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Genetic Analysis of Atherosclerosis and Glucose Homeostasis in an Intercross Between C57BL/6 and BALB/cJ Apolipoprotein E-Deficient Mice

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Abstract

Background—Diabetic patients have an increased risk of developing atherosclerosis and related complications compared to non-diabetic individuals. The increased cardiovascular risk associated with diabetes is due in part to genetic variations that influence both glucose homeostasis and atherosclerotic lesion growth. Mouse strains C57BL/6J (B6) and BALB/cJ (BALB) exhibit distinct differences in fasting plasma glucose and atherosclerotic lesion size when deficient in apolipoprotein E (*Apoe*$^{-/-}$. Quantitative trait locus (QTL) analysis was performed to determine genetic factors influencing the two phenotypes.

Methods and Results—266 female F_2 mice were generated from an intercross between B6.*Apoe*$^{-/-}$ and BALB.*Apoe*$^{-/-}$ mice and fed a Western diet for 12 weeks. Atherosclerotic lesions in the aortic root, fasting plasma glucose, and body weight were measured. 130 microsatellite markers across the entire genome were genotyped. Four significant QTLs, *Ath1* on chromosome (Chr) 1, *Ath41* on Chr2, *Ath42* on Chr5, and *Ath29* on Chr9, and one suggestive QTL on Chr4, were identified for atherosclerotic lesion size. Four significant QTLs, *Bglu3* and *Bglu12* on Chr1, *Bglu13* on Chr5, *Bglu15* on Chr12, and two suggestive QTLs on Chr9 and Chr15 were identified for fasting glucose levels on the chow diet. Two significant QTLs, *Bglu3* and *Bglu13*, and one suggestive locus on Chr8 were identified for fasting glucose on the Western diet. One significant locus on Chr1 and two suggestive loci on Chr9 and Chr19 were identified for body weight. *Ath1* and *Ath42* coincided with *Bglu3* and *Bglu13*, respectively, in the confidence interval.

Conclusions—We have identified novel QTLs that have major influences on atherosclerotic lesion size and glucose homeostasis. The colocalization of QTLs for atherosclerosis and diabetes suggests possible genetic connections between the two diseases.

Keywords

Atherosclerosis; type 2 diabetes; quantitative trait locus; hyperglycemia

Introduction

Type 2 diabetes mellitus (T2DM) is a major risk factor for atherosclerotic cardiovascular disease. Diabetic patients have a two- to four-fold higher risk of developing atherosclerosis and its complications compared with non-diabetic individuals [1]. Part of the increased

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cardiovascular risk associated with diabetes is due to genetic variations that influence both glucose homeostasis and the development of atherosclerosis. A few rare gene mutations result in both early coronary heart disease and T2DM that are observable as Mendelian traits segregatingin families, which include *LRP6*[2], *ABCA1* [3,4], and *APOB* [5]. Recent genome-wide association studies (GWAS) have identified dozens of common genetic variants for both atherosclerosis and T2DM (http://www.genome.gov/GWAStudies/). Several of the variants for coronary heart disease are within genes that are involved in glucose metabolism or are associated with T2DM, such as *MTHFD1L* and *HNF1A*. Thus far few studies have been conducted to examine such genetic variants and in those that have the findings are inconsistent [6, 7, 8, 9,10]. One major challenge for such studies is the difficulties inherent in establishing causality between genetic variants and complex disease in humans due to small gene effects, complex genetic structure, and environmental influences.

A complementary approach to the identification of genetic components in human disease is to use animal models. One commonly usedrodent model of atherosclerosis is the apolipoprotein E-deficient (*Apoe*$^{-/-}$) mouse, which develops all phases of atherosclerotic lesions seen in humans, progressing from the early foam cell stage to the advanced stage with a fibrous cap and necrotic lipid core [11]. We have observed that atherosclerosis susceptible C57BL/6 (B6) *Apoe*$^{-/-}$ mice develop significant hyperglycemia when fed a Western-type diet [12]. In contrast, atherosclerosis-resistant BALB/cJ (BALB) *Apoe*$^{-/-}$ mice are highly resistant to hyperglycemia [13]. The concordant differences between the two *Apoe*$^{-/-}$ strains in susceptibility to atherosclerosis and to hyperglycemia provide an ideal model for investigating genetic connections between the phenotypes. In the present study, we performed quantitative trait locus (QTL) analysis on female mice from an intercross between B6. *Apoe*$^{-/-}$ and BALB. *Apoe*$^{-/-}$ mouse strains to investigate the genetic control of atherosclerosis and glucose homeostasis.

