Copyright © 2002 by the Genetics Society of America

A Phenotype-Sensitizing *Apoe*-Deficient Genetic Background Reveals Novel Atherosclerosis Predisposition Loci in the Mouse

Hayes M. Dansky,*[,1] Pei Shu,[†] M. Donavan,[†] Jill Montagno,[†] Deborah L. Nagle,[†,2] John S. Smutko,[†] Natalie Roy,[†] S. Whiteing,[†] Judith Barrios,* T. J. McBride,[†] Jonathan D. Smith,* Geoffrey Duyk,[†,3] Jan L. Breslow* and Karen J. Moore[†,4]

Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York, New York 10021 and [†]Millennium Pharmaceutical Inc., Cambridge, Massachusetts 02139

Manuscript received August 14, 2001
Accepted for publication January 25, 2002

ABSTRACT

Therapeutic intervention for atherosclerosis has predominantly concentrated on regulating cholesterol levels; however, these therapeutics are not efficacious for all patients, suggesting that other factors are involved. This study was initiated to identify mechanisms that regulate atherosclerosis predisposition in mice other than cholesterol level regulation. To do so we performed quantitative trait locus analysis using two inbred strains that each carry the atherosclerosis phenotype-sensitizing *Apoe* deficiency and that have been shown to have widely disparate predilection to atherosclerotic lesion formation. One highly significant locus on chromosome 10 (LOD = 7.8) accounted for 19% of the variance in lesion area independent of cholesterol. Two additional suggestive loci were identified on chromosomes 14 (LOD = 3.2) and 19 (LOD = 3.2), each accounting for 7–8% of the lesion variance. In all, five statistically significant and suggestive loci affecting lesion size but not lipoprotein levels were identified. Many of these were recapitulated in an independent confirmatory cross. In summary, two independently performed crosses between C57BL/6 and FVB/N *Apoe*-deficient mice have revealed several previously unreported atherosclerosis susceptibility loci that are distinct from loci linked to lipoprotein levels.

GENETIC and environmental factors both contribute to the development of atherosclerotic vascular disease. Studies in twins demonstrate that heredity strongly influences disease susceptibility (Marenberg *et al.* 1994) and segregation patterns in families with a high incidence of coronary heart disease suggest a polygenic mode of inheritance (Lusis *et al.* 1998). Allelic variation in a few genes such as apolipoprotein E can affect blood lipid levels and atherosclerosis susceptibility (Wilson *et al.* 1996). However, the genes responsible for the full range of genetic variation in atherosclerosis susceptibility in the general population have not yet been described. Recently, positional cloning techniques and linkage studies in humans have been undertaken to identify disease susceptibility genes. Nonetheless, in most cases of complex, polygenic disease the identification of genes and even loci has been rendered extremely difficult for a variety of factors including the heterogeneity of disease classification, the genetic heterogeneity of human populations, and the inability to control both environmental and genetic factors. All these issues are particularly pertinent when any single locus is responsible for a small percentage of the phenotypic variance.

The difficulties inherent in human studies suggest that parallel approaches be undertaken using animal models. The laboratory mouse has long been used to study the genetic component of numerous complex human diseases because of its many strengths as a mammalian genetic model organism (Moore 1999). Indeed, previous studies have used the mouse to uncover genetic loci that potentially influence atherosclerosis. Generally, mice fed normal chow do not develop atherosclerosis or even early lesions in the absence of an exacerbating condition such as a high-fat, high-cholesterol diet. Multiple inbred strains of mice have been examined for their predilection to develop early atherosclerotic lesions when fed a high-fat, high-cholesterol diet that contains 0.5% cholic acid (an atherogenic diet; Roberts and Thompson 1977; Paigen *et al.* 1990; Nishina *et al.* 1993; Paigen 1995; Pitman *et al.* 1998; Mu *et al.* 1999), a high-fat, high-cholesterol diet without 0.5% cholic acid (Machleder *et al.* 1997) or a high-fat "Western" diet (Mehrabian *et al.* 2001). Lesions in mice fed these diets are usually limited to fatty streaks in the aortic root. Through the use of either recombinant inbred strains or quantitative trait locus (QTL) analysis 11 loci have been hypothesized to contribute to atherosclerosis in the mouse (Paigen 1995; Mu *et al.* 1999; Mehrabian *et al.* 2001; Welch *et al.* 2001) although

[1]*Present address:* Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY 10029.

[2]*Present address:* Hypnion Inc., Worcester, MA 01605.

[3]*Present address:* Exelixis, Inc., South San Francisco, CA 94083-0511.

[4]*Corresponding author:* Hypnion Inc., Five-Biotech, 381 Plantation St., Worcester, MA 01605. E-mail: kmoore@hypnion.com

only 6 have been genetically mapped. These loci are referred to as *Ath* loci (Paigen *et al.* 1987a,b,c, 1989; Stewart-Phillips *et al.* 1989), *Artles* (Mehrabian *et al.* 2001), and *Athsq1* and *Athsq2* (Welch *et al.* 2001). *Ath1*, *Ath2*, and *Ath3* are all described loci that both change high-density lipoprotein (HDL) levels with consequential changes to lesion formation. *Ath1* maps to mouse chromosome 1 (Paigen *et al.* 1987a), *Ath3* maps to mouse chromosome 7 (Stewart-Phillips *et al.* 1989), and *Ath2* (Paigen *et al.* 1989) is presently unmapped. *Ath4* and *Ath5* remain tentative gene designations as there are no data that resolve them as single Mendelian factors. *Ath6*, *Ath7*, and *Ath8* are all loci that are hypothesized to control lesion formation but not lipid levels. Of these 3, *Ath6* is the only mapped locus; it resides on mouse chromosome 12 (Mu *et al.* 1999). QTL were also identified in an analysis of (C57BL/6J and C3H/HeJ)F_2 mice fed a high-fat, high-cholesterol diet without 0.5% cholic acid (Machleder *et al.* 1997). Additionally, Western diet used in conjunction with measurements of lesion size was used to identify a nonlipoprotein-driven, chromosome 6 atherosclerosis QTL, *Artles* (Mehrabian *et al.* 2001). The CAST/Ei allele of *Artles* conveys atherosclerosis resistance on both a C57BL/6J and an *Ldlr*−/− background (Mehrabian *et al.* 2001). Other atherosclerosis QTL that resulted from measuring lesion size have also been recently identified (Welch *et al.* 2001). *Athsq1* and *Athsq2* are QTL that were identified in a B6.129S7-*Ldlr*−/− × MOLF/Ei cross and map to chromosomes 4 and 6, respectively. No genes that definitively explain any of the reported QTL have yet been identified.

An alternative approach to the phenotype-driven *de novo* discovery of atherosclerotic predisposition loci described above has been to create mouse models of hypercholesterolemia and atherosclerosis via gene manipulation (Plump *et al.* 1992; Van Ree *et al.* 1994; Smith and Breslow 1997). Overexpression of each of the lipoproteins ApoAII, Apo(a), ApoB, and ApoCIII renders mice on a high-cholesterol diet responsive to the development of atherosclerosis. This is also true of mice deficient for either the low-density lipoprotein (LDL) receptor or ApoE in which large human-like foam cell and fibro-proliferative lesions develop. In contrast, overexpression of ApoIV, ApoE, and ApoAI are each atheroprotective for mice on an atherogenic diet. These murine models of atherosclerosis have each, singly and in combination, contributed greatly to the understanding of the role of lipoproteins in atherosclerosis.

