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Quantitative Trait Locus Analysis of Carotid Atherosclerosis in an Intercross Between C57BL/6 and C3H Apolipoprotein E–Deficient Mice

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Abstract

Background and Purpose—Inbred mouse strains C57BL/6J (B6) and C3H/HeJ (C3H) exhibit marked differences in atherosclerotic lesion formation in the carotid arteries on the apolipoprotein E–deficient (apoE$^{-/-}$) background when fed a Western diet. Quantitative trait locus analysis was performed on an intercross between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice to determine genetic factors contributing to variation in the phenotype.

Methods—Female B6.apoE$^{-/-}$ mice were crossed with male C3H.apoE$^{-/-}$ mice to generate F_1 hybrids, which were intercrossed to generate 241 female F_2 progeny. At 6 weeks of age, F_2 mice were started on a Western diet. After being fed the diet for 12 weeks, F_2 mice were analyzed for phenotypes such as lesion size in the left carotid arteries and plasma lipid levels and typed for 154 genetic markers spanning the mouse genome.

Results—One significant quantitative trait locus, named *CAth1* (25 cM, log of the odds score: 4.5), on chromosome 12 and 4 suggestive quantitative trait loci, on chromosomes 1, 5, 6, and 11, respectively, were identified to influence carotid lesion size. One significant quantitative trait locus on distal chromosome 1 accounted for major variations in plasma low-density lipoprotein/very-low-density lipoprotein, high-density lipoprotein cholesterol, and triglyceride levels. Carotid lesion size was not significantly correlated with plasma low-density lipoprotein/very-low-density lipoprotein or high-density lipoprotein cholesterol levels.

Conclusions—These data indicate that the loci for carotid lesions do not overlap with those for aortic lesions as identified in a previous cross derived from the same parental strains, and carotid atherosclerosis and plasma lipids are controlled by separate genetic factors in the B6 and C3H mouse model.

Keywords

carotid atherosclerosis; hyperlipidemia; quantitative trait locus; stroke

Stroke is the leading cause of disability in adults and the third most common cause of death in the United States and other Western countries.[1] A large fraction of ischemic stroke cases are

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caused by atherosclerosis in the carotid arteries. Carotid atherosclerotic lesions may directly or indirectly, through formation of thrombi, result in narrowing of vascular lumen and block the blood flow to the brain. Genetic studies involving twins, siblings, and families have provided strong evidence for the heritability of carotid atherosclerosis, but the underlying genetic factors remain unidentified.[2]

Genetic studies of carotid atherosclerosis in humans are underway, but they have been difficult because of the heterogeneity of human populations and the difficulty for the control of environmental influences on the disease. The difficulties of human genetic studies strongly demand alternative approaches to be undertaken by using animal models. The mouse is the most commonly used mammal for genetic study of atherosclerosis because of the availability of sophisticated genetic and biomedical tools. However, wild-type mice do not develop atherosclerotic lesions in the carotid arteries even when they are fed an atherogenic diet containing high fat/cholesterol and cholate (the Paigen diet) for up to 1 year.[3,4] Apolipoprotein E–deficient (apoE$^{-/-}$) mice represent a mouse model in which spontaneous hyperlipidemia and atherosclerosis occur on a low-fat, low-cholesterol diet.[5,6] Lesions in apoE$^{-/-}$ mice, like in humans, tend to develop at branch points of large and medial arteries and progress from the foam cell stage to the advanced stage with fibrous caps and necrotic lipid core.[7] Recently, Rosenfeld et al[8] reported that apoE$^{-/-}$ mice on a C57BL/6 (B6) background develop advanced lesions in the carotid arteries, exhibiting a highly consistent rate of lesion progression.

We and others have demonstrated that genetic backgrounds have a dramatic influence on the susceptibility of apoE$^{-/-}$ mice to atherosclerosis. When the apoE null allele of B6 mice was transferred onto the genetic background of strain FVB/NT, aortic atherosclerotic lesions of the mice were reduced up to 9-fold.[9] On the C3H background, we found that the influence was even more dramatic.[10] The dramatic differences between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice in the formation of carotid atherosclerotic lesions are ideal for conducting genetics studies of the trait. Thus, in the present study, we performed quantitative trait locus (QTL) analysis using an intercross between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice to search for loci that influence the development of carotid atherosclerosis and its associated traits.