Methods

Mice

B6.*Apoe*$^{-/-}$ mice were purchased from the Jackson Laboratories. BALB. *Apoe*$^{-/-}$ mice at the N10 generation were generated in our laboratory, as previously described [14]. B6. *Apoe*$^{-/-}$ mice were crossed with BALB. *Apoe*$^{-/-}$ mice to generate F_1s, which were intercrossed by brother-sister mating to generate a large F_2 population. Female F_2 mice were weaned at 3 weeks of age onto a rodent chow diet, and male F_2 mice were euthanized at the time of weaning. At 6 weeks of age, F_2 mice were started with a Western diet containing 21% fat, 34.1% sucrose, 0.15% cholesterol, and 19.5% casein (Harlan Laboratories, TD 88137) and maintained on the diet for 12 weeks. All this procedures were in accordance with current National Institutes of Health guidelines and approved by the University Animal Care and Use Committees.

Measurements of plasma glucose

Mice were bled twice: once before initiation of the Western diet and once at the end of the 12 weeks' high-fat feeding period. Mice were fasted overnight before blood was drawn from the retro-orbital venous plexus with the animals under isoflurane anesthesia. Plasma glucose was measured with a Sigma glucose (HK) assay kit, which was adapted for a microplate assay. Briefly, 10 μl of plasma samples (plasma from high-fat diet fed mice was diluted 1:2 in distill water) were mixed with 90 μl of reagent in a 96-well plate. After a 15-min incubation at room temperature, the absorbance at 340 nm was read on a Molecular Devices (Menlo Park, CA) plate reader.

Aortic lesion analysis

Atherosclerotic lesions in aortic root were measured as previously reported [15]. Briefly, the aortic root and adjacent heart were excised *en bloc* and embedded in optimal cutting temperature compound. 10-μm thick cross sections of the vessel were collected, stained with oil red O and hematoxylin, and counterstained with fast green. Atherosclerotic lesion areas were quantified using an ocular lens with a square-micrometer grid on a light microscope. The lesion areas of five 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 phenol/chloroform extraction and ethanol precipitation method. A total of 130 microsatellite markers covering all 19 autosomes and the X chromosome at an average interval of 12 cM were typed. Parental and F_1 DNA served as controls for each marker.

Statistical analysis

QTL analysis was performed using J/qtl and Map Manager QTX software as previously described [12,15, 16]. One thousand permutations of trait values were run to define the genome-wide LOD (logarithm of odds) score threshold required to be significant or suggestive for each specific trait. Loci that exceeded the 95th percentile of the permutation distribution were defined as significant ($P<0.05$) and those exceeding the 37th percentile were suggestive ($P<0.63$) according to the criteria recommended by the genetics community in 2003 [17].

Results

Trait value distributions

Fasting plasma glucose levels of 266 F_2 mice before and after 12 weeks on the Western diet, atherosclerotic lesions in the aortic root, and body weight were measured. As shown in figure 1, values of fasting plasma glucose levels on both chow and Western diets, log-transformed atherosclerotic lesion sizes, and body weight approach normal distributions. These data were analyzed to identify chromosomal regions segregating with the traits. Those loci exhibiting significant linkage and suggestive linkage are presented in Table 1.

Atherosclerotic lesion size

Four significant QTLs, located on chromosomes (Chr) 1, 2, 5, and 9, and one suggestive QTL on Chr4, were identified for atherosclerotic lesion sizes (Figure 2). Details of the QTLs detected, including locus name, LOD score, 95% confidence interval (CI), peak location, genome-wide significance P value, high allele, and mode of inheritance are presented in Table 1. The two significant QTLs on Chr1 and Chr9 replicated the previously reported QTLs, *Ath1* and *Ath29*, respectively [15,18]. The other two significant QTLs were novel. The Chr2 locus had a significant LOD score of 3.77 and a genome-wide significant P value of 0.026. It peaked at 52.2 cM and did not overlap in the confidence interval with known mouse atherosclerosis QTLs. We named it *Ath41* according to the QTL nomenclature for mouse atherosclerotic lesions. The Chr5 locus had a highly significant LOD score of 5.69 and a genome-wide P value of <0.001. Its peak appeared at 54.7 cM. We named it *Ath42*. The Chr4 locus had a suggestive LOD score of 2.8 and peaked at 63.3 cM. This QTL was partially overlapping with *Athsq1*, an atherosclerotic lesion locus identified in a (MOLF/Ei x B6.$Ldlr^{-/-}$) x B6.$Ldlr^{-/-}$ backcross [19]. Paradoxically, the BALB allele was associated with increased lesion size while the B6 allele was associated with decreased lesion size (Table 2). In contrast, for the 4 significant QTLs, the B6 allele was the high allele that increased lesion