We have combined the phenotype- and genotype-driven approaches to studying atherosclerosis in the mouse by creating a series of six inbred strains of mice, each of which are homozygous for the *Apoe* knockout and each of which are 99%, or greater, pure genetic background (Markel *et al.* 1997; P. Shu, J. Montagno, T. McBride, H. M. Dansky, M. Donavan, G. Duyk, J. L. Breslow and K. J. Moore, unpublished data). The absence of the *Apoe* gene sensitizes these strains toward atherosclerosis, allowing any strain-specific atherosclerosis enhancing or protecting modulators to be revealed. The six strains can be divided into two groups, a group that is highly susceptible to atherosclerosis on a normal chow diet, consisting of C57BL/6J *Apoe*−/−, C57BL/KsJ *Apoe*−/−, and 129/SvJ *Apoe*−/− and a group that is far less susceptible, consisting of BALB/cByJ-*Apoe*−/−, C3H/HeJ-*Apoe*−/−, and FVB/NCr-*Apoe*−/− (P. Shu, J. Montagno, H. M. Dansky, M. Donavan, G. Duyk, J. L. Breslow and K. J. Moore, unpublished data). Similar trends were noted for FVB/NJ-*Apoe*−/− *vs.* C57BL/6J-*Apoe*−/− with less pure genetic backgrounds (Dansky *et al.* 1998).

Using the 99.7% pure FVB/NJ-*Apoe*−/− and C57BL/6J-*Apoe*−/− congenic strains we performed a QTL analysis to reveal loci that underscore the difference in atherosclerotic predisposition of these two strains. We identified loci that are responsible for lipid differences and loci that drive lesion predilection yet do not affect lipid levels. The statistical significance of the loci found far exceeds anything yet reported, indicating the discovery of major genetic loci for atherosclerosis predisposition in the mouse.

MATERIALS AND METHODS

Mice: The congenic strain FVB/NCr *Apoe*−/− was created by the speed congenic method as previously described (Markel *et al.* 1997). The congenic strain C57BL/6J *Apoe*−/− was produced from the importation of C57BL/6J.129 –*Apoe*−/− (Zhang *et al.* 1992) at the N_6 backcross from the Jackson Laboratories. Backcrossing to C57BL/6J was continued until the N_{11} generation and then brother-sister mating was used to maintain the strain. The genetic purity of the C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/− strains, which were determined empirically (Markel *et al.* 1997), is 99.5% C57BL/6J and 99.7% FVB/NCr, respectively. C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/− mice were crossed and the resultant F_1 offspring were intercrossed to create a cohort of 197 F_2 mice for the QTL analysis, referred to as cross 1. All parental and subsequent progeny mice were maintained at Millennium Pharmaceutical in a pathogen-free environment and fed a normal chow *ad libitum* diet containing 9% fat. The C57BL/6J.129 *Apoe*−/− and FVB/NJ.129 *Apoe*−/− stocks used in the confirmatory second cross (Dansky *et al.* 1998), which are totally independent of the congenic lines used in cross 1, were 92% C57BL/6J and 91% FVB/NJ, respectively. The genetic purity of these strains was also determined empirically (Dansky *et al.* 1998). These stocks and all subsequent offspring of crosses between them were maintained at Rockefeller University on normal chow, containing 4.5% fat, *ad libitum.* C57BL/6J.129 *Apoe*−/− and FVB/NJ.129 *Apoe*−/− mice were crossed and the resultant F_1 offspring were intercrossed to create a cohort of 186 F_2 mice for the QTL analysis referred to as cross 2.

Plasma cholesterol analysis: Overnight-fasted F_2 mice from cross 1 and the parental strains C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/− were assayed for serum lipid as follows: 60 μl of plasma was overlaid with 60 μl of PBS and spun at 70,000 rpm in a Beckman Optima TL-100 tabletop ultracentrifuge for 3 hr at 4° using a Beckman TLA-100 fixed angle rotor. The upper 60 μl contained the very-low-density lipoprotein (VLDL) predominant fraction. The lower 60 μl was collected and was mixed with 60 μl of potassium bromide (specific

density 1.12) in a new centrifuge tube (final specific density 1.063). Ultracentrifugation for 24 hr at 70,000 rpm using a Beckman TLA-100 fixed angle rotor at 4° was performed. The upper 60 μl is predominantly LDL and intermediate-density lipoprotein and the bottom 60 μl contains the HDL. The various fractions were all assayed using the Sigma (St. Louis) Cholesterol Kit (Cat. 352-100) and quantitated with cholesterol standards from Sigma (Cat. L0524).

F_2 mice from cross 2 and the parental stocks C57BL/6J.129 *Apoe*−/− and FVB/NJ.129 *Apoe*−/− were assayed for serum lipid as described (Dansky *et al.* 1998). Non-HDL cholesterol was determined by subtracting the HDL fraction from the total cholesterol level.

Quantitative atherosclerosis measurements: At 16 wk of age, the F_2 progeny were overnight fasted and anesthetized. Blood was collected from the retro-orbital sinus into capillary tubes containing EDTA. The circulatory system was perfused with 0.9% NaCl by cardiac intraventricular cannulation. The heart and ascending aorta including the aortic arch were removed, and the heart containing the aortic root was fixed in phosphate-buffered formalin. Eight-micrometer sections were cut and every other section was collected for aortic root quantitative atherosclerosis assay as previously described (Dansky *et al.* 1998). A total of five sections were used for quantitation, resulting in an 80-μm coverage of the aortic root.

Genotyping: Genomic DNA from kidney or tail-tip tissue was isolated (Markel *et al.* 1997). The 197 F_2 (C57BL/6J *Apoe*−/− × FVB/NCr *Apoe*−/−) mice of cross 1 were genotyped using 194 markers at a distance no greater than every 10 cM as reported (Markel *et al.* 1997). The 186 (C57BL/6J.129 *Apoe*−/− and FVB/NJ. 129 *Apoe*−/−) F_2 mice were genotyped in a similar fashion with 127 markers.

Statistics: All genotype and phenotypic data were analyzed by the MapManager QT version 3.0b28 (Manly 1993) for QTL analysis. Lander and Kruglyak (1995) propose both verbal definitions and supporting LOD scores to define QTL confidence in mouse crosses. A suggestive linkage is expected to occur one time at random in a genome scan and has an estimated minimum LOD score of 2.0. A significant linkage is expected to occur 0.05 times at random in a genome scan and has an estimated minimum LOD score of 3.4. However, we put even greater constraints on ourselves by using permutation tests to determine significance. Significance was determined by 1000 permutations to provide likelihood ratio statistics (LRS) that are suggestive, significant, and highly significant. LOD scores were calculated from the MapManager results by dividing the LRS by 4.6. QTL analysis was performed using nontransformed cholesterol data and log-transformed atherosclerosis data as lesion area may not be normally distributed. MapMaker (Lander *et al.* 1987; Lincoln and Lander 1992) was also used to analyze the association between the phenotype and genotype and the results were quite similar to that obtained using MapManager QT software (data not shown). Prism 3.0 (Graph Pad) was used for comparisons between genotypic means.

RESULTS

We used QTL analysis of F_2 mice to identify atherosclerosis susceptibility loci underlying the marked difference in atherosclerosis between C57BL/6 and FVB/N *Apoe*−/− mice. Two independent strain intercrosses were performed using different parental strains. Cross 1 was performed using fully congenic C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/− parental strains and cross 2 was performed using C57BL/6J.129 and FVB/NJ.129 *Apoe*−/− mice.

