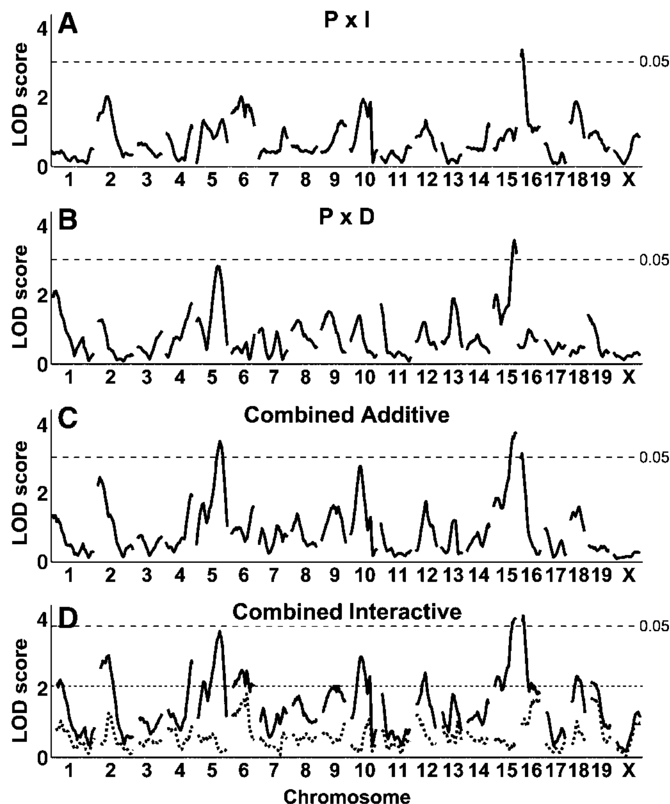

Fig. 4. Genome-wide scan plots for log non-HDL cholesterol concentrations for the P×I intercross (A), the P×D intercross (B), the combined crosses with cross as an additive covariate (C), and the combined crosses with cross as an interactive covariate (D). All scans (A–D) incorporated sex as an additive covariate. The horizontal broken lines (in A–C and the upper line in D) are thresholds for significant ($P < 0.05$) QTLs. LOD scores exceeding 2.9 (A), 2.9 (B), 3.0 (C), and 3.7 (D) were determined by permutation testing to represent thresholds for significant QTLs. The broken curves at the bottom of D depict the ΔLOD scores between scans C and D; ΔLOD > 2.0 (denoted by the lower horizontal broken line in D) indicates allele effects that differ between crosses ($P < 0.05$).

HDL (Fig. 3) and non-HDL (Fig. 4) cholesterol levels in F_2 progeny of the P×I cross (A), the P×D cross (B), the combined crosses with cross as an additive covariate (C), and the combined crosses with cross as an interactive covariate (D). For HDL cholesterol levels, in the P×I cross we detected significant loci on Chr 4 near the SSLP marker *D4Mit111*, on Chr 5 near *D5Mit145*, and on Chr 11 near *D11Mit4*. In the P×D cross, significant loci for HDL cholesterol levels were located on Chr 1 near *D1Mit16* and on Chr 4 near *D4Mit111*. To detect sex-specific QTLs for HDL cholesterol levels in the P×I and P×D intercrosses, we performed additional scans for males and females separately and for males and females combined with sex as an additive or interactive covariate (see supplementary Fig. I). Using the simulation-derived difference of 2 LOD units between the scans with sex as an additive or interactive covariate as criterion for sex-specific QTLs, none of the loci that were significantly associated with HDL cholesterol levels in the P×I and P×D crosses was deemed sex-specific (see supplementary Fig. I). In addition, we repeated our initial analysis using untransformed data and obtained very similar results (data not shown), indicating that the transformation of the data to combine the data sets from the two intercrosses did not alter the outcome of our analysis substantially.