Materials and Methods

Mice

B6.apoE$^{-/-}$ mice at the N10 backcross were purchased from the Jackson Laboratories (Bar Harbor, Maine), and C3H.apoE$^{-/-}$ mice were generated in our laboratory by initially crossing B6.apoE$^{-/-}$ mice with C3H/HeJ mice followed by 12 sequential generations of backcrossing with C3H/HeJ mice. Female B6.apoE$^{-/-}$ mice were crossed with male C3H.apoE$^{-/-}$ mice to generate F_1 hybrids, which were subsequently intercrossed by brother–sister mating to generate 241 female F_2 progeny. Female mice were studied because they develop larger atherosclerotic lesions than their male counterparts. This is opposite to the human condition in which premenopausal women are protected against atherosclerosis. At 6 weeks of age, F_2 mice were started on a Western-type diet containing 42% fat and 0.15% cholesterol without sodium cholate (TD 88137; Teklad, Madison, Wis) and maintained on the diet for 12 weeks. All procedures were carried out in accordance with current National Institutes of Health guidelines and approved by the University Animal Care and Use Committee.

Plasma Lipid Analysis

The measurements of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride were performed as reported previously.[11]

Quantitation of Carotid Atherosclerotic Lesions

The method for isolating the bifurcation of the left common carotid artery was similar to that reported by Rosenfeld et al[8] and von der Thusen et al.[12] Briefly, mice were euthanized by prolonged inhalation of isoflurane. The vasculature of the animals was perfusion-fixed with 10% formalin through the left ventricle of the heart for >10 minutes. Then, the left common carotid artery and its main branches were dissected from the surrounding tissues, harvested en bloc, and embedded in OCT compound (Tissue-Tek). Serial 10-μm-thick cryo-sections were collected every 3 sections and mounted on poly-D-lysine-coated slides. Thirty to 50 sections were collected for each mouse. Sections were stained with oil red O and hematoxylin and counter-stained with light green. Lesion areas were quantified using an ocular with a square-micrometer grid on a light microscope. The lesion areas of 5 sections with the largest readings were averaged for each mouse and this average was used for statistical analysis.

Genotyping

Genomic DNA was isolated from the tails of mice by using the standard phenol/chloroform extraction and ethanol precipitation method. F_2 mice were genotyped initially with 143 microsatellite markers distinguishing strain B6 from strain C3H and covering all chromosomes at an average interval of 12 cM by polymerase chain reaction analysis. Eleven additional markers were then typed for the chromosomal regions that showed linkage with carotid lesion area. Parental and F_1 DNA were used as controls for each marker typed.

Linkage and Statistical Analyses

Linkage analysis of lesion areas and plasma lipid levels was performed by using both Map Manager QTXb20 (http://mapmgr.roswellpark.org/) and J/qtl (www.jax.org/staff/churchill/) software as described previously.[13] Lesion areas were log transformed before QTL analysis was performed. Low-density lipoprotein/very-low-density lipoprotein (LDL/VLDL), HDL cholesterol, and triglyceride levels were directly used for QTL analysis as they were normally distributed. Analysis of variance was used for determining whether the mean phenotype values of progeny with different genotypes at a specific marker were significantly different. Differences were considered statistically significant at $P < 0.05$.

Results

Two hundred forty-one female F_2 mice derived from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice were analyzed to identify the chromosomal regions contributing to carotid atherosclerosis and related traits. As shown in Figure 1, log-transformed lesion areas were distributed in a bimodal manner with approximately 17% (41) of F_2s sitting under the no lesion peak on the left and the remaining 83% (200) falling under the bell-shaped curve on the right. LDL/VLDL, HDL cholesterol, and triglyceride levels of F_2 mice were approximately normally distributed.

A genomewide scan of the F_2 cross revealed one significant QTL on chromosome 12 and 4 suggestive QTLs located on chromosomes 1, 5, 6, and 11, respectively, which influenced carotid lesion areas (Figure 2). Details of the QTLs detected, including peak marker locus, log of the odds (LOD) score, SI, variance, probability value, and mode of inheritance, are presented in Table 1. Effects of B6 and C3H alleles on various traits in different QTLs are presented in Table 2. The chromosome 12 locus exhibited a peak linkage with the microsatellite marker *D12Mit285* (25 cM, LOD: 4.5), which explained 8% of the variation in lesion area of the cross (Figure 3). We designated this locus as *CAth1*. This locus exhibited an additive inheritance pattern in that F_2 mice with the heterozygous BC genotype at *D12Mit285* had a lesion size intermediate between mice with the homozygous BB genotype and those with the CC genotype (Table 2).