size and the BALB allele was the low one that reduced lesion size. *Ath42* affected atherosclerotic lesion size in a dominant mode of inheritance while other QTLs exhibited an additive effect on the trait.

Fasting glucose levels

For fasting glucose on the chow diet, 4 significant QTLs on Chr1, Chr5, and Chr12, and two suggestive QTLs on Chr9 and Chr15 were identified (Figure 3). The significant locus on distal Chr1 and the 2 suggestive loci on Chr9 and Chr15 replicated the previously reported QTLs, *Bglu3*, a suggestive locus on Chr9 (now named *Bglu14*), and *Fbg2*, respectively (Table 1). The significant QTLs on the middle portion of Chr1, Chr5, and Chr12 were novel. The LOD score plot for Chr1 displayed two distinct peaks, located approximately 14 cM apart (Figure 3). The distal QTL peaked at 74.3 CM, overlapping with *Bglu3*, identified in a B6 x C3H *Apoe*$^{-/-}$ F_2 cross [12]. The proximal peak occurred at 60.3 cM with a LOD score reaching 3.94. We named this QTL *Bglu12* to represent a significant mouse QTL for fasting glucose. The Chr5 locus had a highly significant LOD score of 6.72 and peaked at 47.3 cM. We named it *Bglu13*. The QTL on Chr9 had a suggestive LOD score of 3.06, and overlapped with a suggestive locus near *D9mit229* (26.8 cM) for fasting glucose levels identified in a B6 x KK-Ay F2 cross [20]. We designated this QTL as *Bglu14* as it has not been named. The QTL on Chr12, named *Bglu15*, had a significant LOD score of 3.43 and peaked at 10 cM. *Bglu15* is close to the centromere compared to *Fbg-1*, which is located in the middle portion of Chr12 between *D12mit4* (35.5 cM) and *D12mit227* (38.4 cM) [21]. The suggestive QTL on Chr15 overlapped with *Fbg-2* near *D15Mit87* (16.7 cM), identified in (BALB x KK/Ta) x KK/Ta backcross [21]. For fasting glucose on the Western diet, 2 significant QTLs on Chr1 and Chr5 and 1 suggestive QTL on Chr8 were identified (Figure 4). The Chr1 QTL replicated *Bglu3*, and the Chr5 replicated *Bglu13*. The Chr8 locus overlapped with *Giq1*, a locus with a strong influence on the late phase of glucose tolerance test identified in a B6×KK-A cross [22]. *Bglu13* affected fasting glucose levels on both chow and Western diets in a dominant mode of inheritance while all other QTLs exhibited an additive effect on the trait except for the QTL on Chr9 and Chr15 that affected glucose levels in a recessive and a heterosis mode, respectively (Table 2).

Body weight

One significant QTL on distal Chr1 and 2 suggestive QTLs on Chr9 and Chr19 were identified for body weight (Figure 5). The Chr1 QTL overlaps with *Wt3q2* and *Wt6q2* for body weight mapped in two F_2 populations created from a selection and an inbred mouse lines [23], *Bw8q1* originally mapped in a B6×A/J intercross [24] and then replicated in a BxH *Apoe*$^{-/-}$ F_2 cross[12], and *Nob3* for body fat body weight and blood glucose mapped in NZO×B6 F_2 females [25]. The Chr9 locus overlaps with *W10q13* mapped in an M16i x L6 intercross [26], *Do2* for dietary obese mapped in two crosses derived from AKR/J and SWR/J mice [27], and *Obq18* for obesity mapped in B6 x 129 F_2 females [28]. The Chr19 locus corresponds to *Wtmq9* mapped in a B6 x C3H *Apoe*$^{-/-}$ intercross [29], *W3q14* from an M16i x L6 F_2 intercross [26], and *Abfw4* for abdominal fat mapped in DU6i x DBA/2 intercross [30].