Parental strain and F_1 phenotype: Plasma lipids and aortic root atherosclerotic lesion area were measured in the fully inbred parental strains. Mean aortic root lesion area was 6-fold higher in male and 20-fold higher in female C57BL/6J *Apoe*−/− mice when compared to gender- and age-matched FVB/NCr *Apoe*−/− mice (Table 1). Total cholesterol, VLDL-C, LDL-C, and HDL-C were higher in FVB/NCr mice; however, the differences were more prominent in male mice (Table 1). Both the male and female F_1s had intermediate lesion areas. Mean male F_1 lesion area was significantly different from that of FVB/NCr *Apoe*−/− male mice, but was not significantly different from that of C57BL/6J *Apoe*−/− male mice. Mean lesion area in the F_1 females was significantly different from that of both C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/− female parental mice (Table 1).

In parental mice from cross 2, mean aortic root lesion area was seven- to ninefold higher in C57BL/6.129 *Apoe*−/− mice compared to FVB/NJ.129 *Apoe*−/− as previously reported (Dansky *et al.* 1998).

Quantitative trait analysis: *Lesion area:* A highly significant locus on chromosome 10 was obtained from both crosses with a LOD score of 7.8 in cross 1 (Table 2) and 11.9 in cross 2 (Table 3). This locus accounted for 19% of the log lesion variance in cross 1 (Table 2) and 25% of the log lesion variance in cross 2 (Table 3). Significant LOD scores were still obtained when the analysis was limited to a single gender in both crosses, except for the female cohort in cross 1 where this locus was suggestive (Tables 2 and 3). The markers with the highest LOD scores were D10Mit213 in cross 1 and D10Mit214 in cross 2 but as these markers are <3 cM apart the simplest hypothesis is that they are representing the same QTL. Indeed, interval mapping of chromosome 10 in cross 1 (Figure 1) revealed a peak that included both D10Mit213 and D10Mit214. These data suggest that the same gene on chromosome 10 affects lesion area in the F_2 progeny of both crosses. We have termed this locus *Ath11*, in keeping with the previously established system for naming atherosclerosis susceptibility loci in the mouse. Highly significant LOD scores were obtained for the chromosome 10 locus using either a dominant or additive model. When lesion area in F_2 mice (cross 1) was plotted according to D10Mit213 genotype, there was a twofold difference between mean lesion area in mice homozygous for the D10Mit213 FVB/NCr (FF) allele compared to mice homozygous for the C57BL/6J allele (BB, Figure 2A). Unexpectedly, homozygosity for the FVB/NCr allele (FF) was associated with a significant increase in lesion area when compared to heterozygotes (BF) and mice homozygous for the C57BL/6J allele (BB).

An additional significant lesion QTL was also seen at D10Mit49 (Table 2) in both crosses with male-only data.

TABLE 1

Total cholesterol, VLDL, HDL, LDL, and lesion area for the progenitor strains and the F_1 offspring at 16 weeks of age

	Gender	Lesion area (mm^2)	Total cholesterol (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Weight (g)
C57BL/6J Apoe−/−	M	100,281 ± 46,933 (5)	652 ± 33 (5)	411 ± 33 (5)	151 ± 25 (5)	23.0 ± 4 (5)	30 ± 2 (5)
	F	221,006 ± 24,960 (3)	596 ± 114 (5)	341 ± 93 (5)	204 ± 102 (5)	14 ± 2 (5)	23 ± 0 (3)
FVB/NCr Apoe−/−	M	17,445 ± 6,533 (5)**	976 ± 99 (5)**	747 ± 115 (5)*	208 ± 47 (5)*	32.0 ± 9.9 (7)	30 ± 2 (7)
	F	10,371 ± 5,551 (6)**	821 ± 228 (5)	487 ± 92 (6)*	258 ± 56 (6)	18.7 ± 7.1 (6)	27 ± 1 (7)
F_1 Apoe−/−	M	56,046 ± 25,065 (5)	895 ± 59 (5)**	602 ± 81 (5)**	161 ± 21 (5)	30 ± 9 (5)	40 ± 6 (5)
	F	98,965 ± 19,805 (3)**	797 ± 125 (3)	512 ± 56 (3)**	154 ± 18 (3)	21 ± 6 (3)	25 ± 3 (3)

Total cholesterol, VLDL, HDL, and LDL levels are expressed as milligrams/deciliter. The lesion quantitation is expressed as the cumulative morphometric analysis of five cross sections, expressed as square micrometers per aortic root per mouse. All data are expressed as mean and standard error. The number of animals used for each analysis is indicated in parentheses. A Student's *t*-test with two-tail, unequal variance measured significance between any two samples. *$P < 0.05$; **$P < 0.001$.

LOD scores of 5.3 and 8.8 were seen in crosses 1 and 2, respectively. However, interval mapping controlling for D10Mit213 and D10Mit214 diminished the peak at D10Mit49 to nonsignificance. It is unlikely therefore that there is a separate QTL at D10Mit49. Additional loci were suggestive for linkage with lesion area in cross 1, some of which were also seen in cross 2. Interval mapping indicated a linkage peak on chromosome 14 that was suggestive for linkage in the entire F_2 cohort (peak markers: D14Mit60 and D14Mit63 in cross 1 and 2, respectively). Given the small genetic interval (1.5 cM) containing these markers, the simplest hypothesis is that both crosses are detecting the same QTL, which we have termed *Ath13*. The dominantly inherited, with respect to C57BL/6J, *Ath13* locus accounted for 6–9% of the variance in log lesion area. Mean lesion area in male F_2 mice from cross 1 that were homozygous for the D14Mit60 FVB/NCr allele (FF) was significantly smaller when compared to D14Mit60 (BF) heterozygotes (Figure 2B). Since there were no significant differences in mean lesion area in male F_2 D14Mit60 heterozygote mice (BF) and homozygous BB mice (Figure 2B), lesion areas were combined from these two genotypes and compared to male F_2 homozygotes (FF). There was a 34% decrease in mean lesion area in male F_2 homozygotes (FF) compared to the combined group (65,400 ± 5141, $n = 60$ *vs.* 43,360 ± 4982, $n = 28$; $P = 0.0029$).

Two loci (D14Mit55 and D14Mit158) from cross 1 male F_2 mice and one locus (D14Mit63) from cross 2 male F_2 gave suggestive linkages. Interval mapping in cross 1 controlling for D14Mit55 still gave a peak at D14Mit158 (LOD of 2.5). Likewise, controlling for D14Mit158 gave suggestive peaks at D14Mit55 (LOD of 2.4). Given this analysis it is possible that this region of chromosome 14 (from 5 to 30 cM) contains multiple QTL. However, such complexities of QTL analysis cannot be confirmed with theoretical modeling alone, especially with closely linked QTL and only suggestive LOD scores. Empirical data, based on lines derived from recombination breakpoints, are the best way to confirm multiple, closely linked QTL. No chromosome 14 markers showed significance for lesion for female F_2 mice (Tables 2 and 3).