The chromosome 1 locus had a suggestive LOD score of 2.9 and displayed a peak linkage with the marker *D1Mit45* (58.5 cM) (Figure 3; Table 1). This locus explained 5% of the variation in lesion area of the cross and exhibited an additive effect on the trait. The chromosome 5 locus near marker *D5Mit210* (64 cM) had a suggestive LOD score of 3.2 and explained 7% of the variation in lesion area. The C3H allele was associated with a paradoxical increase in lesion area. The locus on chromosome 6 near marker *D6Mit102* (38.5 cM) had a suggestive LOD score of 3.1 and accounted for 6% of the variation in lesion area. The locus at marker *D11Mit36* on chromosome 11 had a suggestive LOD score of 2.4 and explained 5% of the variation in lesion area. The last 2 QTLs displayed an additive inheritance pattern with the B6 allele associated with an increase in lesion area.

As shown in Figure 4 and Table 1, plasma LDL/VLDL, HDL cholesterol, and triglyceride levels were each controlled by multiple QTLs. However, one QTL on chromosome 1 was associated with major variations in plasma levels of LDL/VLDL, HDL cholesterol, and triglyceride of the cross. LOD score plots for chromosome 1 revealed the colocalization of QTL peaks for these traits in the distal region (Figure 5), suggesting a possibility that these traits were controlled by the same gene within the region. This QTL had LOD scores of 10.1 for LDL/VLDL, of 7.2 for HDL cholesterol, and of 8.8 for triglyceride and explained 13% to 19% of the variations in the traits.

QTLs on other chromosomes also contributed to the variations in plasma lipid levels. For LDL/VLDL, the locus near *D3Mit21* (19.2 cM, LOD: 2.7) corresponded to *Hdlq19*, previously identified in a B6×DBA/2 cross.[14] The locus at *D6Mit243* (30.4 cM, LOD: 2.2) corresponded to *Pnhdlc1*, a QTL mapped in a B6×CASA intercross.[15] The QTLs near *D9Mit274* (38 cM, LOD: 2.7) and *D12Mit97* (47 cM, LOD: 4.6) overlapped with 2 loci near *D9Mit26* (28 cM) and *D12Mit5* (37 cM), respectively, previously mapped in a NZB×SM F_2 cross.[16] These 2 QTLs were named *Nhdlq11* and *Nhdlq12*, respectively, because they have not been named.

For HDL, the QTL near *D2Mit263* (92 cM, LOD: 4.3) corresponded to *Hdlq19*, which was mapped in a B6×129 intercross.[17] The QTL near *D17Mit244* (55.7 cM, LOD: 3.0) overlapped with *Hdl4*, mapped in a CAST/Ei×B6 cross,[18] and *Hdlq39*, mapped in PERA/EiJ×I/LnJ and PERA/EiJ×DBA/2J intercrosses.[19] For triglyceride, the locus at *D5Mit95* (68 cM, LOD: 3.2) overlapped with a QTL at *D5Mit65* (68 cM) mapped using a NZB×SM F2 cross.[16] The QTL at *D9Mit260* (38 cM, LOD: 2.5) overlapped with *Trigq1* mapped in a KK×RR F_2 mice.[20] The QTL at *D2Mit263* is novel.

The relationships between carotid atherosclerotic lesions and plasma lipid levels were analyzed using the F_2 population. No significant correlations were observed between carotid lesion areas and LDL/VLDL cholesterol levels (R=0.042, P=0.54) nor between carotid lesion areas and HDL cholesterol levels ($R = -0.123$ and $P = 0.06$; Figure 6).

Discussion

In the present study, we used the apoE$^{-/-}$ mouse model to search for QTLs that contribute to the differences between B6 and C3H mice in carotid atherosclerosis and plasma lipid levels. We identified one significant QTL on chromosome 12 and 4 suggestive QTLs on chromosomes 1, 5, 6, and 11, respectively, for carotid atherosclerosis, and replicated 12 QTLs for plasma lipids, including one QTL on distal chromosome 1 contributing to the major variations in plasma lipid levels of the mice.