The analysis of combined data from both crosses increased the detection power of our analysis and identified additional significant loci for HDL cholesterol levels on Chrs 6, 7, 15, and 17 (Fig. 3D). To distinguish loci that were specific for one cross and absent in the second cross from those loci that were common to both crosses, we performed the statistical analysis including cross as an additive (Fig. 3C) or as an interactive (Fig. 3D) covariate. A difference in the LOD scores (ΔLOD) between the two statistical analyses exceeding a simulation-derived threshold of 2.0 LOD units indicates QTLs that are cross-specific (i.e., present in one cross and absent in the other) (Fig. 3D; ΔLOD is represented by the broken curve, and the ΔLOD threshold is indicated by the lower broken line). This analysis revealed no significant differences in LOD units for the HDL QTLs on Chrs 1, 4, 11, and 17; therefore, we conclude that these loci were shared between both crosses. In contrast, for the loci on Chrs 5, 6, 7, and 15, ΔLOD exceeded the threshold of 2.0 LOD units; therefore, these loci were found in one cross but not in the other. For example, the Chr 5 QTL was detected in the P×I cross (Fig. 3A) but not in the P×D cross (Fig. 3B).

For non-HDL cholesterol levels, we detected significant loci on Chr 16 in the P×I cross near marker *D16Mit122* (Fig. 4A) and on Chr 15 near marker *D15Mit171* in the P×D cross (Fig. 4B). We performed additional analyses to assess whether any of the QTLs for non-HDL cholesterol levels were sex-specific (see supplementary Fig. II). The difference in LOD scores between the scans using sex as additive and interactive covariates did not exceed the simulation-derived difference of 2 LOD units, indicating that the QTLs for non-HDL cholesterol in the P×I and P×D crosses were not sex-specific (see supplementary Fig. II). Repeating the QTL analysis using nontransformed data yielded similar results compared with our initial findings using individual values after a logarithmic transformation (data not shown), and we proceeded to combine the data sets of log-transformed data from both crosses for the conjoint analysis. This analysis located an additional significant QTL on Chr 5 (Fig. 4C). Because ΔLOD did not exceed the threshold of 2.0 LOD units between the analyses using cross as either an additive or an interactive covariate (Fig. 4D), all three loci were shared between both crosses.

Allele effects at peak markers in individual and combined crosses

Figure 5 displays the effects of the different alleles at those chromosomal locations that were significantly associated with lipoprotein cholesterol levels in the QTL analysis of the combined crosses. In addition, for these loci the allele effects in the individual crosses are shown and

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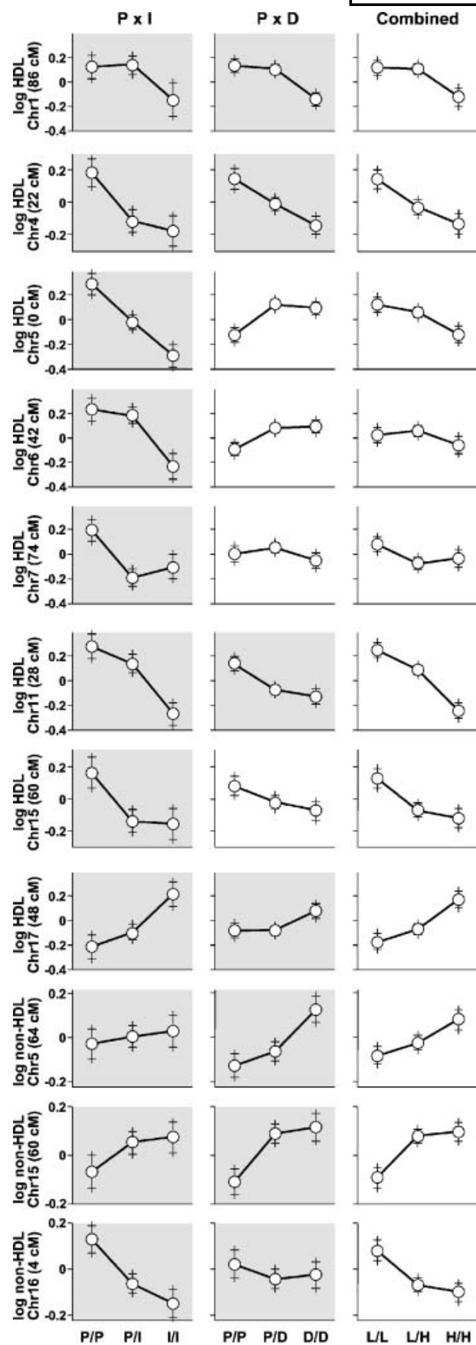

