Quantitative Trait Loci Analysis for Plasma HDL-Cholesterol Concentrations and Atherosclerosis Susceptibility Between Inbred Mouse Strains C57BL/6J and 129S1/SvImJ

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Objective—The C57BL/6 (B6) and 129 mouse inbred strains differ markedly in plasma HDL-cholesterol concentrations and atherosclerosis susceptibility after a high-fat diet consumption. To identify loci controlling these traits, we performed quantitative trait loci (QTL) analysis.

Methods and Results—We fed a high-fat diet to 294 (B6x129S1/SvImJ)F_2 females for 14 weeks, measured plasma HDL concentrations and size of aortic fatty-streak lesions, genotyped F_2 females, and performed QTL analysis. HDL concentrations were affected by six loci: *Hdlq14* and *Hdlq15* on chromosome 1 (peaks cM 80 and cM 104, logarithm of odds [LOD] 5.3 and 9.7, respectively); *Hdlq16* on chromosome 8 (cM 44, LOD 2.6); *Hdlq17* on chromosome 9 (cM 24, LOD 2.9); *Hdlq18* on chromosome 12 (cM 20, LOD 5.9); and *Hdlq19* on chromosome 2 (cM 90), which interacted with *Hdlq15*. Atherosclerosis susceptibility was affected by five loci: *Ath17* on chromosome 10 (cM 34, LOD 6.6); *Ath18* on chromosome 12 (cM 16, LOD 3.7); *Ath19* (chromosome 11, cM 60), which interacted with *Ath18*; and *Ath20* (chromosome 10, cM 10), which interacted with *Ath21* (chromosome 12, cM 50).

Conclusions—We identified six loci for HDL and five loci for atherosclerosis susceptibility in a (B6x129S1/SvImJ)F_2 intercross. **(*Arterioscler Thromb Vasc Biol*. 2004;24:161-166.)**

Key Words: atherosclerosis ■ HDL cholesterol ■ inbred strain ■ mice ■ quantitative trait loci

Atherosclerosis is a complex, multifactorial disease controlled by both environmental and genetic factors. Human population studies show that atherosclerosis correlates with LDL cholesterol levels and inversely with HDL levels.[1,2] Knowledge of the primary genetic determinants of plasma lipoprotein levels will enhance our understanding of lipoprotein metabolism and likely provide novel molecular targets for intervention. In addition, although substantial gains in heart disease prevention can be achieved through ameliorating known risk factors, some patients have no obvious risk factor, suggesting the presence of unidentified modifiers. Thus, analyses of the genetic determinant of atherosclerosis susceptibility itself may reveal new insights into mechanisms and potential therapy. The use of mouse models of atherosclerosis has facilitated genetic analysis of atherosclerosis.[3] When exposed to a high-fat diet, different inbred mouse strains exhibit great variation in plasma lipoproteins and atherosclerosis susceptibility.[4] To uncover the genetic basis of these differences, quantitative trait loci (QTL) mapping may be used to locate the genes.[5] These QTL genes generally encode regulating proteins or rate-limiting enzymes for the polygenic trait; furthermore, the QTL for a trait are located in homologous regions in mice and humans.[6]

Female B6 mice have low plasma HDL levels and are susceptible to atherosclerosis; in contrast, female 129 mice have high plasma HDL levels and are relatively resistant. We report here the results of our investigation of plasma HDL concentrations and size of aortic atherosclerotic lesions after feeding F_2 progeny a high-fat diet containing high cholesterol and cholic acid for 14 weeks.

Materials and Methods

Animals

C57BL/6J (B6) and 129S1/SvImJ (129) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and mated to produce the (B6x129)F_1 progeny, which were intercrossed to produce 294 female F_2 progeny. Mice were maintained in a temperature- and humidity-controlled environment with a 14-hour light:10-hour dark cycle and given unrestricted access to food and acidified water. The cages were covered with polyester filters and contained pine shavings bedding. Experiments were approved by the Institutional Animal Care and Use Committee of The Jackson Laboratory.

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Quantitative Phenotype Measurements

Six-week-old mice were fed a high-fat diet[7,8] containing 15% dairy fat, 1% cholesterol, and 0.5% cholic acid for 14 weeks, after which they were euthanized by cervical dislocation. Their hearts and upper aortic sections were removed and fixed. The average size (±SEM) of atherosclerotic lesions of five aortic root cross sections/mouse was determined as described previously.[9] The numbers of mice are listed in tables and figures.