Coincident QTLs for atherosclerosis and fasting glucose

LOD score plots for chromosome 5 show that the QTLs for atherosclerosis (*Ath42*) coincided with the QTL for fasting glucose (*Bglu13*) in the confidence interval (Figure 6). Both loci exhibited a dominant effect from the B6 allele on atherosclerotic lesions or fasting glucose levels (Table 2). LOD score plots for chromosome 1 show partial overlapping of the QTL for atherosclerosis (*Ath1*) with the QTLs for fasting glucose (*Bglu3*) and body weight in the confidence interval (Figure 7). The B6 allele was associated with increased atherosclerotic lesions and decreased glucose levels and body weight while the BALB allele was associated with decreased lesions and increased glucose levels and body weight (Table

2). *Ath29* was also partially overlapping with *Bglu14* in the confidence interval on the Chr9 (Table 1).

Discussion

In this study, we have identified five loci contributing to the development of atherosclerosis, six loci to fasting glucose levels on the chow diet, three loci contributing to fasting glucose levels on the Western diet, and three loci for body weight in an intercross between B6 and BALB *Apoe*$^{-/-}$ mouse strains. Moreover, we have observed the colocalization of QTLs for atherosclerotic lesions and for plasma glucose levels on chromosomes 1, 5, and 9.

B6 and BALB are prototype mouse strains for genetic studies of atherosclerosis. In pioneering studies of recombinant inbred strains derived from the two strains, as well as from B6 and C3H/HeJ, Paigen et al [18] identified the first atherosclerosis susceptibility locus, *Ath-1*. Subsequent studies of female F_2 and N_2 progeny derived from the B6 and BALB strains demonstrates the segregation of *Ath-1* with HDL cholesterol levels [31]. However, there are several limitations in those studies: First, the number rather than the size of atherosclerotic lesions was measured. Thus, atherosclerotic lesions were treated as a "qualitative trait" rather than a "quantitative trait". Second, the mapping was performed using a rather small numbers of animals, especially the recombinant inbred strains, thus the power for detecting susceptibility loci was low. Third, there were fewer polymorphic markers available at the time when the studies were conducted. Lastly, the diet-induced mouse model of atherosclerosis develops only small fatty streak lesions that are largely limited to the aortic root [32]. In contrast, *Apoe*-deficient mice develop all phases of atherosclerotic lesions in large and medium sized arteries seen in humans [11]. Our present work has extended the prior studies by finding five atherosclerosis QTLs, including *Ath1*. Among the five QTLs, *Ath1*, *Athsq1*, and *Ath29*, have been previously reported [15,18,33]. *Tnfsf4* has been identified to be the causal gene of *Ath1* [34]. We recently have identified *Rcn2*, a calcium-binding protein in the endoplasmic reticulum, as a key regulator in oxidized phospholipid-induced cytokine production and a probable candidate gene of *Ath29* [35]. The confidence interval of *Athsq1* is corresponding to human chromosome 9p21, a region that is associated with coronary heart disease [36, 37,38].

The QTL on chromosome 2, named *Ath41*, is close to *Athla1*, an atherosclerosis susceptibility locus mapped in a (PERA×B6-*Ldlr*$^{-/-}$)×B6-*Ldlr*$^{-/-}$ N_2 backcross mice [39]. *Athla1* is located in a more distal region (69 cM), and it increases lesion size only when homozygous for the B6 allele. Candidate genes for *Ath41* include *Dab2ip, Tfpi*, and *Slc38a11*, which have been shown to be associated with coronary heart disease in humans [40,41,42].