The mouse is the most commonly used animal model for genetic study of atherosclerosis. Because the aortic root is easy to study, previous genetic studies of atherosclerosis are entirely based on the aortic root, a site of less clinical significance in humans. The findings from the aortic root may not reflect the situation elsewhere in the vascular tree. It has been shown that

the aortic root responds differently to probucol as compared with more distal regions of the aorta,[21] and the aortic root and the innominate artery respond differently to immune deficiency in terms of atherosclerotic lesion formation.[22] Because of the close association with the brain and the ready accessibility, the carotid arteries are the most extensively studied vessels in humans with ultrasonography. Several prospective studies with ultrasound demonstrate that carotid artery stenosis is an important risk factor for stroke. In patients with noticeable carotid stenosis, there are severely impaired cerebral blood flow and markedly increased risk for ipsilateral stroke.[23,24] In patients with no obvious carotid stenosis, perspective studies also show a close association between the intima media thickening and the risk for stroke.[24–28] Intima media thickening of the carotid arteries is considered an early marker of atherosclerosis.[29,30]

The carotid arteries have been shown to develop athero-sclerotic lesions in apoE$^{-/-}$ mice by 27 weeks of age, even if these mice are maintained on a chow diet,[22] and lesion formation could be shortened if the mice are fed a Western-type diet. Using a F_2 cross between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice, in this study, we identified one significant QTL and 4 suggestive QTLs affecting carotid lesion formation. We have designated the chromosome 12 locus as *CAth1* to represent the first QTL that affects carotid lesion formation in the mouse. The chromosome 11 locus (37 to 54 cM) for carotid atherosclerosis was different from the locus for aortic atherosclerosis (11 to 33 cM) because the 2 QTLs did not overlap in the support interval.[13] The QTL mapped to chromosome 6 corresponded to the position of *Artles* (46 to 71 cM) previously mapped in a CAST×B6 intercross[31] and of *Athsq2* (52 to 70 cM) mapped in a (B6.*Ldlr*$^{-/-}$ × MOLF) × B6.*Ldlr*$^{-/-}$ N_2 cross.[32] The QTLs mapped to chromosomes 1 and 5 did not overlap with any previously mapped QTLs for aortic atherosclerosis.

The QTLs for carotid atherosclerosis are different from those for aortic atherosclerosis previously identified using a separate cross between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ strains.[13] In the previous cross, which was done under identical conditions, aortic lesion area was affected by a significant QTL near *D9Mit360* (35 cM) with a LOD score of 3.7 and a suggestive QTL near *D11Mit236* (20 cM) with a LOD score of 2.4.[13] In contrast, carotid lesion area was affected by one significant QTL near *D12Mit285* and 4 suggestive QTLs near *D1Mit45*, *D5Mit210*, *D6Mit102*, and *D11Mit36*. The distribution pattern of lesion size in the F_2s and the difference between the 2 parental strains in susceptibility to aortic atherosclerosis versus carotid atherosclerosis also suggest that carotid atherosclerosis and aortic atherosclerosis are controlled by separate genetic factors in the apoE$^{-/-}$ mice. The carotid lesion area of F_2 mice exhibited a bimodal distribution with 41 animals having no lesion, which is in contrast to the bell-shaped normal distribution of aortic lesion area in the previous cross.[13] C3H.apoE$^{-/-}$ mice were completely absent of carotid lesions after being fed a Western diet for 12 weeks (data not shown), but these mice develop aortic lesions under the same condition.[10] The bimodal distribution of carotid lesions suggests a genetic control of carotid atherosclerosis by a recessive-resistant gene from C3H.

In the present study, we found that a QTL in distal chromosome 1 was responsible for the major variations in plasma LDL/VLDL, HDL cholesterol, and triglyceride levels of the cross. This finding is consistent with our observation in the previous F_2 cross between B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice.[13] Elevation in plasma LDL/VLDL levels or reduction in HDL levels have been considered to be major risk factors for atherosclerotic vascular disease. However, the present study of F_2 mice has demonstrated that carotid atherosclerosis susceptibility was independent of plasma lipids because the size of carotid lesions was not correlated with LDL/VLDL or HDL cholesterol levels. The studies of atherosclerotic lesions at the aortic root in several genetic crosses also demonstrated no significant associations between atherosclerosis susceptibility and plasma lipoprotein levels.[13,32,33] Previously, we have found that cultured B6 aortic endothelial cells are highly responsive to oxidized LDL, exhibiting induction of

chemokines and adhesion molecules, whereas C3H endothelial cells are relatively unresponsive.[34] Recently, direct evidence supporting the arterial wall involvement in control of atherosclerosis susceptibility has been found when B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ aortic segments were transplanted into F1 mice, and the donor aortic segments of B6 mice developed significantly larger lesions than those of C3H mice.[35] Blood pressure of the 2 strains was normal and comparable when fed chow or a high-fat diet; thus, it could not explain their difference in carotid lesion formation.