Blood samples from mice fasted for 4 hours were collected in plasma separator tubes containing EDTA, placed on ice, and centrifuged. Plasma HDL concentrations from each blood sample were measured using an enzymatic assay (Beckman, Fullerton, Calif) as previously described.[8]

Genotyping

We genotyped 294 F_2 progeny initially with 88 simple sequence length polymorphic (SSLP) markers (Research Genetics, Huntsville, Ala) spaced approximately 20 cM apart and later added 23 additional SSLP markers in the QTL regions (Table I; available online at http://atvb.ahajournals.org). DNA isolation, polymerase chain reaction amplifications, and subsequent gel electrophoreses were previously described.[10] We reported map positions in cM according to the 2003 Mouse Genome Informatics database.[11]

Statistics

One-way ANOVAs with Tukey multiple composition post-test were used to determine statistically significant differences in plasma lipid levels and lesion sizes between mouse groups. Data were analyzed using Graphpad Prism (Windows v3.00, GraphPad Software). Phenotypes were associated using Pearson product moment correlation. As described previously,[6,12,13] a three-step QTL analysis searched for main effects and pairwise gene interactions, then integrated all the main and interacting QTL-phenotype associations into a multiple regression. The traits were log transformed prior to analysis. This resulted in approximate normality for HDL. Lesion sizes are complicated by the presence of a "zero" class for mice with no lesions. The nonzero lesion sizes were log transformed, and lesions were analyzed using a two-part model that took into consideration both qualitative and quantitative aspects of this trait. QTL were deemed significant if they either met or exceeded the 95% genome-wide threshold, which was assessed by permutation analysis; they were deemed suggestive if they either met or exceeded the 90% genome-wide threshold but were not significant. QTL confidence intervals (CI) were calculated according to the posterior probability density of QTL locations, as described previously.[13] Analyses were carried out using Pseudomarker 0.9 open source software (Sen and Churchill, http://www.jax.org/staff/churchill/labsite).

Results

Inheritance of Plasma HDL Concentrations and Aortic Lesions in an F_2 Intercross between Strains B6 and 129

Plasma HDL concentrations were measured after animals were fed the high-fat diet for 14 weeks (Figure 1A). Compared with B6, 129 displayed significantly increased plasma HDL levels. The F_1 mice displayed plasma HDL levels intermediate between and significantly different from the parental strains; thus, high HDL was inherited in an additive fashion. HDL levels are high in 129, which is consistent with the resistance of 129 to aortic lesion formation.[4] Indeed, 129 mice and F_1 progeny did not develop fatty-streak aortic lesions when fed the high-fat diet, but B6 mice did. Of the 301 F_2 females produced, 294 survived the 14-week high fat diet. Log transformed HDL in the F_2 progeny was approximately normally distributed (Figure 1B). Log-transformed lesions in the F_2 progeny were distributed into two separate groups that resembled either the 129 parental strain with no or very small lesions or the B6 parental strain with large lesions (Figure 1C).

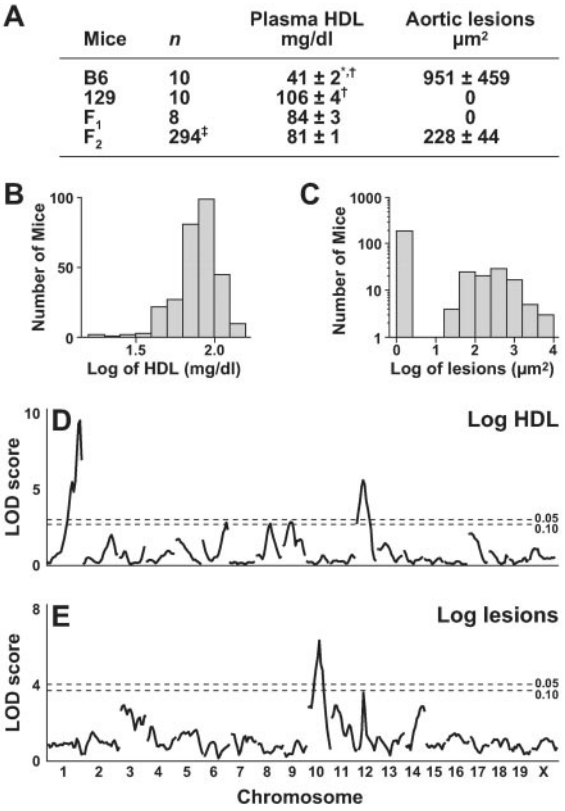

Figure 1. Plasma HDL concentrations and aortic lesion sizes of 10 female B6, 10 female 129, 8 female F_1, and 294 female F_2 progeny fed a high-fat diet for 14 weeks. A, Plasma HDL concentrations were measured in mice fasted for 4 hours. Aortic lesion sizes were determined by averaging the lesion sizes of five aortic-root cross sections from the progeny. Data are presented as the mean±SEM. *Significant difference ($P<0.001$, by ANOVA) versus 129. †Significant difference ($P<0.001$, by ANOVA) versus F_1. ‡Number of F_2 mice is 292 for plasma HDL concentrations. Because it is the distribution and not the mean among the F_2 population that is most important for detecting genetic linkage to a phenotype, we did not test for significant differences between F_2 progeny and either the parental strains or F_1 progeny. B, Distribution of log transformed plasma HDL concentrations of 292 female F_2 progeny. C, Distribution of log transformed lesion sizes in the aortas of 294 female F_2 progeny. D and E, Genome-wide scans for log transformed HDL concentrations and lesions, respectively, in (B6x129)F_2 progeny fed a high-fat diet for 14 weeks. Chromosomes 1 through X are represented numerically on the ordinate. The relative width of the space allotted for each chromosome reflects the relative length of each chromosome. The abscissa represents the LOD score, the traditional metric of genetic linkage. The significant ($P<0.05$) and suggestive ($P<0.10$) levels of linkage were determined by permutation testing.[12]