We identified a major locus on chromosome 5, approximately between 40 and 60 cM, which affected both atherosclerotic lesion size and fasting plasma glucose levels. We named it *Ath42* for atherosclerotic lesions and *Bglu13* for fasting glucose. As the two loci overlap significantly in the confidence interval, it is plausible to postulate that they share the same underlying causal gene. The present observation that both QTLs exhibited the same dominant B6 allele effect on the two different traits supports this speculation. Nevertheless, it is also likely that the two phenotypes are affected by two linked but unique genes residing in the QTL interval. The region from 40 to 60 cM on chromosome 5 in the mouse corresponds to chromosomal regions of 4q13, 4q21, and 12q24 in humans. The 4q13 region has been shown to be associated with variations in metabolic traits, including blood glucose [43,44], and the 12q24 region is associated with coronary heart disease [45,46], metabolic syndrome [47,48], type 1 and type 2 diabetes [49,50,51]. One promising candidate gene in the region is *Hnf1a*, which encodes hepatocyte nuclear factor 1α. One A/G SNP in exon 9

between B6 and BALB leads to amino acid substitution (P580R) in the *Hnf1a* protein. In humans, *Hnf1a* mutations are the most common cause of maturity-onset diabetes of the young (MODY) [52]. Polymorphisms in the *Hnf1a* gene are associated with risk for T2DM and coronary heart disease [49, 53].

The QTLs for plasma glucose and body weight on distal chromosome 1 have been reported previously in three separate mouse intercrosses, including two B6×C3H crosses deficient in *Apoe* [12, 29], and a cross between New Zealand obese (NZO) and B6 mice [25]. The confidence interval of the QTLs overlaps with a region of linkage to type 2 diabetes found in multiple human populations that has been extensively examined by the International Chromosome 1q Type 2 Diabetes Consortium [54]. In the current cross, we have observed two distinct peaks of the linkage curve for plasma glucose on the chow diet with the distal peak at 74.3 cM and the proximal peak at 60.3 cM. The bootstrap test, an effective statistical method for defining the confidence interval of QTLs using simulation [55], also indicated the existence of two QTLs for the trait on chromosome 1. We have named the proximal QTL *Bglu12* to represent a new locus for fasting glucose in the mouse.

The QTLs for atherosclerosis (*Ath1*), fasting glucose (*Bglu3*), and body weight (*BW8q1*) overlap in the confidence interval on the distal chromosome 1 region. The B6 allele was associated with increased atherosclerosis but decreased glucose levels and body weight. *Apoa2* is a major gene in the region that may contribute to variations in the traits. The QTL effect on body weight disappeared when the influence from the *Apoa2* allele was eliminated [56]. On the other hand, transgene expression of *Apoa2* in mice results in several phenotypes observed in T2DM, including glucose intolerance, insulin resistance, hypertriglyceridemia, and obesity [57,58]. *Apoa2* is also a major gene in the mouse that has a dramatic influence on plasma HDL cholesterol levels [59]. BALB mice have an $Apoa2^b$ allele that elevates HDL cholesterol levels and B6 mice have an $Apoa2^a$ allele that decreases HDL cholesterol levels [60]. High HDL cholesterol levels protect against atherosclerosis. *Apcs*, encoding serum amyloid P (SAP), is another candidate in the distal chromosome 1 region that may contribute to T2DM and atherosclerosis. Plasma SAP levels, which are primarily regulated by the *Apcs* gene, are correlated with blood glucose and body weight in a segregating F_2 population derived from B6 and C3H $Apoe^{-/-}$ mice[12]. In humans SAP is significantly correlated with obesity, blood pressure, lipids, common and internal carotid wall thickness, and ankle-brachial index [61]. *Cxcr4*, *Pask*, *Cntnap5a*, *Lct*, and *Pik3c2b* are positional candidate genes for *Bglu12*. Variants of these genes have been found to be associated with susceptibility to T2DM, fasting glucose, or insulin resistance in humans [62, 63,64, 65, 66, 67].

The QTL for fasting glucose on chromosome 9 was partially overlapping with the QTL for atherosclerosis. *Sorl1*, *Rcn2*, and *Apoc3* are potential candidate genes in the region that may affect both atherosclerosis and T2DM [35, 68, 69]. For *Bglu15*, *Adam17*, encoding a disintegrin and metallopeptidase domain 17, and *Ahr*, encoding aryl-hydrocarbon receptor, are two likely candidate genes. *Adam17* is involved in the shedding of the extracellular domain of cytokines, growth factors, receptors or adhesion molecules [70]. *Ahr* signaling affects molecular clock genes associated with glucose metabolism, and *Ahr* deficiency enhances insulin sensitivity and reduces PPAR-α pathway activity [71]. In the present study, we have found some QTLs, such as *Bglu3* and *Bglu13*, that influenced glucose levels when mice had normoglycemia on a chow diet also affected glucose levels when mice developed hyperglycemia on a high-fat diet. However, some other QTLs, such as *Bglu12*, *Bglu14*, and *Bglu15* only exerted effect under a specific condition (the chow diet). Six QTLs were found for fasting plasma glucose when mice were fed the chow diet while only three QTLs were detected when fed the Western diet. The reasons for the discrepancy in the results are unknown. One probable explanation is that the Western diet has a significant influence on