The mouse has been a powerful force in elucidating the genetic basis of human physiology and pathophysiology.[36] Finding QTL genes in mice is much more cost-effective, less time-consuming, and less fraught with ethical issues than finding them in humans because many new genetic, genomic, and bioinformatics tools for the mouse are available. After a QTL gene is identified in the mouse, it can be tested in human association studies for its role in human diseases. Among the human atherosclerosis QTLs reported, 63% are located in regions homologous to mouse QTLs,[37] although further studies are needed to determine whether mouse and human atherosclerosis QTLs have the same underlying genes.

In summary, this study has identified several new QTLs for carotid atherosclerosis, which do not overlap with loci for aortic atherosclerosis as identified from a separate F_2 cross derived from the same parental strains. The demonstration that the variation in carotid atherosclerosis between mouse strains B6 and C3H is not due to genetic differences in plasma lipid levels provides an explanation for the clinical observation that a large fraction of patients develop ischemic strokes despite normolipidemia.[38]

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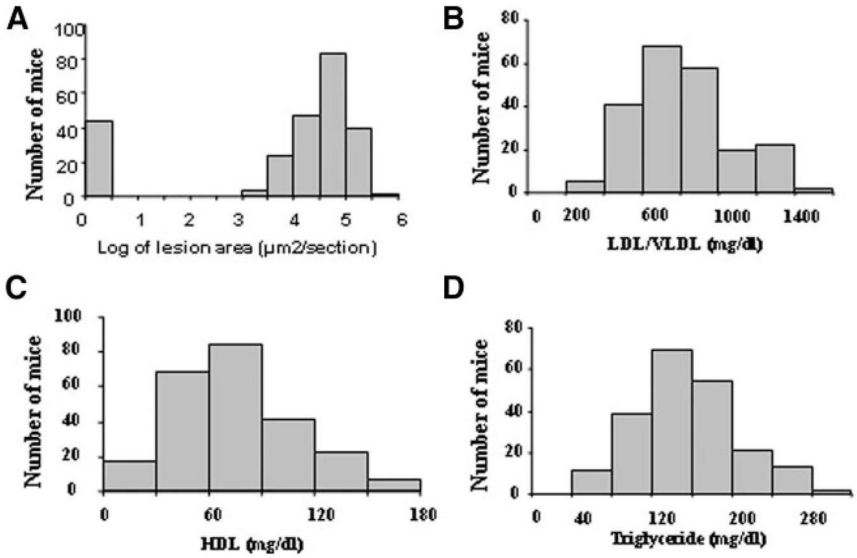

Figure 1.

Distributions of log-transformed carotid lesion areas (A), LDL/VLDL (B), HDL cholesterol (C), and triglyceride levels (D) in 241 female F_2 mice derived from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. Mice were fed a Western diet for 12 weeks.

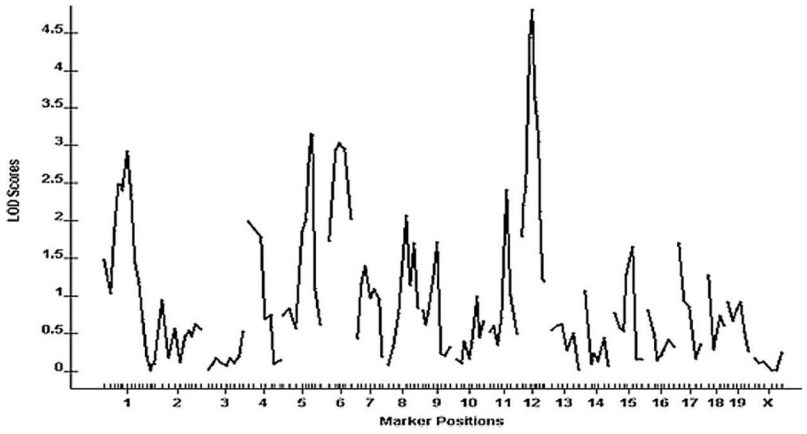

Figure 2.