Fig. 5. Effects of different alleles in F_2 offspring at the peak markers for QTLs for log HDL cholesterol and log non-HDL cholesterol concentrations from the analysis of combined data sets from both crosses. Allele effects in the P×I cross, the P×D cross, and in the data sets from combined crosses are shown in the first, second, and third columns, respectively. Homozygous P, I, and D alleles are denoted P/P, I/I, and D/D, respectively. Accordingly, heterozygous alleles are denoted P/I and P/D. In combined crosses, alleles from parental strains that conferred higher lipoprotein levels are denoted H/H and alleles from parental strains that conferred lower lipoprotein levels are denoted L/L. Accordingly, heterozygous alleles are denoted H/L. The allele effects are shown at the peak location in centimorgan (cM; means ± SEM). The *y* axis shows log-transformed lipoprotein cholesterol levels centered on the cross-specific mean. Allele effects at loci that contributed significantly to the variance of the phenotype in the multiple regression models of individual crosses are shaded.

the panels are shaded if the respective locus contributed significantly to the variance of lipoprotein levels in multiple regression models of the individual crosses (data not shown). Use of this criterion enables us to determine whether a QTL contributes to the variance of the phenotype while accounting for all other QTLs in a cross, regardless of the significance level in the initial genome-wide QTL analysis.

For the QTLs influencing HDL cholesterol levels on Chrs 1, 4, and 11 that were shared between both crosses, the P allele was associated with higher HDL cholesterol levels. In contrast, at the shared locus on Chr 17, the allele inherited from strains I and D conferred higher HDL cholesterol levels compared with that inherited from strain P. The QTLs for HDL cholesterol on Chrs 5, 6, 7, and 15 did not display a simple additive pattern in the analysis of the combined data sets from the two intercrosses. These QTLs were detected in the P×I but not the P×D cross, and higher HDL cholesterol levels were associated with the P allele. The QTLs influencing non-HDL cholesterol levels were all shared between both crosses. At these QTLs, higher levels of non-HDL cholesterol were associated with the alleles from strains I and D at the loci on Chrs 5 and 15 and with the allele from strain P at the locus on Chr 16.

The multiple regression models (**Table 1**) summarize the contribution of each single locus, sex, cross, and all interactions in combination with all other factors that contributed to HDL and non-HDL cholesterol levels in the analysis of combined crosses. The combination of all significant factors in our final model explained 53.0% of the variance in HDL cholesterol levels and 22.7% of the variance in non-HDL cholesterol levels in the combined F_2 progeny from both crosses.

Haplotype and sequence analysis

Of all the QTLs detected, only the QTLs for HDL cholesterol levels on Chrs 6 and 17 overlapped with significant QTLs for cholesterol gallstone formation in the same crosses. The QTL on Chr 6 was shared between both crosses for gallstone susceptibility (15) but was associated with HDL cholesterol levels in the P×I cross only (Fig. 3). Furthermore, evidence from another of our QTL studies suggested that this locus comprises two or more overlapping QTLs (17). Because of the apparent complexity of the locus on Chr 6, we focused our analysis on the QTL on distal Chr 17, which displayed an association of the P allele with lower HDL cholesterol levels but higher cholesterol gallstone susceptibility, as shown in our previous study (15). The 95% confidence interval of the HDL QTL on Chr 17 extended from 42 to 58 cM. The size of this region corresponds on the physical map to a 24 Mb interval (69.5–93.5 Mb). The analysis of the parental strains' inferred haplotypes in this region narrowed the QTL interval to 9.26 Mb, thereby excluding >60% of the original 95% confidence intervals. The regions that shared alleles between strains D and I but displayed a different allele in strain P met our criteria for harboring the underlying

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TABLE 1. Multiple regression analysis for the QTLs influencing HDL cholesterol levels and non-HDL cholesterol levels in the combined F_2 progeny from the P×I and P×D intercrosses