Another suggestive locus observed is on chromosome 19 and fits a recessive model, with respect to C57BL/6J. This locus, named *Ath16*, was suggestive for linkage in cross 1 but not observed in cross 2. Genotype analysis, of the F_2 mice from cross 2 (data not shown) showed that most markers on chromosome 19 showed triallelism, indicating non-FVB/NJ and non-C57BL/6J alleles were segregating. This precluded confirmation of the chromosome 19 locus revealed by cross 1. Single marker and interval mapping of F_2 mice from cross 1 revealed a peak at D19Mit120 (Table 3 and Figure 2C). The LOD score was 3.8 in male F_2 mice (Table 3), but no linkage was found in female mice on chromosome 19. Consistent with a recessive model (Figure 2C), there was a 50%

TABLE 2

MapManager QT analysis for the F_2s from cross 1

Chromosome marker	Phenotype (log transformed)	Gender	LOD	% variance	*P* value	Significance	Model with respect to C57BL/6J allele
D1Mit359	Total cholesterol	M + F	3.5	7	0.00035	Suggestive	Additive/recessive
D1Mit359	Total cholesterol	F	7.1	29	7.5e-8	Highly sig.	Additive/recessive
D1Mit359	HDL	M + F	3.6	7	0.0002	Sig.	Additive/recessive
D1Mit359	HDL	M	6.2	23	1.6e-7	Highly sig.	Additive
D1Mit359	LDL	M + F	2.9	6	0.00144	Suggestive	Additive
D1Mit359	LDL	F	6.0	25	9.1e-7	Highly sig.	Additive
D1MIT359	VLDL	M + F	2.3	4	0.00529	Suggestive	Additive/recessive
D1Mit359	VLDL	F	3.4	14	0.00044	Suggestive	Additive/recessive
D1Mit203	HDL	M	2.4	8	0.00411	Suggestive	Dominant
D1Mit78	HDL	M	3.2	13	0.00064	Suggestive	Recessive
D1Mit231	Lesion	M + F	2.3	5	0.00443	Suggestive	Additive/recessive
D4Mit41	Total cholesterol	M + F	2.4	5	0.00371	Suggestive	Additive
D4Mit41	Total cholesterol	F	2.8	11	0.00147	Suggestive	Recessive
D6Mit10	LDL	M + F	2.2	4	0.00589	Suggestive	Additive
D9Mit90	LDL	M + F	2.7	5	0.00197	Suggestive	Dominant
D10Mit80	HDL	M + F	2.6	5	0.00264	Suggestive	Additive/dominant
D10MIT133	VLDL	M + F	2.3	5	0.00443	Suggestive	Recessive
D10Mit49	Lesion	M	5.3	22	5.7e-6	Sig.	Additive
D10Mit213	Lesion	M + F	7.8	19	1.7e-8	Highly sig.	Additive/dominant
D10Mit213	Lesion	M	5.1	21	7.8e-6	Sig.	Dominant
D10Mit214	Lesion	F	3.5	16	0.00035	Suggestive	Additive/dominant
D10Mit233	VLDL	F	2.4	10	0.00397	Suggestive	Recessive
D14Mit55	Lesion	M	2.4	10	0.00417	Suggestive	Dominant
D14Mit60	Lesion	M + F	3.2	8	0.00062	Suggestive	Dominant
D14Mit63	Lesion	M	2.5	9	0.00495	Suggestive	Dominant
D14Mit158	Lesion	M	2.4	10	0.00420	Suggestive	Dominant
D16Mit103	Lesion	M + F	2.5	6	0.00319	Suggestive	Additive/dominant
D17Mit164	Total cholesterol	M + F	2.4	5	0.00393	Suggestive	Additive/recessive
D17Mit164	LDL	M	2.5	9	0.00326	Suggestive	Additive
D17MIT164	VLDL	M + F	2.6	5	0.00223	Suggestive	Additive/recessive
D19Mit120	Lesion	M + F	3.2	7	0.00135	Suggestive	Recessive
D19Mit120	Lesion	M	3.8	16	0.00014	Suggestive	Recessive

The phenotypes analyzed are total cholesterol, HDL, LDL, and VLDL and cross-sectional lesion data and are indicated in the second column. Each data set is log transformed. The first column indicates the chromosome and marker on which the linkage was seen. The fourth column gives the LOD score that was derived by dividing the LRS by 4.6. The fifth column indicates the percentage of the total phenotypic variance detected in the F_2 cohort with which each marker showing linkage was associated. The next two columns indicate the statistical significance of the linkage, first as a numerical *P* value and then verbally. The designation of suggestive, significant, and highly significant is determined by 1000 permutation tests for each data set. The last column indicates the best model for the mode of inheritance, with respect to the C57BL/6J allele. This was determined using the MapManager QT program. If two models, such as recessive and additive, appear equally likely by MapManager QT analysis, it is indicated as additive/recessive. Each analysis was done for the sex-combined, the male-only, and the female-only data. Only suggestive or significant linkages are listed in the table. Sig., significant.

decrease in mean lesion area in F_2 mice homozygous for the D19Mit120 (BB) when compared to heterozygotes or to F_2 mice homozygotes for the FVB/NCr allele (FF).

Weaker but still suggestive, QTL for lesions observed in cross 1 include D1Mit231 and D16mit103, both of which were seen in the full F_2 cohort and each of which accounted for ~5% of the phenotypic variance. When the chromosome 10, 14, and 19 QTL were controlled for, the D16MIT103 QTL showed a significant LOD score of 4.4 ($P = 3.5e\text{-}5$) and explained 9% of the variance.

Lipid measurements: Blood lipids were measured in individual F_2 mice of both crosses and linkage analysis was performed using MapManager QT software. A highly significant locus at the distal end of chromosome 1 (peak marker D1Mit359) was associated with total cholesterol (TC), HDL, and LDL in cross 1 with LOD scores exceeding 3.5 in the combined gender groups (Table 2). A suggestive linkage of D1Mit359 to VLDL was also seen. Upon gender splitting the data, the linkage to TC, LDL, and VLDL became statistically stronger in females but yielded nonsignificant results in males. Contrarily the D1Mit359 linkage to HDL in males was increased but was not significant in females. These gender differences may reflect the presence of more than one QTL in this genetic region. Overall, this region accounted for

TABLE 3

The MapManager QT analysis of the lesion and log-lesion data from the confirmatory cross 2

Chromosome marker	Phenotype	Gender	LOD	% variance	*P* value	Significance	Model with respect to C57BL/6J allele
D1MIT359	Lesion	M + F	3.3	7	0.00049	Suggestive	Additive
D1Mit50	Total chol.	M + F	7.6	18	2.5E-08	Highly sig.	Additive
D1Mit50	Total chol.	M	5.9	26	1.4E-06	Sig.	Additive
D1Mit50	Non-HDL	M + F	7.0	17	1.1E-07	Highly sig.	Additive
D1Mit50	Non HDL	M	5.4	24	4.0E-06	Highly sig.	Additive
D1Mit50	HDL	M + F	5.2	12	7.0E-06	Highly sig.	Additive
D1Mit50	HDL	M	2.7	11	1.93E-03	Suggestive	Additive
D1Mit50	HDL	F	4.1	18	8.4E-05	Sig.	Additive
D10MIT49	Lesion	M	8.8	37	1.6E-09	Highly sig.	Dominant/additive
D10MIT214	Lesion	M + F	11.9	25	1.3E-12	Highly sig.	Dominant/additive
D10MIT214	Lesion	M	5.8	24	1.5E-06	Highly sig.	Dominant/additive
D10MIT214	Lesion	F	6.8	27	1.5E-07	Highly sig.	Dominant/additive
D14MIT63	Lesion	M + F	2.6	6	0.00245	Suggestive	Dominant
D14MIT63	Lesion	M	3.1	13	0.00076	Suggestive	Dominant
D14MIT158	Lesion	M	3.3	14	0.00047	Suggestive	Dominant

Chol., cholesterol; Sig., significant.