A genomewide scan to search for loci influencing carotid lesions. Chromosomes 1 through X are represented numerically on the x-axis. The relative width of the space allotted for each chromosome reflects the relative length of each chromosome. The y-axis represents the LOD score. Carotid lesion areas were determined by averaging the lesion areas of 5 cross-sections with the largest readings for each of 241 female F_2 mice.

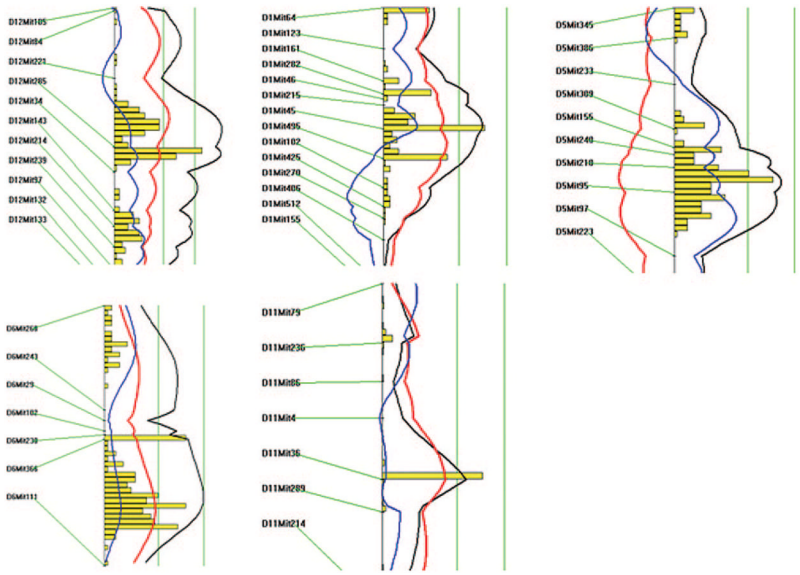

Figure 3.

Likelihood plots for carotid lesion QTLs on chromosomes 12, 1, 5, 6, and 11. Plots were created using the interval mapping function of Map Manager QTX, including a bootstrap test shown as a histogram to estimate the confidence interval a QTL. Two straight vertical lines on the plot represent significance thresholds for suggestive and significant QTLs from left to right, respectively. Black plots reflect the likelihood ratio statistic calculated at 1-cM intervals. The red plot represents the additive effect, whereas the blue plot represents the dominant effect.

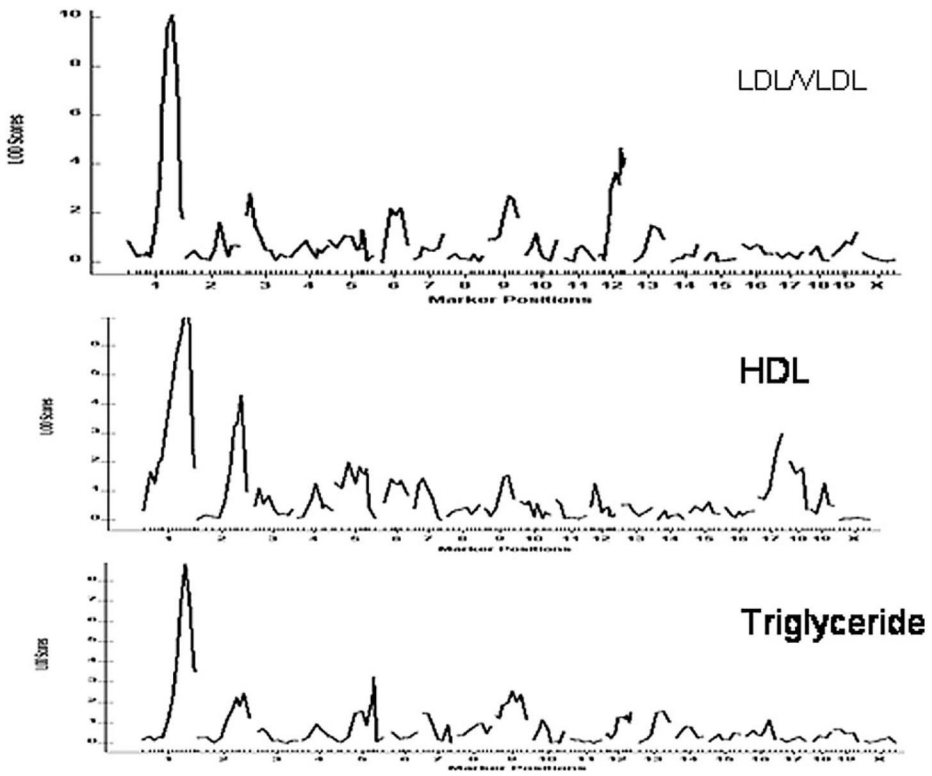

Figure 4.
Genomewide scans for loci affecting plasma levels of LDL/VLDL, HDL cholesterol, and triglyceride in F_2 mice. Chromosomes 1 through X are represented numerically on the x-axis and the y-axis represents the LOD score.