Locus	Degrees of Freedom	Type III Sums of Squares	Percentage Variance[a]	*F* Value	*P*
HDL cholesterol					
Sex	1	31.8	10.2	124.7	<0.001
Cross	9	96.2	30.8	41.8	<0.001
Chr 1 (86 cM)	2	5.4	1.7	10.6	3×10^{-5}
Chr 4 (22 cM)	2	7.7	2.5	15.0	4.3×10^{-7}
Chr 5 (0 cM)	4	12.2	3.9	11.9	2.6×10^{-9}
Chr 6 (42 cM)	4	5.9	1.9	5.8	0.0001
Chr 7 (74 cM)	4	5.2	1.7	5.1	0.0005
Chr 11 (28 cM)	2	8.3	2.6	16.2	1.5×10^{-7}
Chr 15 (60 cM)	4	5.5	1.8	5.4	0.0003
Chr 17 (48 cM)	2	2.6	0.8	5.0	0.007
Chr 5 (0 cM) × cross	2	8.3	2.7	16.2	1.4×10^{-7}
Chr 6 (42 cM) × cross	2	4.5	1.4	8.8	0.0002
Chr 7 (74 cM) × cross	2	3.5	1.1	6.9	0.001
Chr 15 (60 cM) × cross	2	1.8	0.6	3.4	0.03
Model	26	165.7	53.0		
Error	576	147.1			
Total	602	312.8			
Non-HDL cholesterol					
Sex	1	28.3	18.7	146.2	<0.001
Cross	1	0.2	0.1	0.8	0.37[b]
Chr 5 (64 cM)	2	2.8	1.9	7.3	0.0007
Chr 15 (60 cM)	2	3.2	2.1	8.2	0.0003
Chr 16 (4 cM)	2	2.5	1.6	6.3	0.002
Model	8	34.4	22.7		
Error	606	117.2			
Total	614	151.6			

Chr, chromosome; cM, centimorgan; D, inbred mouse strain DBA/2J; I, inbred mouse strain I/LnJ; P, inbred mouse strain PERA/EiJ; QTL, quantitative trait locus.

[a] Percentage of variance of the phenotypes in F_2 progeny explained by the QTL identified on the indicated Chr.

[b] Although nonsignificant, cross was kept in the final model because it is a design factor.

quantitative trait gene. These regions contain 69 transcripts that are likely to be translated into proteins (see supplementary Table III). *Abcg5* and *Abcg8* are located in a head-to-head configuration within the QTL region. Their encoded proteins form a heterodimer that functions as an intestinal and biliary cholesterol transporter (25). These transcripts were investigated previously as candidate genes for cholesterol gallstone formation in the same crosses (14, 15). **Table 2** displays the haplotype analysis for the QTL region that harbors the *Abcg5* and *Abcg8* genes. Among the three parental strains, the distribution of SNP alleles surrounding *Abcg5* and *Abcg8* supports these genes as candidate genes for the HDL cholesterol locus on Chr 17. To discern the allele distribution pattern of *Abcg5* and *Abcg8* among strains P, I, and D, the coding and intergenic regions of these genes were sequenced in strain D. We thereby complemented our previous sequencing of these genes in strains P and I (14). The coding regions of *Abcg5* and *Abcg8* in strain D each exhibited several polymorphisms relative to the coding regions of both genes in strain P. The intergenic region separating the translation start sites of the genes was identical between strains D and P. The *Abcg5/Abcg8* polymorphisms detected between strains P and D were the same as those between strains P and I (14). Therefore, strains D and I carry identical *Abcg5* and *Abcg8* alleles (denoted "allele B" in Table 2), and these differ from the alleles in strain P (denoted "allele A").

DISCUSSION

In this study, we performed QTL mapping for lipoprotein cholesterol levels in two intercrosses of inbred mouse strain P with either strain I or D, respectively, which

TABLE 2. Haplotype analysis for the QTL region on Chr 17 that harbors the *Abcg5* and *Abcg8* genes

SNP	Base Pair	P	I	D	Comment[a]
rs6263123	81971402	G	G	G	IBS
rs3662869	82041104	A	A	A	IBS
rs13483135	82051202	G	G	G	IBS
rs13483136	82320445	G	A	A	
rs3706971	82357907	A	C	C	
mCV22838407	82364401	G	G	G	
Abcg5/Abcg8[b]	82487682	Allele A	Allele B	Allele B	
rs3707550	82567640	A	T	T	
rs3701338	82579890	G	G	G	
rs3091223	82615519	A	C	C	
rs13483137	82765248	A	C	C	
rs13483138	83014820	G	G	G	IBS
rs3694880	83063710	A	A	A	IBS
mCV25276476	83163111	G	G	G	IBS
rs13483139	83294259	G	G	G	IBS
rs6361015	83316881	A	A	A	IBS

IBS, identical by state; SNP, single nucleotide polymorphism.