4–29% of the variance in these lipid parameters (Table 2). Interval mapping of chromosome 1 revealed a peak LOD score of 6 at D1Mit359 for HDL in male-specific data (Figure 1), named *Ath9*. The FVB/NCr allele was associated with increasing HDL and best fit the additive model (Figure 2D). In cross 2, the same region of chromosome 1 (peak marker D1Mit50) was associated with TC, non-HDL cholesterol, and HDL (Table 3). The chromosome 1 locus accounted for 4–11% of the variance in these lipid parameters in the combined gender groups in cross 2. As D1Mit150 and D1Mit359 co-localize on the genetic map they can be regarded as equivalent for comparing crosses 1 and 2. Unexpectedly, cross 2 also revealed a suggestive linkage for lesion formation at D1Mit359 whereas cross 1 did not. This difference cannot be explained by the genetic contamination of the cross 2 stocks because these stocks are carrying only FVB and C57BL/6 alleles in this region of chromosome 1.

Other suggestive QTL for lipid levels were seen at D1Mit203, D1Mit78, D4Mit41, D6Mit10, D9Mit90, D10Mit80, D10Mit133, D10Mit233, and D17Mit164 (Table 2). None of these were recapitulated in cross 2, likely due to the less genetically clean background of cross 2 compounding difficulties of phenotypic and genotypic analysis.

Cross 1 was also analyzed by the MapMaker QTL (Lander *et al.* 1987; Lincoln and Lander 1992) program. All suggestive, significant, and highly significant QTL detected by MapManager (Table 2) were also detected by MapMaker QTL with one exception. This was the very weak, LOD2.3, D10Mit133 linkage to VLDL (Table 2). Furthermore, MapMaker detected an additional suggestive linkage (LOD 2.7) to D17Mit164 in the male-only data for total cholesterol**.** D17Mit164 had already been linked, using MapManager to total cholesterol and VLDL in the full cohort and LDL in male-only data. This QTL therefore seems to be very consistently associated with cholesterol regulation.

DISCUSSION

Atherosclerosis is a complex pathological process involving a multitude of cell types and gene products (Lusis 2000; Glass and Witztum 2001). The evaluation of candidate genes using mutant mouse models has yielded important insights into the role of apolipoproteins (Plump *et al.* 1994), adhesion molecules (Dong *et al.* 2000), immune mediators (Dansky *et al.* 1999), receptors (Gupta *et al.* 1997), and signaling molecules (Gu *et al.* 1998). However, this approach requires *a priori* knowledge of the identity of the candidate gene.

Genetic approaches to gene discovery afford greater *de novo* discovery of novel genes and pathways because they do not require prior knowledge of a candidate gene for a disease. Many genes for single-gene traits in both man and mouse have been discovered this way (Moore 1999). More recently, studies using crosses between inbred strains of mice and QTL analysis have identified disease susceptibility loci for many complex diseases, including obesity, lupus, diabetes, and hypertension (Moore and Nagle 2000)

In this study, we have identified multiple atherosclerosis susceptibility loci in F_2 progeny derived from two crosses, each of which are a mix of C57BL/6 *Apoe*−/− and FVB/N *Apoe*−/− mice. Blood lipids did not correlate with lesion area in the F_2 progeny of either cross, suggesting the segregation of atherosclerosis susceptibility genes that do not affect plasma cholesterol levels. Two such atherosclerosis susceptibility loci, located on chromosomes 10 and 14, were identified in both crosses.

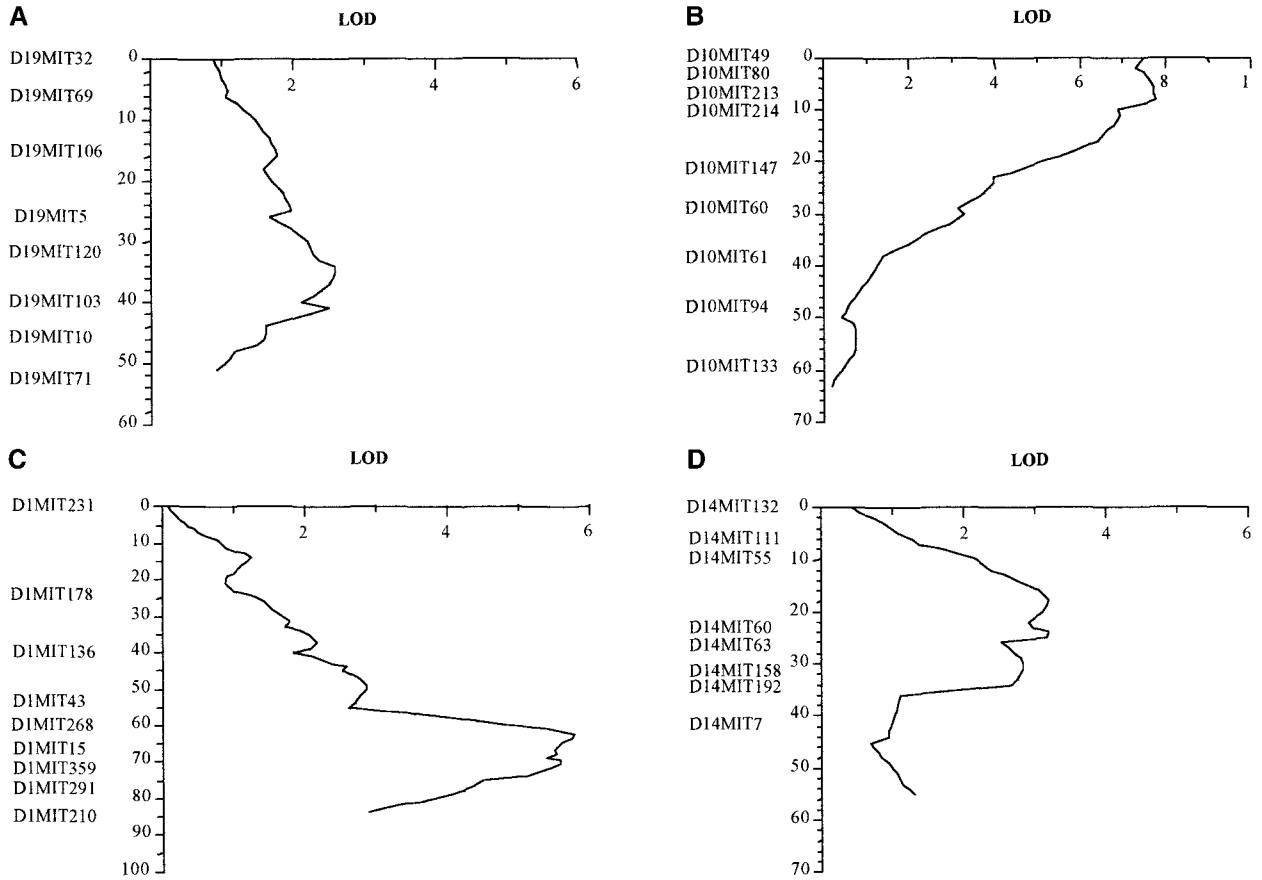

FIGURE 1.—The chromosome interval maps for chromosome 10 (B), chromosome 14 (D), and chromosome 19 (A) for lesion area and the chromosome interval map for chromosome 1 for HDL cholesterol (C). All data shown are from the F_2 cohort of cross 1.