Identification of Genetic Loci Contributing to Increased Plasma HDL Concentrations and Aortic Lesions

The genome-wide scans for single QTL are presented in Figures 1D and 1E and summarized in Table 1, which provides the QTL peak, 95% confidence interval, allele conferring increased HDL concentrations or lesions, nearest SSLP marker from QTL peak, logarithm of odds (LOD) score, variance, and overlapping QTL. The QTL were named, if they were significant, either as single QTL or interacting

TABLE 1. QTL Identified for Single Gene or Pairwise Genome-Wide Scan of 294 (B6x129)F_2 Females*

Traits	Location Chr (cM)†	Locus Name	95% CI (cM)†	High Allele	Nearest Marker	LOD Score‡	Variance (%)§	Overlapping QTL‖	
								Name	Reference
HDL¶	Chr 1 (80)**	*Hdlq14*	50–82	129	*D1Mit159*	5.3	7.9	*Hdlq13, Cq1*	14, 15
	Chr 1 (104)	*Hdlq15*	98–105	129	*D1Mit406*	9.7	14.0	*Cq2, Hdlq6, Hdlq20*	15, 16††
	Chr 8 (44)	*Hdlq16*	37–50	129	*D8Mit248*	2.6	3.7	Unnamed QTL	17
	Chr 9 (24)	*Hdlq17*	15–32	129	*D9Mit129*	2.9	4.9	Unnamed QTL	15, 17
	Chr 12 (20)	*Hdlq18*	15–24	129	*D12Mit172*	5.9	8.9		
	Chr 2 (90)**	*Hdlq19*	70–110	—	*D2Mit285*	—	—	Unnamed QTL	17, 19
Lesions¶	Chr 10 (34)	*Ath17*	30–36	129	*D10Mit31*	6.6	9.8	*Artles2*	19
	Chr 12 (16)	*Ath18*	13–17	B6 or 129	*D12Mit243*	3.7	5.6		
	Chr 11 (60)**	*Ath19*	55–70	—	*D11Mit333*	—	—		
	Chr 10 (10)**	*Ath20*	5–40	129	*D10Mit213*	—	—	*Ath11*	25
	Chr 12 (50)**	*Ath21*	10–70	—	*D12Mit7*	—	—	*Ath7*	‡‡

*Number of F_2 mice is 292 for plasma HDL concentrations.

†From Mouse Genome Informatics (MGI) database at http://www.informatics.jax.org.

‡LOD scores only for a single QTL are shown. Suggestive QTL LOD≥2.6, significant QTL LOD≥2.9 for HDL; suggestive QTL LOD≥3.6, signfiicant QTL LOD≥3.9 for lesions as defined by permutation testing.

§Variance (%) indicates the percentage of the total F_2 phenotypic variance associated with each marker. Variances only for a single QTL are shown.

‖Overlapping QTL identified in previous studies.

¶Each data set is log transformed.

**Interacting QTL.

††Unpublished observations by R. Korstanje and B. Paigen, 2003.

‡‡Unpublished observations by K. Svenson and B. Paigen, 1997 and 1998.

QTL. Suggestive QTL in this cross that were found previously were also named. We named the loci *Hdlq* for high-density lipoprotein QTL or *Ath* for atherosclerosis susceptibility followed by a number. Figure 2A through 2E shows the allele effects, which demonstrate the magnitude of the effect and the inheritance pattern (dominant, recessive, or additive).

For HDL, we found two significant loci on chromosome 1: *Hdlq14* (LOD 5.3) and *Hdlq15* (LOD 9.7; Figure 2F), which interacted with each other (Table 2). Both loci confirmed QTL identified earlier using different crosses.[14–16] A suggestive QTL on chromosome 8 colocalized with a locus identified earlier on a chow diet using a different cross;[17] thus, we named it *Hdlq16* since it has been confirmed. *Hdlq17* on chromosome 9 (Figure 2G) with higher HDL caused by a dominant 129 allele (Figure 2B) colocalized with loci found previously on a chow diet using different crosses.[15,17] *Hdlq18* on chromosome 12 (Figure 2H) had higher HDL caused by a dominant 129 allele (Figure 2C). The pairwise genome scan revealed that two interactions, *Hdlq14-Hdlq15* (Figure 3A) and *Hdlq15*, the *D2Mit285* locus (Figure 3B), which we named *Hdlq19*, affected plasma HDL concentrations. In both cases, the combination of homozygous B6 alleles at one locus with homozygous 129 alleles at the second locus led to dramatically low HDL levels.