[a] IBS is interpreted as a minimum of three sequential identical SNPs.

[b] See data from sequencing the *Abcg5* and *Abcg8* genes in the results section of the manuscript.

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TABLE 3. QTLs for cholesterol levels identified in the P×I cross, the P×D cross, and combined crosses

	P×I F_2		P×D F_2		Combined Crosses[a]		
Chr	LOD	Loc	LOD	Loc	LOD	Loc	Homologous Human Regions[b]
HDL cholesterol							
1	2.0	98 (70–116)	6.5	84 (78–92)	5.1	86 (78–104)	1q23-25
4	3.2	20 (8–42)	3.4	22 (12–40)	6.6	22 (12–32)	9p21-13, 9q22-23
5	5.6	0 (0–8)	1.5	0 (0–54)	9.9[c]	0 (0–6)	7q11, 7q22
6	3.0	42 (32–54)	1.3	48 (30–64)	5.2[c]	42 (36–54)	2p14-12, 3p26-25, 3p14-12, 3q21, 10p11-q11, 12p13
7	2.7	72 (46–74)	0.7	74 (20–74)	4.7[c]	74 (68–74)	1p15, 11q13-14, 16q11-12, 10q26
11	3.5	28 (8–52)	2.8	28 (4–44)	7.9	28 (18–38)	5q23-31, 5q33-35
15	2.3	50 (24–62)	0.7	56 (44–62)	4.6[c]	60 (32–62)	8q24, 12q12-13, 22q12-13
17	1.6	46 (28–52)	1.6	46 (43–52)	3.7	48 (42–58)	2p23-16, 18p11
Non-HDL cholesterol							
5	1.3	72 (50–84)	2.7	62 (40–78)	3.6	64 (40–80)	1p22, 4p16, 4p12-q22, 7p22, 7p11, 7q11, 7q22, 12q24, 22q11-12
15	1.1	62 (55–62)	3.3	56 (44–62)	4.0	60 (36–62)	8q24, 12q12-13, 22q12-13
16	3.2	4 (0–14)	0.9	36 (0–50)	3.9	4 (0–16)	1q44, 2q21, 3q27, 12p11, 16p13, 22q11

Loc, location of the QTL [peak (95% confidence interval) in cM]; LOD, logarithm of the odds.
[a] From the analysis using cross as an interactive covariate.
[b] According to http://www.informatics.jax.org/reports/homologymap/mouse_human.shtml.
[c] Cross-specific QTLs.

we examined previously for genetic determinants of gallstone susceptibility (14, 15). For each of these analyses, the offspring were fed a high-fat diet, which contains high proportions of dairy fat, cholesterol, and cholic acid and promotes atherosclerosis and the formation of cholesterol gallstones in mice (26). Consumption of cholic acid influences lipoprotein levels in some inbred mouse strains (17), but in all strains it replaces most endogenous murine tauro-β-muricholate with taurocholate (27), thus rendering the bile salt pool composition more similar to that of humans (28). Importantly, QTLs detected in studies that used a high-fat diet display substantial overlap with QTLs derived from similar studies in which mice were fed a regular chow diet (29). Accordingly, some of the loci that were associated with HDL cholesterol levels in this analysis overlapped QTLs detected in studies using a regular chow diet (29).

The QTLs that were associated with HDL cholesterol levels in this study are summarized in **Table 3**. The QTL for HDL on Chr 5 was identified only in the P×I cross and is novel; therefore, it was named *Hdlq34* (**Table 4**; see legend for QTL nomenclature). The loci on Chrs 6, 7, and 15 were also specific for the P×I cross and confirmed QTLs for HDL cholesterol detected in previous studies using inbred mice (Table 4). In contrast, additional QTLs on Chrs 1, 4, 11, and 17 were shared between the P×I and the P×D intercrosses. These loci colocalized with chromosomal regions that were associated with HDL cholesterol levels in previous QTL studies using inbred mice (Table 4). The importance of considering the influence of sex on QTLs related to lipoprotein cholesterol levels is well accepted, and previous QTL studies similar to ours have reported QTLs that had an effect in males or females only (24). Although it is valuable to look at the separate analysis of males and females, using sex as a covariate is the appropriate method to identify sex-specific QTLs (22, 23). Interestingly, even though sex influenced lipoprotein cholesterol levels in the parental strains of our crosses, using this method, none of the significant QTLs for HDL or non-HDL lipoprotein cholesterol levels from the two intercrosses in our study was found to be sex-specific (see supplementary Figs. I, II).