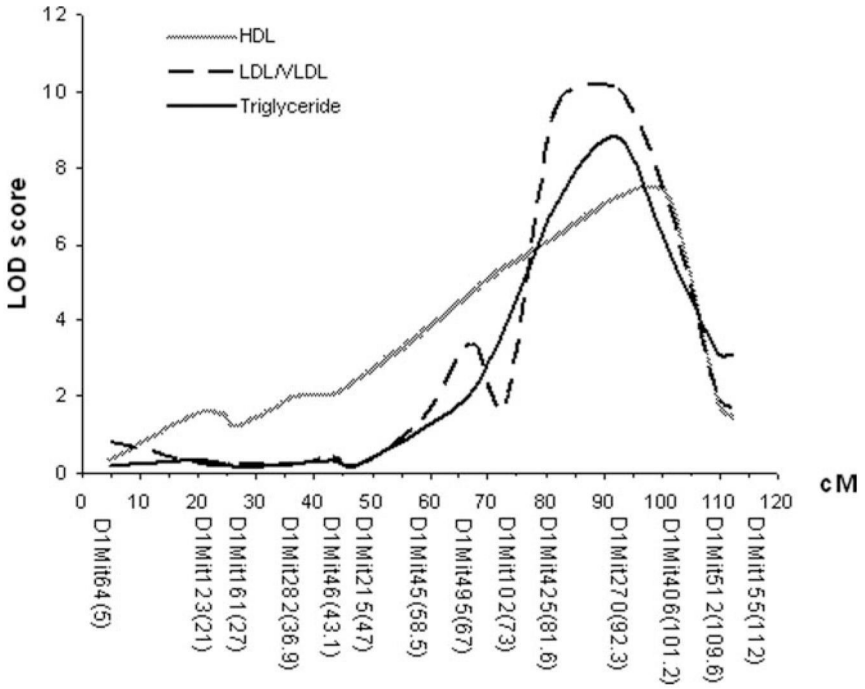

Figure 5.

LOD score plots for LDL/VLDL, HDL cholesterol, and triglyceride levels on chromosome 1. The x-axis depicts the marker positions in centimorgans, and the y-axis depicts the LOD score. The micro-satellite markers typed are listed below the x-axis corresponding to their map locations on the chromosome.

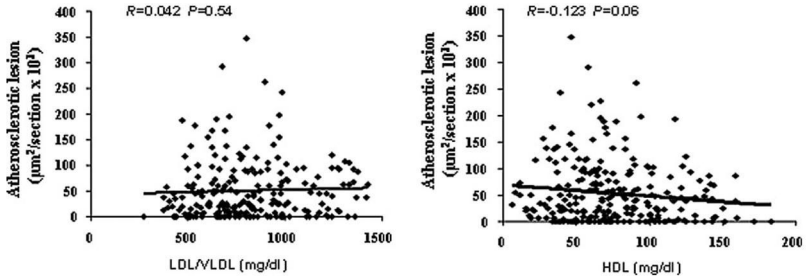

Figure 6.

Scatterplots showing relationships between carotid lesion sizes and plasma lipid levels in the F_2 cross. Each point represents an individual value of a F_2 mouse. The correlation coefficient (*R*) and significance (*P*) are shown. Plasma levels of LDL/VLDL or HDL cholesterol were not correlated with the sizes of atherosclerotic lesions in the cross.