For atherosclerosis, the genome scan is shown in Figure 1E. The significant chromosome 10 QTL (Figure 2I), named *Ath17*, had a dominant B6 allele for atherosclerosis resistance (Figure 2D). The locus on chromosome 12 was suggestive as a single QTL but was significant in a gene interaction; thus, we named it *Ath18* (Figure 2J). At *Ath18*, both B6/B6 and 129/129 genotypes were associated with significantly higher atherosclerosis susceptibility (Figure 2E). *Ath18* interacted with the *D11Mit333* locus, which we named *Ath19*. *Ath19* was not shown to affect atherosclerosis susceptibility by itself (data not shown), but its combined effect with *Ath18* on lesion size was dramatic (Figure 3C). When the *Ath18* genotype was B6/B6, strain 129 contributed a significant additive allele for atherosclerosis susceptibility at *Ath19*; when the *Ath18* genotype was 129/129, strain B6 contributed a recessive allele for atherosclerosis susceptibility at *Ath19*. A second interaction for lesions was found between the *D10Mit213* locus and the *D12Mit7* locus, which we named *Ath20* and *Ath21*, respectively (Figure 3D). A 129/129 *Ath20* genotype, regardless of the *Ath21* genotype, was associated with significantly increased atherosclerosis susceptibility. When the *Ath20* genotype was B6/B6, strain 129 contributed an additive allele for atherosclerosis susceptibility at *Ath21*; when the *Ath20* genotype was either B6/129 or 129/129, strain B6 contributed a recessive or additive allele for atherosclerosis susceptibility at *Ath21*, respectively.

The multiple regression analysis confirmed that the individual QTL and interacting QTL contributed jointly to the effects on each trait and revealed that six QTL and two interactions accounted for 48% of the variance in HDL levels (Table 2) and that five QTL and two interactions accounted for 35% of the variance in atherosclerosis susceptibility (Table 3).

Discussion

In the present study, the genome-wide scans of 294 (B6x129S1/SvImJ)F_2 progeny for associations between marker genotypes and the quantitative traits of plasma HDL concentrations and atherosclerosis susceptibility resulted in the localization of six QTL for HDL and five QTL for atherosclerosis.

For HDL concentrations, we identified five main-effect QTL (*Hdlq14–Hdlq18*) and one additional QTL by a gene interaction (*Hdlq19*). *Hdlq14* and *Hdlq15*, both on chromosome 1, interacted with each other; likewise, *Hdlq15* and *Hdlq19* interacted with each other. These gene interactions