TABLE 4. Concordance of QTLs for lipoprotein levels from the current analysis with loci from human studies

Chr	QTL Name	Previously Named	Overlapping QTLs for the Same Phenotype (References)	Concordant Loci from Genome-Wide Human Studies (References)
HDL cholesterol				
1	*Hdlq33*	*Hdlq5*	(24, 41–48)	1q23-25 (49, 50)
4	*Hdlq10*	*Hdlq10*	(41, 47, 51)	9p21-13 (52)
5	*Hdlq34*		None detected	7q11-22 (52, 53)
6	*Hdlq35*	*Hdlq11*	(17, 54)	3p26-25 (55, 56), 3p14-12 (57), 10p11-q11 (58), 12p13 (59)
7	*Hdlq36*	*Chol-1* (chow)	(54, 60)	None detected
11	*Hdlq37*		(47)	5q35 (61)
15	*Hdlq38*	*Hdlq27, Lipq3* (chow)	(24, 62)	8q24 (55, 58, 61), 22q12-13 (59, 63), 12q12-13 (64, 65)
17	*Hdlq39*		(48)	None detected
Non-HDL cholesterol				
5	*Nhdlq8*		(47, 66)	None detected
15	*Nhdlq9*		None detected	None detected
16	*Nhdlq10*		None detected	1q44 (65)

According to the international standardized genetic nomenclature for mice, QTLs are assigned with a name only if significant or, when suggestive, if there is substantial overlap with a previously identified suggestive QTL. If a QTL has been reported previously, the old name is used if the crosses share one parental strain, and a new name is chosen if crosses do not share one parental strain.

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This study confirmed a number of QTLs that were both detected and investigated in more detail previously. For example, in some inbred mouse strains, variations in *Apoa2* (encoding apolipoprotein A-II) underlie the locus on Chr 1; however, an analysis of *Apoa2* haplotypes revealed that in the crosses of strain P with strains I and D, this is not the case (30). Thus, another gene affecting HDL must reside in this region of Chr 1. A region-specific combined analysis for the QTL located on Chr 4 for HDL cholesterol levels revealed a shared QTL in each of four different crosses, including the two crosses from our analysis (16). Because of the increased number of crossover events in the combined analysis, the 95% confidence interval of this QTL was narrowed to 12 cM (16) and contained *Abca1* (encoding an ATP binding cassette transporter for phospholipids and cholesterol) as a positional candidate gene. Mutations in *ABCA1* cause Tangier disease, which is associated with very low HDL cholesterol levels (4). Targeted mutations of *Abca1* confirmed the importance of the gene product for the control of HDL cholesterol levels in mice (31). Ongoing work will reveal whether polymorphisms in *Abca1* underlie the QTL on Chr 4.

Recently, a systematic review revealed a highly significant concordance of loci controlling HDL cholesterol levels in mouse and human (29). Accordingly, for the majority of QTLs for HDL cholesterol levels in this study, we identified homologous human regions that were associated with HDL cholesterol levels in human genome-wide genetic analyses (Table 4). For these loci on human Chrs 1, 3, 5, 7, 8, 9, 10, 12, and 22, the identification of the genes that underlie the concordant QTLs in inbred mice may facilitate the detection of the corresponding quantitative trait genes in humans. Regarding the mouse QTL on Chr 4 for HDL cholesterol and its positional candidate gene *Abca1*, the orthologous human gene *ABCA1* is located on human Chr 9q, a region that was not associated with HDL cholesterol levels in human genome-wide genetic studies (29). However, human association studies confirmed a contribution of *ABCA1* polymorphisms to the variation of plasma HDL cholesterol levels (32–34), supporting the concept of quantitative trait gene identification in mice and the translation of these findings into directed studies in humans.