plasma glucose levels, which might overwhelm the influence from genetic factors on the trait. In addition, the western diet induces some metabolic changes that suppress gene expression. We recently have found that there are much more differentially expressed genes in the aorta of two strains when fed a chow diet than a Western diet [72].

In summary, we have identified multiple QTLs contributing to the development of atherosclerosis and glucose homeostasis in a segregating F_2 population. The finding on the colocalization of QTLs for atherosclerosis and glucose has laid the basis for further study to determine whether they are controlled by the same genes or different unique genes in the QTL intervals.

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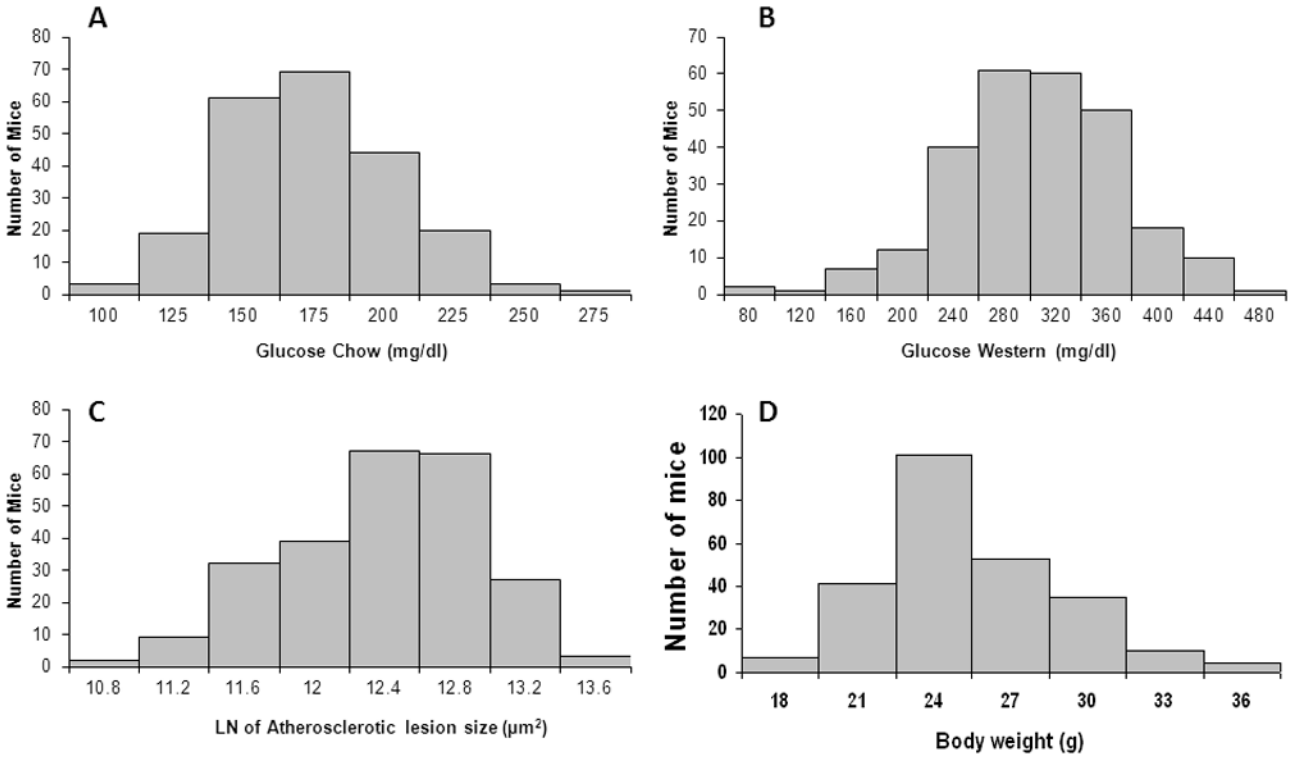

Figure 1.