We have termed these loci *Ath11* and *Ath13*, respectively. The reproducibility of the findings in two independently performed crosses greatly increases the likelihood that atherosclerosis susceptibility genes are located within the chromosome 10 and 14 QTL intervals and that these associations are not statistical phenomena. In particular, the chromosome 10 *Ath11* locus was highly significant (LOD $>$ 7) in both crosses. To our knowledge, this is the first time that an atherosclerosis susceptibility locus has been identified in the mouse with LOD scores of this magnitude and additionally confirmed in a second cross.

The *Ath11* interval (0–19 cM) contains several candidate genes and is evolutionary conserved with human chromosome 6q22-24. The following candidate gene map, sequence, and annotation information is obtained from publicly available sources. The strongest candidate gene for the chromosome 10 QTL is the interferon gamma receptor (*Ifngr*), which is located at 15 cM on mouse chromosome 10.

The role that *Ifng* may play in atherosclerosis is complicated as there are both proatherogenic and antiatherogenic induced functions (GUPTA *et al.* 1997; ROSS 1993, 1999). Interferon gamma is secreted by both CD4 and CD8 TH1 cells, which are often co-localized within atherosclerotic lesions. *In vitro*, interferon gamma exerts numerous effects on the cell types present in atherosclerotic lesions. These effects include the induction of class II histocompatibility antigens on macrophages, induction of macrophage metalloproteinase secretion (SCHONBECK *et al.* 1997), downregulation of lipoprotein receptors on macrophage (HUSSAINI *et al.* 1996), downregulation of macrophage scavenger receptor A (LI *et al.* 1995) and ABC-1 (PANOUSIS and ZUCKERMAN 2000), induction of endothelial VCAM-1 (DE CATERINA *et al.* 2001), and inhibition of smooth muscle proliferation (SELZMAN *et al.* 1998). Evidence that the *Ifngr* plays a proatherogenic role is derived from studies using mutant mice. Atherosclerotic lesion area decreased by 60% when interferon gamma receptor knockout mice were bred onto the *Apoe*-deficient background (GUPTA *et al.* 1997). *Ifngr* coding polymorphisms that affect receptor expression and immune function have been identified between the C57BL/6 and BALB/c inbred strains of mice (CHOU *et al.* 2000). *Ifng* is clearly an excellent candidate within the QTL region on mouse chromosome 10 although initial results have shown no coding variant difference between C57BL/6J and FVB/NJ (data not shown). Ex-

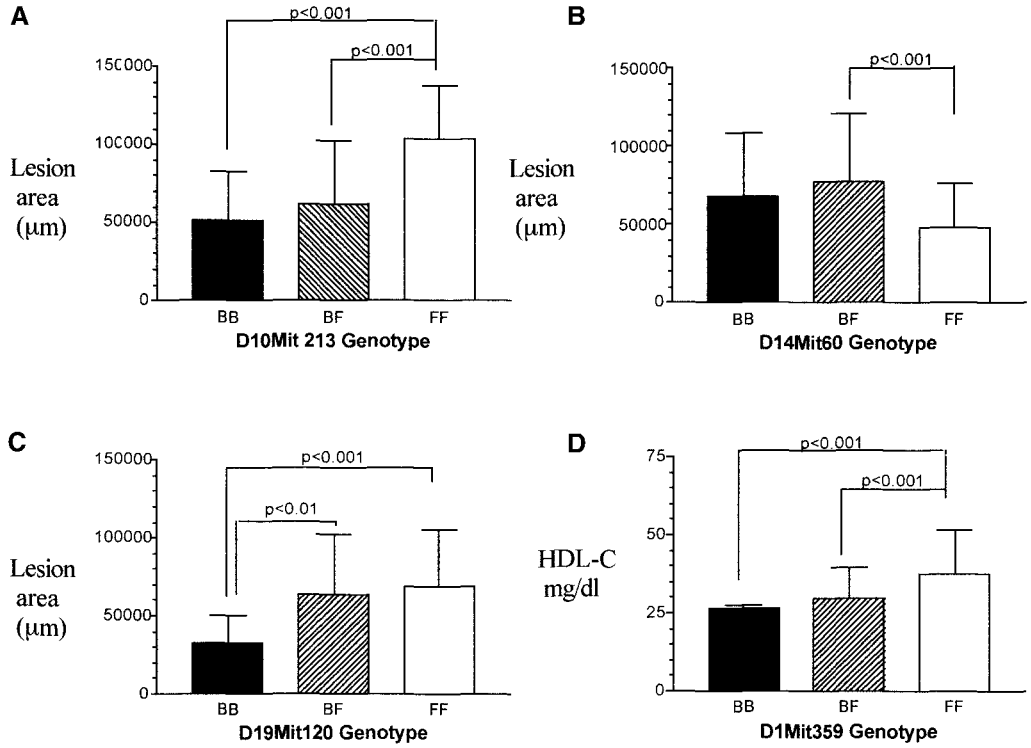

FIGURE 2.—The allele distributions in the F_2 cohort of cross 1 at D10Mit213 (A), D14Mit60 (B), and D19Mit-120 (C) for the male and female atherosclerotic lesion area data and the allele distribution in the F_2 cohort of cross 1 at D1Mit359 (D) for HDL cholesterol. The pairwise comparison bars note statistically significant differences.

periments are in progress to determine whether there are differences in macrophage *Ifngr* expression in C57BL/6 *Apoe*−/− and FVB/NCr-*Apoe*−/− mice.

Other candidate genes on chromosome 10 include the connective tissue growth factor gene (*CNF* in human and *Fisp12* in mouse), the estrogen receptor-α (*Esr1*) and tumor-necrosis-factor-induced protein 3 (*Tnfip3*). CNF has been shown to be upregulated in atherosclerotic lesions, being expressed predominantly at the shoulder of fibrous caps but also at the lipid core margins and in the necrotic core (OEMAR *et al.* 1997). Preliminary experiments looking at the expression of *Fisp12* in the aortic root of C57BL/6J and FVB/NJ mice have indicated that *Fisp12* is upregulated in C57BL/6J (data not shown), making *Fisp12* an excellent candidate for further studies.

The chromosome 14 interval (10–35 cM) has homology with human 14q11.2, 8p11.2, and 13q11-12. Candidate genes within this region of chromosome 14 embrace many proteases, including a family of mast cell proteases (*Mcpt1*, *2*, *4*, *5*, and *9*), two metalloproteases (*Mmp14* and *Adam13*), cathepsins G (*Ctsg*) and B (*Ctsb*), and clusterin (*Clu*), also known as apolipoprotein J.

The interval spanning 15–45 cM on mouse chromosome 19 that contains the QTL *Ath16* is evolutionarily conserved with human chromosomal regions of 9q12-21, 9p24, and 10q23-26. Candidates of some note within this interval include the very-low-density lipoprotein receptor (*Vldlr*), fibroblast growth factor 8 (*Fgf8*), the colony-stimulating factor, granulocyte macrophage, receptor-α (*Csfgmra*), and antioxidant protein 1 (*Apo1*).