Table 1

Significant and Suggestive QTLs for Carotid Atherosclerotic Lesions, LDL/VLDL, HDL Cholesterol, and Triglyceride Identified in an Intercross Between B6.apoE$^{-/-}$ and C3H. $^{-/-}$ Mice

Chromosome Marker (cM)	Traits	LOD*	SI (cM)†	Variance (%)‡	*P* Value	Model of Inheritance
D1Mit45 (58.5)	Lesion	2.9	32–70	5	1.3×10^{-3}	Additive
D5Mit210 (64)	Lesion	3.2	56–72	7	1.2×10^{-4}	Dominant/C3H
D6Mit102 (38.5)	Lesion	3.1	21–60	6	6.9×10^{-4}	Additive
D11Mit36 (47.64)	Lesion	2.4	37–54	5	3.4×10^{-3}	Additive
D12Mit285 (25)	Lesion	4.5	21–28	8	6.0×10^{-5}	Additive
D1Mit270 (92.3)	LDL/VLDL	10.1	79–95	19	$<10^{-5}$	Dominant/C3H
D3Mit21 (19.2)	LDL/VLDL	2.7	11–28	6	1.6×10^{-3}	Additive
D6Mit243 (30.4)	LDL/VLDL	2.2	25–59	5	4.5×10^{-3}	Additive
D9Mit274 (38)	LDL/VLDL	2.7	35–67	5	2.7×10^{-3}	Dominant/C3H
D12Mit97 (47)	LDL/VLDL	4.6	38–56	8	3.0×10^{-5}	Additive
D1Mit406 (101.2)	HDL	7.2	88–103	13	$<10^{-5}$	Additive
D2Mit263 (92)	HDL	4.3	87–98	8	4.0×10^{-5}	Dominant/B6
D17Mit244 (55.7)	HDL	3	45–56	5	1.2×10^{-3}	Dominant/B6
D1Mit270 (92.3)	Triglyceride	8.8	85–99	18	$<10^{-5}$	Dominant/C3H
D2Mit263 (92)	Triglyceride	2.5	89–103	6	2.6×10^{-3}	Dominant/C3H
D5Mit95 (68)	Triglyceride	3.2	65–70	9	3.0×10^{-5}	Additive
D9Mit260 (38)	Triglyceride	2.5	20–65	5	3.2×10^{-3}	Dominant/C3H

*LOD scores were obtained by dividing the LRS by 4.6. For lesion area: suggestive QTL LOD ≥2.2, significant LOD ≥3.6; for LDL/VLDL: suggestive QTL LOD ≥2.2, significant LOD ≥3.6; for HDL: suggestive QTL LOD ≥2.2, significant LOD ≥3.5; for triglyceride: suggestive QTL LOD ≥2.2, significant LOD ≥3.5.

†Support interval (SI) was defined as a one-unit drop in LOD score on both sides of the peak marker.

‡Variance (%) indicates the percentage of the total trait variance, which could be explained by a QTL at this locus.

Table 2

Effects of C3H (C) and B6 (B) Alleles in Different QTLs on Carotid Lesions and Plasma Lipid Levels in the B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ Intercross

Traits	Marker	BB*	BC†	CC‡	*P* Value
Lesion	D1Mit45	70806±71304	56184±59943	33538±40300	2.1×10^{-3}
	D5Mit210	38895±55573	59679±62504	56884±56415	9.3×10^{-2}
	D6Mit102	70106±71556	53846±55193	29524±42929	8.9×10^{-4}
	D11Mit36	70233±67856	54724±58816	30107±41469	1.1×10^{-3}
	D12Mit285	84347±76076	51338±53111	26563±33529	1.8×10^{-7}
LDL/VLDL	D1Mit270	645±153	859±306	934±306	1.2×10^{-10}
	D3Mit21	895±270	818±268	722±191	1.8×10^{-3}
	D6Mit243	875±276	810±240	713±234	4.1×10^{-3}
	D9Mit274	709±200	838±266	860±270	2.6×10^{-3}
	D12Mit97	924±276	818±246	709±220	3.9×10^{-5}
HDL	D1Mit406	59±28	78±34	99±47	7.1×10^{-8}
	D2Mit263	65±33	65±26	87±42	4.8×10^{-5}
	D17Mit244	75±38	69±34	92±42	1.3×10^{-3}
Triglyceride	D1Mit270	120±37	168±45	175±50	2.0×10^{-9}
	D2Mit263	134±43	163±50	160±46	2.8×10^{-3}
	D5Mit95	141±48	148±42	172±48	1.6×10^{-4}
	D9Mit260	132±31	163±48	166±55	3.4×10^{-3}

Measurements are given as means ± SD. The units for these measurements are: lesion (μm^2 per section), LDL/VLDL, HDL cholesterol, and triglyceride (mg/dL).

*BB, homozygous for C57BL/6 alleles,

†CC, homozygous for C3H alleles, and

‡BC, heterozygous for C57BL/6 and C3H alleles at the linked markers.