The QTLs that were associated with non-HDL cholesterol levels are summarized in Table 3. The analysis of combined data sets from our two crosses increased the detection power for QTLs for non-HDL cholesterol levels and identified two significant novel loci on Chrs 15 and 16 that were shared between both crosses and that were named *Nhdlq9* and *Nhdlq10*, respectively (Table 4). Another QTL for non-HDL cholesterol levels on Chr 5 overlapped QTLs for the same trait detected in previous studies of different inbred mouse strains that also used a high-fat diet. Genetic linkage in these studies, however, did not exceed the significance threshold (Table 4). The plasma non-HDL cholesterol fraction in mice contains cholesterol derived from both LDL and triacylglycerol-rich VLDL subpopulations. The non-HDL cholesterol locus on murine Chr 16 was concordant with a locus on human Chr 1q44 that was associated with LDL cholesterol levels in a human study (Table 4). In summary, the results of QTL mapping in the current study contribute to the knowledge of genetic control of non-HDL cholesterol levels in mice and may aid the identification of the underlying gene for the LDL locus on Chr 1q44 in humans.

Our analysis of gallstone susceptibility and lipoprotein cholesterol levels detected an association between higher non-HDL cholesterol levels and gallstone formation in the P×D cross. The correlation between higher HDL cholesterol levels and gallstone formation was weak in both crosses and not significant. Furthermore, most of the loci that were associated with lipoprotein cholesterol levels did not overlap QTLs associated with cholesterol gallstone formation in the two crosses presented here (15). Therefore, susceptibility to cholesterol gallstone formation and lipoprotein levels are most likely controlled by polymorphisms in different genes, at least in these crosses of inbred mice. One exception to the contention that cholesterol gallstones and lipoprotein cholesterol levels are controlled by independent loci is the QTL on Chr 17, which contributed to the control of HDL cholesterol levels in this study and was associated with cholesterol gallstone formation in the same crosses (15). The vast majority of genetic variation between inbred mouse strains is ancestral (35). Therefore, it appears reasonable to assume that chromosomal regions that are IBS are unlikely to contain the polymorphism underlying the QTL. We performed a region-specific haplotype analysis for the Chr 17 locus that narrowed the QTL to 9.3 Mb that contained 69 transcripts (see supplementary Table III). Two of these transcripts that were supported by our haplotype analysis (Table 2) encode the ATP binding cassette half-transporters ABCG5 and ABCG8. These proteins form a heterodimer (36) and function as a cholesterol and plant sterol exporter in epithelial cells of the small intestine as well as a biliary cholesterol and plant sterol transporter (25, 37, 38). We showed previously that higher mRNA expression levels of both genes in strain P were associated with increased biliary cholesterol levels and gallstone formation (14, 15) and that the expression levels of both genes were *cis*-regulated in the P×I cross (14). Sequencing of the coding regions of both genes revealed the same haplotypes of *Abcg5* and *Abcg8* in strains I and D that differed from the haplotype in strain P. This strain distribution pattern is consistent with either or both of these genes underlying this QTL. Additional support for an association of *Abcg5/Abcg8* haplotypes and HDL cholesterol levels is derived from the analysis of plasma lipoproteins in *ABCG5/ABCG8* transgenic mice, which displayed reduced cholesterol absorption and increased biliary cholesterol secretion (37). At least in female *ABCG5/ABCG8* transgenic animals, plasma HDL cholesterol levels were lower compared with those of wild-type littermates (37).

The homologous region of murine distal Chr 17 on human Chr 2p was not linked to HDL cholesterol levels in any of the genome-wide genetic studies performed to date (6). However, different haplotypes of *ABCG5/ABCG8* were associated with differences in plant sterol levels, supporting the functional consequences of genetic differences

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in the cholesterol transporter genes (39). Furthermore, lower cholesterol absorption rates were associated with significantly lower HDL levels (40). From our genetic studies, we cannot ascertain how different *Abcg5/Abcg8* alleles affect plasma HDL cholesterol levels, but these findings indirectly support the hypothesis that *ABCG5/ABCG8* polymorphisms influence intestinal cholesterol absorption and consequently plasma HDL cholesterol levels. However, to date, no human study was designed to directly test this hypothesis. In summary, the results of our genetic studies are in accordance with alleles of *Abcg5* and *Abcg8* from strain P that contribute to lower serum HDL cholesterol levels but increase the risk of cholesterol gallstone formation in inbred mice and therefore warrant directed studies to investigate a possible association of *ABCG5/ABCG8* polymorphisms and HDL cholesterol levels in humans.

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