A QTL on the distal end of chromosome 1 was strongly associated with total cholesterol, non-HDL cholesterol, and HDL cholesterol in both crosses. This interval from 60 to 90 cM is homologous to human 1q21-32 and encompasses the previously identified *Ath1* locus (PAIGEN *et al.* 1987a,b,c). We have no data to support that *Ath1* and the QTL we identified are the same. Lusis and co-workers (PURCELL-HUYNH *et al.* 1995; MACHLEDER *et al.* 1997) have also analyzed F_2 progeny derived from strains with high and low HDL levels and identified a QTL on chromosome 1 with peak markers close to the *Apoa2* gene, which is within the interval we defined in these studies. There are many reasons to think that the *Apoa2* gene is an excellent candidate for the QTL, *Ath9*, although it has been excluded as a candidate for *Ath1* (PAIGEN *et al.* 1987a,b,c). *Apoa2* expression levels have effects on both HDL and non-HDL cholesterol levels in mice; HDL is reduced in the *Apoa2* knockout mouse (WENG and BRESLOW 1996) and increased in mouse *Apoa2* transgenic mice (HEDRICK *et al.* 1993). Coding differences in the *Apoa2* gene sequence appear to play a major role in the differences in HDL and APOA2 levels in inbred strains of mice such as NZB and SM (PURCELL-HUYNH *et al.* 1995). We previously reported that plasma *Apoa2* levels in 129.FVB/NJ *Apoe*−/− mice are much higher than those in 129.C57BL/6J *Apoe*−/− mice (DANSKY *et al.* 1998). The *Apoa2* gene may therefore be responsible for the chromosome 1 QTL, *Ath9*. However, it is also possible that other genes such as the antioxidant protein 2 (*Aop2*) that lie in the interval may play a role in the strain differences in plasma lipids.

An unexpected finding was that mean lesion area was greater in F_2 mice homozygous for the D10Mit213 FVB/N

(FF) allele compared to F_2 mice homozygous for the C57BL/6 allele (BB). The increased lesion size that the F allele drives is not intuitively obvious given that FVB/NCr *Apoe*−/− mice have smaller lesions than do C57BL/6J *Apoe*−/− mice. Nevertheless, this locus accounted for ~20% of the variance in lesion area in the F_2 progeny. Likewise, homozygosity for the D19Mit120 C57BL/6 allele (BB) was associated with a smaller mean lesion area in the F_2 progeny (Figure 2C). However, it should be noted that a QTL analysis detects loci that regulate the phenotypic variance seen within the F_2 cohort and does not necessarily detect loci that are responsible for the phenotypic variance between the two parental strains. There are several possible explanations for these findings. The most likely scenario is that gene-gene interactions (epistasis) may be present. The (F) allele of the chromosome 10 QTL, *Ath11*, is proatherogenic but may require the presence of other genes in the C57BL/6J background. In this way, it would be proatherogenic in the F_2 progeny that have the interacting C57BL/6J alleles of the epistatic genes and not be proatherogenic in FVB/N Apoe−/− parental mice. Another possibility is that the (F) allele of this gene is proatherogenic but its effects are overshadowed by the actions of antiatherogenic genes in FVB/N *Apoe*−/− parental mice. Since the presence of the (F) allele of chromosome 14 locus was associated with decreases in atherosclerosis when compared to mice homozygous for the C57BL/6J allele (BB), this locus may represent one such antiatherogenic FVB gene.

The interval maps (Figure 2) for the chromosome 10, 14, and 19 QTL encompass regions that span 20–30 cM, which is fairly typical for most QTL. Interval mapping of chromosome 14 revealed a few peaks and valleys surrounding the peak locus (Figure 1B). This suggests, although does not prove, the possibility of multiple QTL and, therefore, multiple atherosclerosis susceptibility genes in this region. Indeed, the subsequent dissection of initially identified mouse QTL peaks has often resulted in the identification of multiple loci (Moore and Nagle 2000).

The next steps for this research are to identify the genes responsible for the predisposition loci identified. A two-pronged approach toward this will be taken. The production of interval-specific congenic strains has been started for the most significant, non-lipid-regulating loci, including the proximal region of chromosome 10, the broad region of D14Mit55-158, and the D19Mit120 interval. These interval-specific congenics will be used to reduce the genetic intervals defining each of the QTL and assist in further dissection into subregions and multiple QTL where necessary. Subphenotyping, classical genetic crosses, and gene identification approaches will then be pursued. In parallel the candidate gene approach will be used to look for DNA polymorphism and expression differences between FVB/NJ and C57BL/6J. We have started the candidate gene approach using both broad sweep expression profiling with gene arrays and mass spectrometer protein profiling of serum as well as the gene-by-gene approach for all the genes mentioned in the text above.

The intent of this long-term study was to use an ApoE null sensitized genetic background to identify loci that regulate the predisposition to atherosclerosis in the mouse but do not necessarily regulate cholesterol. Indeed, we have identified three such novel atherosclerosis susceptibility loci in the F_2 progeny from an intercross of C57BL/6J *Apoe*−/− and FVB/NCr *Apoe*−/−mice. The identification of these QTL is a prerequisite to further genetic and molecular analysis that will result in the identification of mouse atherosclerosis susceptibility genes. Given the striking similarities in atherosclerotic lesions present in humans and in mutant mouse models of hypercholesterolemia and atherosclerosis, it is likely that human atherosclerosis susceptibility genes will subsequently be isolated. In turn, the identification of these human susceptibility genes may lead to the discovery of new molecular pathways involved in atherogenesis and provide therapeutic targets aimed at treatment and prevention of human atherosclerotic vascular disease.

LITERATURE CITED

Chou, S. R., A. Brownell, M. S. Ko and J. Kaplan, 2000 Interferon-gamma receptor polymorphisms determine strain differences in accessibility of activated lymphocyte NK-triggering antigens to recognition by self-reactive NK cells. Cell. Immunol. **200:** 88–97.

Dansky, H. M., S. A. Charlton, J. L. Sikes, S. Heath and R. Simantov *et al.*, 1998 Genetic background determines the extent of atherosclerosis in apolipoprotein E deficient mice. Arterioscler. Thromb. Vasc. Biol. **999:** 1960–1968.

Dansky, H. M., S. A. Charlton, M. M. Harper and J. D. Smith, 1999 T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. Proc. Natl. Acad. Sci. USA **94:** 4642–4646.

De Caterina, R., T. Bourcier, U. Laufs, V. La Fata, G. Lazzerini *et al.*, 2001 Induction of endothelial-leukocyte interaction by interferon-gamma requires co-activation of nuclear factor-kappaB. Arterioscler. Thromb. Vasc. Biol. **21:** 227–232.

Dong, Z. M., A. A. Brown and D. D. Wagner, 2000 Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. Circulation **101:** 2290–2295.

Glass, C. K., and J. L. Witztum, 2001 Atherosclerosis. The road ahead. Cell **104:** 503–516.

Gu, L., Y. Okada, S. K. Clinton, C. Gerard, G. K. Sukhova *et al.*, 1998 Absence of monocyte chemo-attractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol. Cell **2:** 275–281.

Gupta, S., A. M. Pablo, X. C. Jiang, N. Wang, A. R. Tall *et al.*, 1997 IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. J. Clin. Invest. **99:** 2752–2761.

Hedrick, C. C., L. W. Castellani, C. H. Warden, D. L. Puppione and A. J. Lusis, 1993 Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice. J. Biol. Chem. **268:** 20676–20682.

Hussaini, I. M., J. LaMarre, J. J. Lysiak, L. R. Karns, S. R. VandenBerg *et al.* 1996 Transcriptional regulation of LDL receptor-related protein by IFN-gamma and the antagonistic activity of TGF-beta(1) in the RAW 264.7 macrophage-like cell line. J. Leukocyte Biol. **59:** 733–739.

Lander, E. S., and L. Kruglyak, 1995 Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. **11:** 241–247.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics. **1:** 174–181.

Li, H., M. W. Freeman and P. Libby, 1995 Regulation of smooth muscle cell scavenger receptor expression in vivo by atherogenic diets and in vitro by cytokines. J. Clin. Invest. **95:** 122–133.