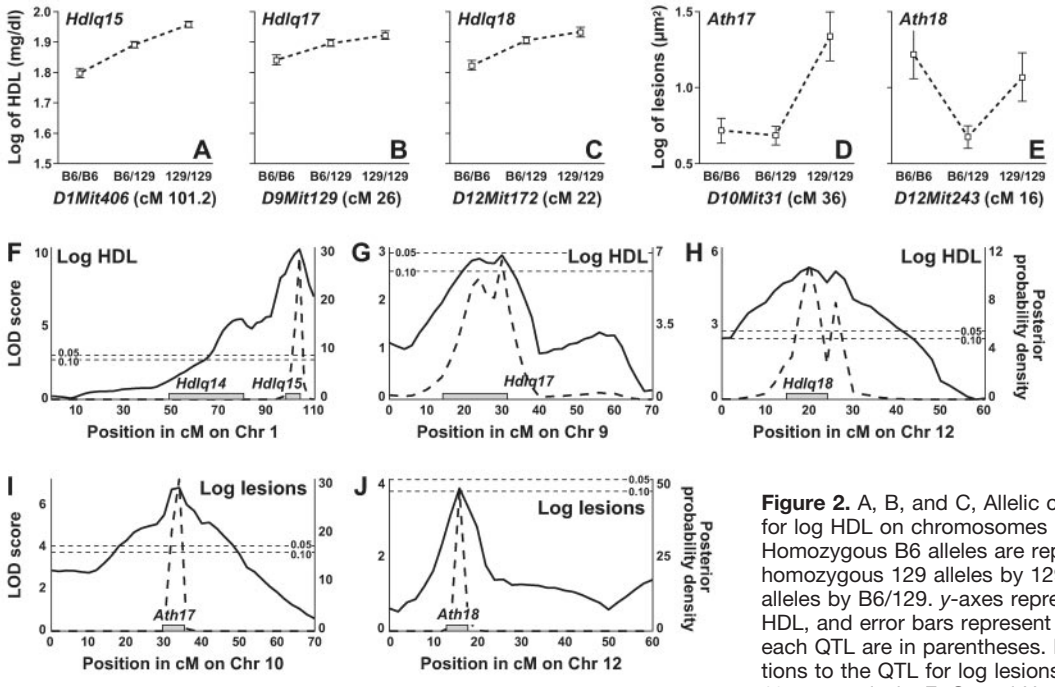

Figure 2. A, B, and C, Allelic contributions to the QTL for log HDL on chromosomes 1, 9, and 12, respectively. Homozygous B6 alleles are represented by B6/B6, homozygous 129 alleles by 129/129, and heterozygous alleles by B6/129. *y*-axes represent mean values of log HDL, and error bars represent SE. Marker locations for each QTL are in parentheses. D and E, Allelic contributions to the QTL for log lesions on chromosomes 10 and 12, respectively. F, G, and H, Genome wide scans (solid lines) and posterior probability densities (broken lines) for log HDL for chromosomes 1, 9, and 12, respectively. The posterior probability densities are a likelihood statistics that give rise to the 95% confidence intervals indicated by gray bars.[13] I and J, Genome wide scans (solid lines) and posterior probability densities (broken lines) for log lesions for chromosomes 10 and 12, respectively.

may give clues as to the candidate genes. *Hdlq18* appears to be a novel QTL, but the remaining *Hdlq* genes may have been discovered previously in other crosses.[18] For example, QTL for HDL have been reported many times at the distal region of chromosome 1 (Table 1).[14–16] One cross using advanced intercross lines was able to clearly separate two QTL on distal chromosome 1 located approximately 10 cM apart.[16] Although we do not have clear statistical evidence that *Hdlq14* and *Hdlq15* are separate main-effect QTL, we have nevertheless given them separate names. *Hdlq15* interacts with both *Hdlq14* and *Hdlq19*. One candidate gene for *Hdlq15* is *Apoa2* (cM 92.6), which encodes ApoA-II, a major constituent lipoprotein in HDL. *Hdlq19* colocalized with two QTL found previously using a (B6xCAST)F_2 intercross[17] and a (B6xDBA/2)F_2 intercross.[19] A candidate gene for *Hdlq19* is the gene (*Pltp*; cM 93.0) coding for plasma phospholipid transfer protein, which is bound to HDL and mediates the net transfer and exchange of phospholipids among different lipoproteins and participates in the transformation of larger HDL_3 into smaller HDL_2.[20] *Hdlq16* colocalized with a QTL found previously using a (B6xCAST)F_2 intercross.[17] Interestingly, *Hdlq16* colocalized with syntenic regions of QTL for serum cholesterol previously identified using a rat intercross[21] or a rabbit intercross.[22] The candidate gene for these QTL is the gene (*Lpl*; cM 33.0) coding for lipoprotein lipase (LPL), which plays a major role in lipoprotein metabolism.[23] It was reported that LPL mutations in humans are associated with dyslipidemia and atherosclerosis.[24] *Hdlq17* colocalized

TABLE 2. Multiple Regression Analysis of Variance for Log HDL in 292 (B6x129)F_2 Females

Location Chr (cM*)	Nearest Marker	DF	Type III SS	Variance (%)†	F Value	*P* Value	Locus Name
Chr 1 (80)‡	*D1Mit159*	6	0.280	4.76	3.66	0.00164	*Hdlq14*
Chr 1 (104)‡	*D1Mit406*	10	0.981	16.68	7.70	7.7×10^{-11}	*Hdlq15*
Chr 8 (44)	*D8Mit248*	2	0.095	1.62	3.75	0.02483	*Hdlq16*
Chr 9 (24)	*D9Mit129*	2	0.249	4.24	9.78	0.00008	*Hdlq17*
Chr 12 (20)	*D12Mit172*	2	0.272	4.63	10.70	0.00003	*Hdlq18*
Chr 2 (90)‡	*D2Mit285*	6	0.428	7.28	5.60	0.00002	*Hdlq19*
Chr 1 (80):Chr 1 (104)	*D1Mit159:D1Mit406*	4	0.278	4.73	5.46	0.00030	*Hdlq14:Hdlq15*
Chr 1 (104):Chr 2 (90)	*D1Mit406:D2Mit285*	4	0.258	4.39	5.07	0.00059	*Hdlq15:Hdlq19*
Total		293	5.879	48.33			

DF indicates degrees of freedom, includes main effect and any interactions; SS, sums of squares.
*From Mouse Genome Informatics (MGI) database at http://www.informatics.jax.org.
†Variance (%) indicates the percentage of the total F_2 phenotypic variance associated with each marker.
‡Interacting QTL.