Distributions of fasting plasma glucose before (A) and after (B) 12 weeks on the Western diet, LN (natural log)-transformed atherosclerotic lesion sizes (C), and body weight (D) in 266 female F_2 mice derived from B6. *Apoe*$^{-/-}$ and BALB. *Apoe*$^{-/-}$ mice.

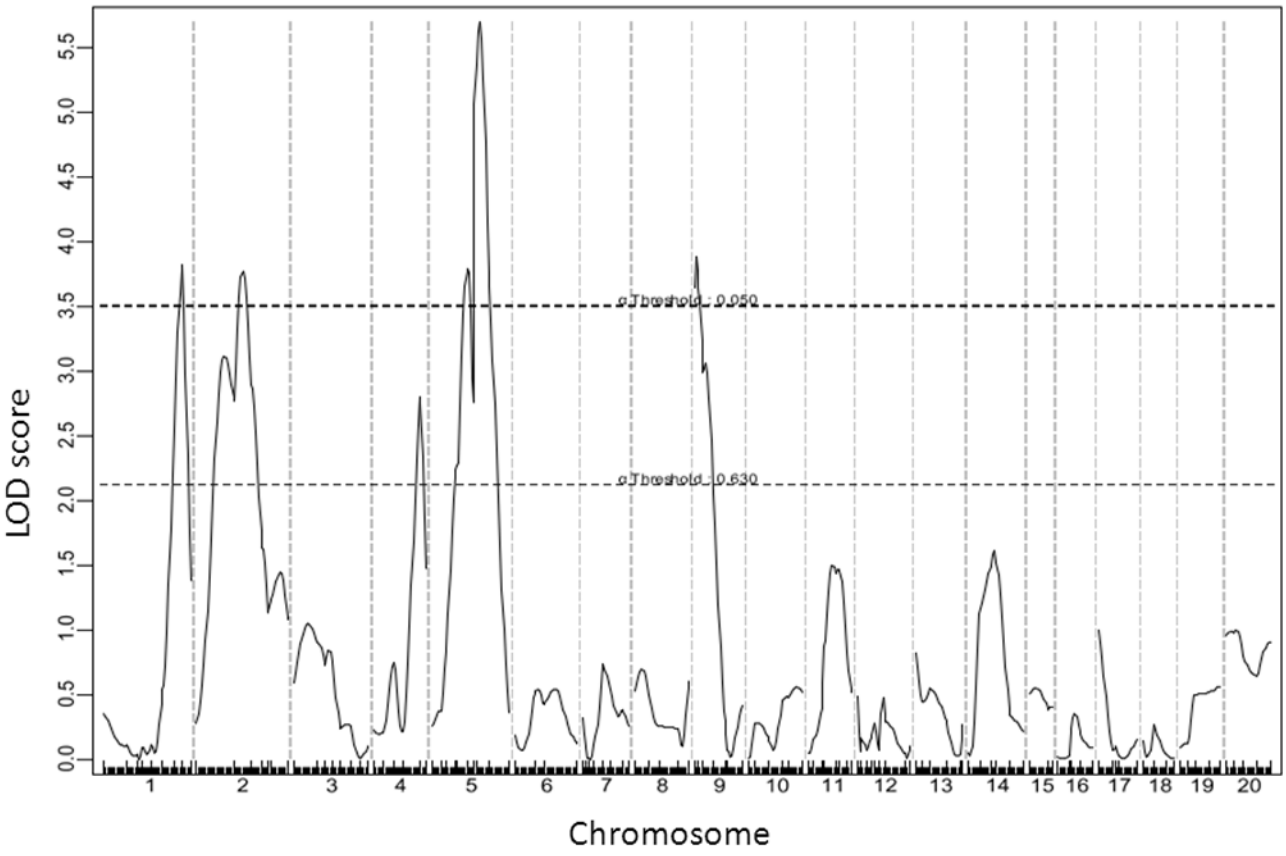

Figure 2.

A genome-wide scan to search for loci influencing atherosclerotic lesion sizes of the F_2 mice. Chromosomes 1 through 20 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. Atherosclerotic lesion sizes were determined by averaging the lesion areas of 5 cross-sections with the largest readings for each F_2 mouse. Two horizontal dashed lines denote genome-wide empirical thresholds for suggestive (P=0.63) and significant (P=0.05) linkage.