Lincoln, S. E., and E. S. Lander, 1992 Systematic detections of errors in genetic linkage data. Genomics **14:** 604–610.

Lusis, A. J., 2000 Atherosclerosis. Nature **407:** 233–241.

Lusis, A., A. Weinreb and T. A. Drake, 1998 Genetics of atherosclerosis, pp. 2389–2413 in *Textbook of Cardiovascular Medicine*, edited by E. J. Topol. Lipincott-Raven Publishers, Philadelphia.

Machleder, D., B. Ivandic, C. Welch, L. Castellani, K. Reue *et al.*, 1997 Complex genetic control of HDL levels in mice in response to an atherogenic diet. J. Clin. Invest. **99:** 1406–1419.

Manly, K. A., 1993 Macintosh program for storage and analysis of experimental genetic mapping data. Mamm. Genome **4:** 303–313.

Marenberg, M. E., N. Risch, L. F. Berkman, B. Floderus and U. de Faire, 1994 Genetic susceptibility to death from coronary heart disease in a study of twins. N. Engl. J. Med. **330:** 1041–1046.

Markel, P., P. Shu, G. A. Carlson, D. L. Nagle, J. S. Smutko *et al.*, 1997 Theoretical and empirical issues for marker assisted breeding of congenic mouse strains. Nat. Genet. **17:** 280–284.

Mehrabian, M., J. Wong, X. Wang, Z. Jiang, W. Shi *et al.*, 2001 Genetic locus in mice that blocks development of atherosclerosis despite extreme hyperlidemia. Circ. Res. **89:** 125–130.

Moore, K. J., 1999 Utilization of mouse models in the discovery of human disease genes. Drug Discov. Today **4:** 123–128.

Moore, K. J., and D. L. Nagle, 2000 Complex trait analysis in the mouse: the strengths, the limitations and the promise yet to come. Annu. Rev. Genet. **34:** 653–686.

Mu, J. L., J. K. Naggert, K. L. Svenson, G. B. Collin, J. H. Kim *et al.*, 1999 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. **40:** 1328–1335.

Nishina, P. M., J. Wang, W. Toyofuku, F. A. Kuypers, B. Y. Ishida *et al.*, 1993 Atherosclerosis and plasma and liver lipids in nine inbred strains of mice. Lipids **28:** 599–605.

Oemar, B. S., A. Werner, J. M. Garnier, D. D. Do, N. Godoy *et al.*, 1997 Human connective tissue growth factor is expressed in advanced atherosclerotic lesions. Circulation **95:** 831–839.

Paigen, B., 1995 Genetics of responsiveness to high-fat and high-cholesterol diets in the mouse. Am. J. Clin. Nutr. **62:** 458S–462S.

Paigen, B., D. Albee, P. A. Holmes and D. Mitchell, 1987a Genetic analysis of murine strains C57BL/6J and C3H/HeJ to confirm the map position of Ath-1, a gene determining atherosclerosis susceptibility. Biochem. Genet. **25:** 501–511.

Paigen, B., D. Mitchell, P. A. Holmes and D. Albee, 1987b Genetic analysis of strains C57BL/6J and BALB/cJ for Ath-1, a gene determining atherosclerosis susceptibility in mice. Biochem. Genet. **25:** 881–892.

Paigen, B., D. Mitchell, K. Reue, A. Morrow, A. J. Lusis *et al.*, 1987c *Ath-1*, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc. Natl. Acad. Sci. USA **84:** 3763–3767.

Paigen, B., M. N. Nesbitt, D. Mitchell, D. Albee and R. C. LeBoeuf, 1989 Ath-2, a second gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Genetics **122:** 163–168.

Paigen, B., B. Y. Ishida, J. Verstuft, R. B. Winters and D. Albee, 1990 Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. Atherosclerosis **10:** 316–323.

Panousis, C. G., and S. H. Zuckerman, 2000 Interferon-gamma induces down regulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells. Arterioscler. Thromb. Vasc. Biol. **20:** 1565–1571.

Pitman, W. A., M. H. Hunt, C. McFarland and B. Paigen, 1998 Genetic analysis of the difference in diet-induced atherosclerosis between the inbred mouse strains SM/J and NZB/BINJ. Atheroscler. Thromb. Vasc. Biol. **18:** 615–620.

Plump, A. S., J. D. Smith, Y. Hayek, K. Aalto-Setala, A. Walsh *et al.*, 1992 Severe hypercholesterolemia and atherosclerosis in apoliprotein E-deficient mice created by homologus recombination in ES cells. Cell **71:** 343–353.

Plump, A. S., C. J. Scott and J. L. Breslow, 1994 Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. Proc. Natl. Acad. Sci. USA **91:** 9607–9611.

Purcell-Huynh, D. A., A. Weinraub, L. W. Castellani, M. Mehrabian, M. H. Doolittle *et al.*, 1995 Genetic factors in lipoprotein metabolism: analysis of a genetic cross between inbred mouse strains NZB/BINJ and SM/J using a complete linkage map approach. J. Clin. Invest. **96:** 1845–1858.

Roberts, A., and J. Thompson, 1977 Genetic factors in the development of atheroma and in serum total cholesterol levels in inbred mice and their hybrids. Prog. Biochem. Pharmacol. **13:** 298–308.

Ross, R., 1993 The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature **362:** 801–809.

Ross, R., 1999 Mechanisms of disease: atherosclerosis—an inflammatory disease. N. Engl. J. Med. **340:** 115–126.

Schonbeck, U., F. Mach, G. K. Sukhova, C. Murphy, J. Y. Bonnefoy *et al.*, 1997 Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: A role for CD40 signaling in plaque rupture? Circ. Res. **81:** 448–454.

Selzman, C. H., B. D. Shames, T. A. Whitehill, A. H. Harken and R. C. McIntyre, Jr., 1998 Class II cytokine receptor ligands inhibit human vascular smooth muscle proliferation. Surgery **124:** 318–326.

Smith, J. D., and J. L. Breslow, 1997 The emergence of mouse models of atherosclerosis and their relevance to clinical research. J. Intern. Med. **242:** 99–109.

Stewart-Phillips, J. L., J. Lough and E. Skamene, 1989 *Ath-3*, a new gene for atherosclerosis in the mouse. Clin. Invest. Med. **12:** 121–126.

Van Ree, J. H., W. J. van den Broek and V. E. Dahlmans, 1994 Diet-induced hypercholesterolemia and atherosclerosis in heterozygous apolipoprotein E-deficient mice. Atherosclerosis **111:** 25–37.

Welch, C. L., S. Bretschger, N. Latib, M. Bezouevski, Y. Guo *et al.*, 2001 Localization of atherosclerosis susceptibility loci to chromosomes 4 and 6 using the Ldlr knockout mouse model. Proc. Natl. Acad. Sci. USA **98:** 7946–7951.

Weng, W., and J. L. Breslow, 1996 Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. Proc. Natl. Acad. Sci. USA **93:** 14788–14794.

Wilson, P. W., E. J. Schaefer, M. G. Larson and J. M. Ordovas, 1996 Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler. Thromb. Vasc. Biol. **16:** 1250–1255.

Zhang, S. H., R. L. Reddick, J. A. Piedrahita and N. Maeda, 1992 Spontaneous hypercholesterolemia and arterial lesions in mice lacking apoliprotein E. Science **258:** 468–471.

Communicating editor: N. A. Jenkins