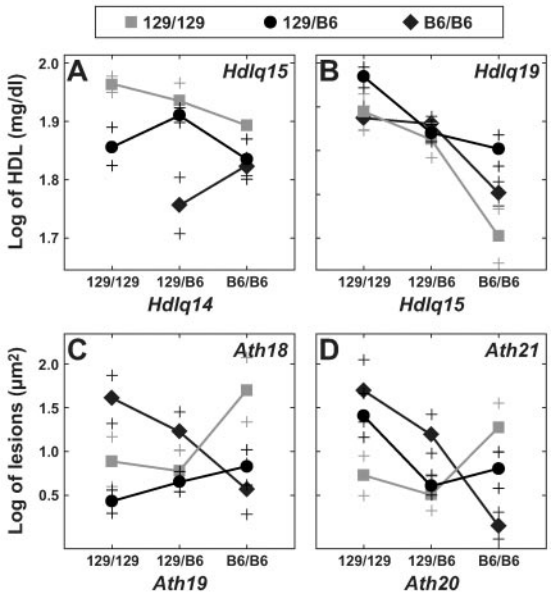

Figure 3. The effects of gene interactions contributing to increased plasma HDL concentrations and aortic lesions detected by the pairwise genome scan in female (B6x129)F_2 progeny fed a high-fat diet for 14 weeks. Homozygous B6 alleles are represented by B6/B6, homozygous 129 alleles by 129/129, and heterozygous alleles by 129/B6. *y*-axes represent mean values of log transformed HDL (A and B) and log transformed aortic lesion sizes (C and D). Error bars represent SE. There were no mice that represented both a 129/129 genotype at *Hdlq14* and a B6/B6 genotype at *Hdlq15*.

with QTL previously identified in a (B6xKK-A^y)F_2 intercross[15] and a (B6xCAST)F_2 intercross,[17] and the candidate gene is *Apoa1* (cM 27.0), which constitutes 70% of HDL protein and is clustered with *Apoa4* and *Apoc3*.

For size of aortic fatty-streak lesions, we identified two main-effect QTL (*Ath17* and *Ath18*) and three additional QTL by gene interactions (*Ath19*, *Ath20*, and *Ath21*). *Ath18* and *Ath19* interacted with each other; likewise, *Ath20* and *Ath21* interacted with each other. These gene interactions may give clues as to the candidate genes. *Ath18* and *Ath19* appear to be novel QTL, but the remaining QTL may have been discovered previously in other crosses. Dansky and colleagues[25] reported that an atherosclerosis susceptibility locus, *Ath11*, identified in a B6.129P2-*Apoe*^{tm1Unc}×FVB/N *Apoe*^{tm1Unc} cross, lies on chromosome 10 (cM 0 to 19). Our gene-interaction analysis indicated that *Ath20* overlaps *Ath11*. It would be interesting if these two loci proved to be the same, because *Ath11* was discovered in a sensitized cross with the *Apoe* knockout,[25] whereas *Ath20* was discovered in mice fed a high-fat diet. It is unknown whether loci discovered using the high-fat diet on a sensitizing background[26,27] (apoE or LDL receptor deficiency) will be the same. *Ath21* overlaps *Ath7*, an atherosclerosis susceptibility locus we identified in a SWR/J×SJL/J cross (K. Svenson and B. Paigen, unpublished observations, 1997–1998). *Ath18* is very near our previously discovered *Ath6* (chromosome 12, cM 2 to 4), which we identified in a B6xC57BLKS/J cross.[10,28] However, we think these loci are not identical because B6 contributes the susceptible allele for *Ath18* but the resistant allele for *Ath6*. And finally, *Ath17* appears to partly overlap an atherosclerosis susceptibility locus, *Artles2*, recently reported in a cross between B6 and DBA/2;[19] however, the peak of *Ath17* is cM 34 and the resistance is dominant, whereas the peak of the B6xDBA/2 QTL is cM 24 and the resistance phenotype displays an additive inheritance. Additional evidence is needed to determine whether they are the same QTL. It is interesting that the B6 allele of *Ath17* conferred smaller lesion size, since the strain B6 is susceptible to atherosclerosis, whereas the atherosclerosis-resistant strains 129 and DBA/2 conferred the susceptible allele. However, a susceptible strain carrying some resistant alleles is often found in QTL crosses.[29]

The number of different *Ath* loci that differ between B6 and 129 underscore the importance of strain background when evaluating the impact of a gene deficiency created by homologous recombination. In most cases, targeted mutant mice are derived from embryonic stem cells of 129 mouse substrains. A target gene in these cells is "knocked out" by homologous recombination, and resulting cells are microinjected into C57BL/6 (B6) blastocysts, which develop into B6/129 chimeras. These in turn are mated to B6 mice to produce mice heterozygous between B6 and 129 at all loci. These mice are intercrossed to generate mice homozygous for 129 alleles at the target locus (−/−) and a small region surrounding it, but

TABLE 3. Multiple Regression Analysis of Variance for Log Lesions in 294 (B6x129)F_2 Females

Location Chr (cM*)	Nearest Marker	DF	Type III SS	Variance (%)†	F Value	*P* Value	Locus Name
Chr 10 (34)	*D10Mit31*	2	13.89	3.25	5.64	0.0040	*Ath17*
Chr 12 (16)‡	*D12Mit243*	6	29.36	6.86	3.98	0.0008	*Ath18*
Chr 11 (60)‡	*D11Mit333*	6	19.49	4.56	2.64	0.0166	*Ath19*
Chr 10 (10)‡	*D10Mit213*	6	22.59	5.28	3.06	0.0065	*Ath20*
Chr 12 (50)‡	*D12Mit7*	6	22.81	5.33	3.09	0.0061	*Ath21*
Chr 12 (16):Chr 11 (60)	*D12Mit243:D11Mit333*	4	18.61	4.35	3.78	0.0052	*Ath18:Ath19*
Chr 10 (10):Chr 12 (50)	*D10Mit213:D12Mit7*	4	21.65	5.06	4.40	0.0018	*Ath20:Ath21*
Total		293	427.86	34.69			

DF indicates degrees of freedom, includes main effect and any interactions; SS, sums of squares.
*From Mouse Genome Informatics (MGI) database at http://www.informatics.jax.org.
†Variance (%) indicates the percentage of the total F_2 phenotypic variance associated with each marker.
‡Interacting QTL.

the remainder of their genomes are a random mix of B6 and 129 genes.[30] If littermates differ in the mix of *Ath* genes in such mixed background knockouts, the results could differ. Indeed, it has been reported that plasminogen activator inhibitor-1 deficiency protects against atherosclerosis progression in a B6 background,[31] but has the opposite effect, resulting in larger lesions throughout the vasculature, in a mixed B6;129 background.[32] Thus, to evaluate gene function in targeted mutant mice, the genetic background must be carefully controlled by constructing B6.129 congenic mice. This is carried out by successively backcrossing the knockout to B6 mice until the only 129 genes left on a nearly pure B6 background are the deleted target locus (−/−) and the surrounding genetic materials.[33]

In summary, by performing a QTL analysis of a (B6x129)F_2 cohort, we identified chromosomal regions that affect atherosclerosis susceptibility and plasma HDL concentrations in mice with backgrounds that are a combination of B6 and 129. Knowledge of the primary genetic determinants of plasma HDL concentrations and atherosclerosis susceptibility will enhance our understanding of lipoprotein metabolism and likely provide novel molecular targets for atherosclerotic disease. Advantages of this phenotype-driven method are (1) detection of rate-limiting genetic defects, (2) discrimination of rate-limiting defects from secondary (downstream) effects, and (3) identification of novel genes or of known genes with novel functions. To date, including the present study, more than 20 loci for either HDL levels or atherosclerosis susceptibility have been identified.[18,29] Identifying the underlying genes of these QTL will greatly improve our understanding of the complex atherosclerotic process.

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References

1. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann Intern Med.* 1979;90:85–91.
2. Gordon DJ, Rifkind BM. High-density lipoprotein–the clinical implications of recent studies. *N Engl J Med.* 1989;321:1311–1316.
3. Breslow JL. Mouse models of atherosclerosis. *Science* 1996;272:685–688.
4. Paigen B, Ishida BY, Verstuyft J, Winters RB, Albee D. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis* 1990;10:316–323.
5. Korstanje R, Paigen B. From QTL to gene: the harvest begins. *Nat Genet.* 2002;31:235–236.
6. Sugiyama F, Churchill GA, Higgins DC, Johns C, Makaritsis KP, Gavras H, Paigen B. Concordance of murine quantitative trait loci for salt-induced hypertension with rat and human loci. *Genomics* 2001;71:70–77.
7. Nishina PM, Verstuyft J, Paigen B. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J Lipid Res.* 1990;31:859–869.
8. Nishina PM, Lowe S, Verstuyft J, Naggert JK, Kuypers FA, Paigen B. Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J Lipid Res.* 1993;34:1413–1422.
9. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis* 1987;68:231–240.
10. Mu JL, Naggert JK, Svenson KL, Collin GB, Kim JH, McFarland C, Nishina PM, Levine DM, Williams KJ, Paigen B. Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57BLKS/J. *J Lipid Res.* 1999;40:1328–1335.
11. Mouse Genome Database (MGD), Mouse Genome Informatics Web Site (http://www.informatics.jax.org). Bar Harbor,ME:Jackson Laboratory.
12. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994;138:963–971.
13. Sen S, Churchill GA. A statistical framework for quantitative trait mapping. *Genetics* 2001;159:371–387.
14. Lyons MA, Wittenburg H, Li R, Walsh KA, Leonard MR, Churchill GA, Carey MC, Paigen B. New quantitative trait loci that contribute to cholesterol gallstone formation detected in an intercross of CAST/Ei and 129S1/SvImJ inbred mice. *Physiol Genomics* 2003;14:225–239.
15. Suto J, Matsuura S, Yamanaka H, Sekikawa K. Quantitative trait loci that regulate plasma lipid concentration in hereditary obese KK and KK-Ay mice. *Biochim Biophys Acta.* 1999;1453:385–335.
16. Wang X, Le Roy I, Nicodeme E, Li R, Wagner R, Petros C, Churchill GA, Harris S, Darvasi A, Kirilovsky J, Roubertoux PL, Paige B. Using advanced intercross lines for high-resolution mapping of HDL cholesterol quantitative trait loci. *Genome Res.* 2003;13:1654–1664.
17. Mehrabian M, Castellani LW, Wen PZ, Wong J, Rithaporn T, Hama SY, Hough GP, Johnson D, Albers JJ, Mottino GA, Frank JS, Navab M, Fogelman AM, Lusis AJ. Genetic control of HDL levels and composition in an interspecific mouse cross (CAST/Ei × C57BL/6J). *J Lipid Res.* 2000;41:1936–1946.
18. Wang X, Paigen B. Quantitative trait loci and candidate genes regulating HDL cholesterol: a murine chromosome map. *Arterioscler Thromb Vasc Biol.* 2002;22:1390–1401.
19. Colinayo VV, Qiao JH, Wang X, Krass KL, Schadt E, Lusis AJ, Drake TA. Genetic loci for diet-induced atherosclerotic lesions and plasma lipids in mice. *Mamm Genome* 2003;14:464–471.
20. Huuskonen J, Olkkonen VM, Jauhiainen M, Ehnholm C. The impact of phospholipid transfer protein (PLTP) on HDL metabolism. *Atherosclerosis* 2001;155:269–281.
21. Bonne AC, den Bieman MG, Gillissen GF, Lankhorst A, Kenyon CJ, van Zutphen BF, van Lith HA. Quantitative trait loci influencing blood and liver cholesterol concentration in rats. *Arterioscler Thromb Vasc Biol.* 2002;22:2072–2079.
22. Van Haeringen WA, Den Bieman M, Gillissen GF, Lankhorst AE, Kuiper MT, Van Zutphen LF, Van Lith HA. Mapping of a QTL for serum HDL cholesterol in the rabbit using AFLP technology. *J Hered.* 2001;92:322–326.
23. Goldberg IJ, Merkel M. Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci.* 2001;6:D388–D405.
24. Ukkola O, Garenc C, Perusse L, Bergeron J, Despres JP, Rao DC, Bouchard C. Genetic variation at the lipoprotein lipase locus and plasma lipoprotein and insulin levels in the Quebec Family Study. *Atherosclerosis* 2001;158:199–206.
25. Dansky HM, Shu P, Donavan M, Montagno J, Nagle DL, Smutko JS, Roy N, Whiteing S, Barrios J, McBride TJ, Smith JD, Duyk G, Breslow JL, Moore KJ. A phenotype-sensitizing apoe-deficient genetic background reveals novel atherosclerosis predisposition Loci in the mouse. *Genetics* 2002;160:1599–1608.
26. Knowles JW, Maeda N. Genetic modifiers of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol.* 2000;20:2336–2345.
27. Matin A, Nadeau JH. Sensitized polygenic trait analysis. *Trends Genet.* 2001;17:727–731.
28. Purcell MK, Mu JL, Higgins DC, Elango R, Whitmore H, Harris S, Paigen B. Fine mapping of Ath6, a quantitative trait locus for atherosclerosis in mice. *Mamm Genome* 2001;12:495–500.
29. Moore KJ, Nagle DL. Complex trait analysis in the mouse: The strengths, the limitations and the promise yet to come. *Annu Rev Genet.* 2000;34:653–686.
30. Smithies O, Maeda N. Gene targeting approaches to complex genetic diseases: atherosclerosis and essential hypertension. *Proc Natl Acad Sci U S A* 1995;92:5266–5272.
31. Eitzman DT, Westrick RJ, Xu Z, Tyson J, Ginsburg D. Plasminogen activator inhibitor-1 deficiency protects against atherosclerosis progression in the mouse carotid artery. *Blood* 2000;96:4212–4215.
32. Luttun A, Lupu F, Storkebaum E, Hoylaerts MF, Moons L, Crawley J, Bono F, Poole AR, Tipping P, Herbert JM, Collen D, Carmeliet P. Lack of plasminogen activator inhibitor-1 promotes growth and abnormal matrix remodeling of advanced atherosclerotic plaques in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol.* 2002;22:499–505.
33. Sigmund CD. Viewpoint: are studies in genetically altered mice out of control? *Arterioscler Thromb Vasc Biol.* 2000;20:1425–1429.

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