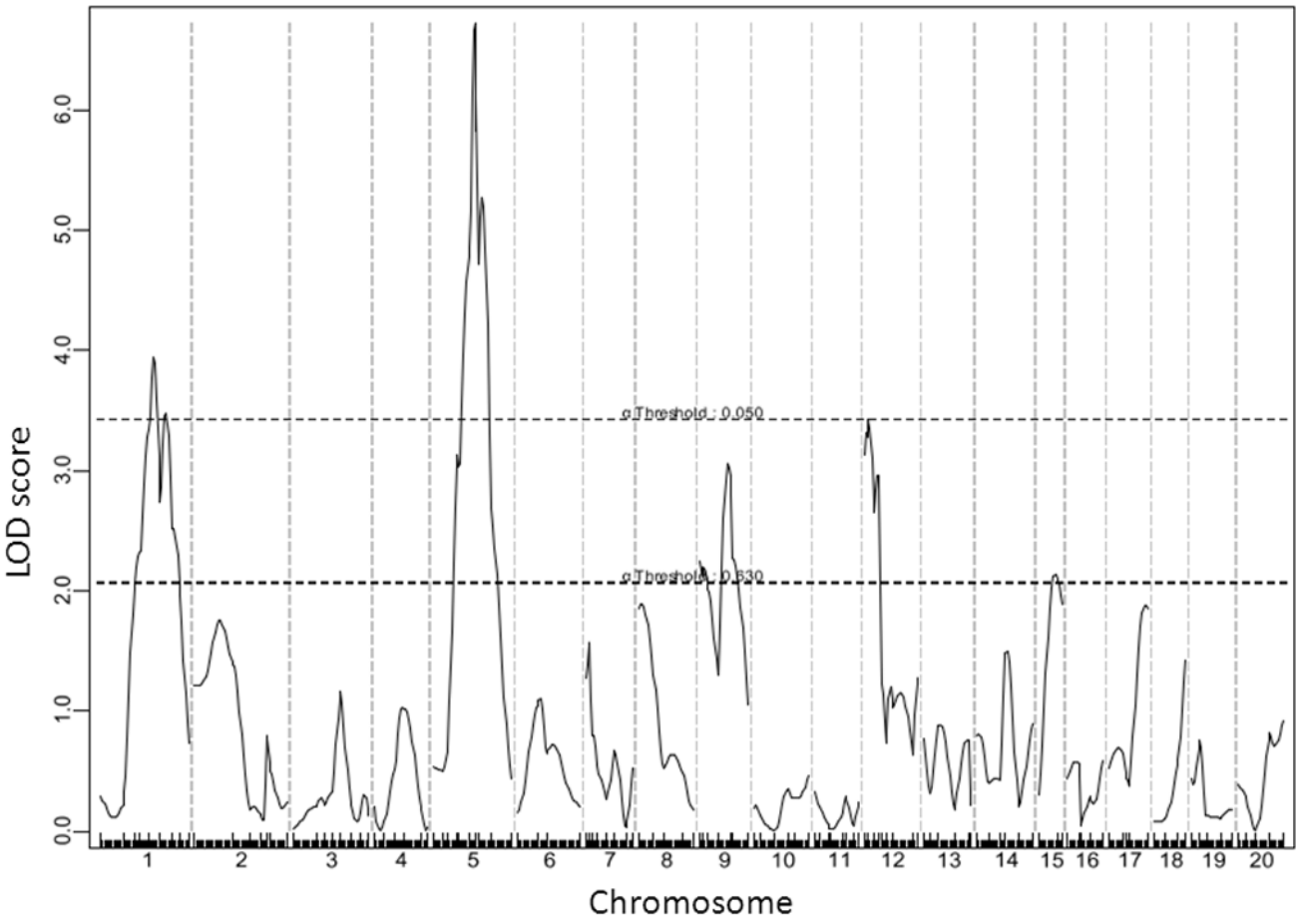

Figure 3.
A genome-wide scan to search for loci for fasting plasma glucose levels when the F_2 mice were fed the chow diet. Blood was collected from overnight fasted F_2 mice the day before being started on the Western diet.

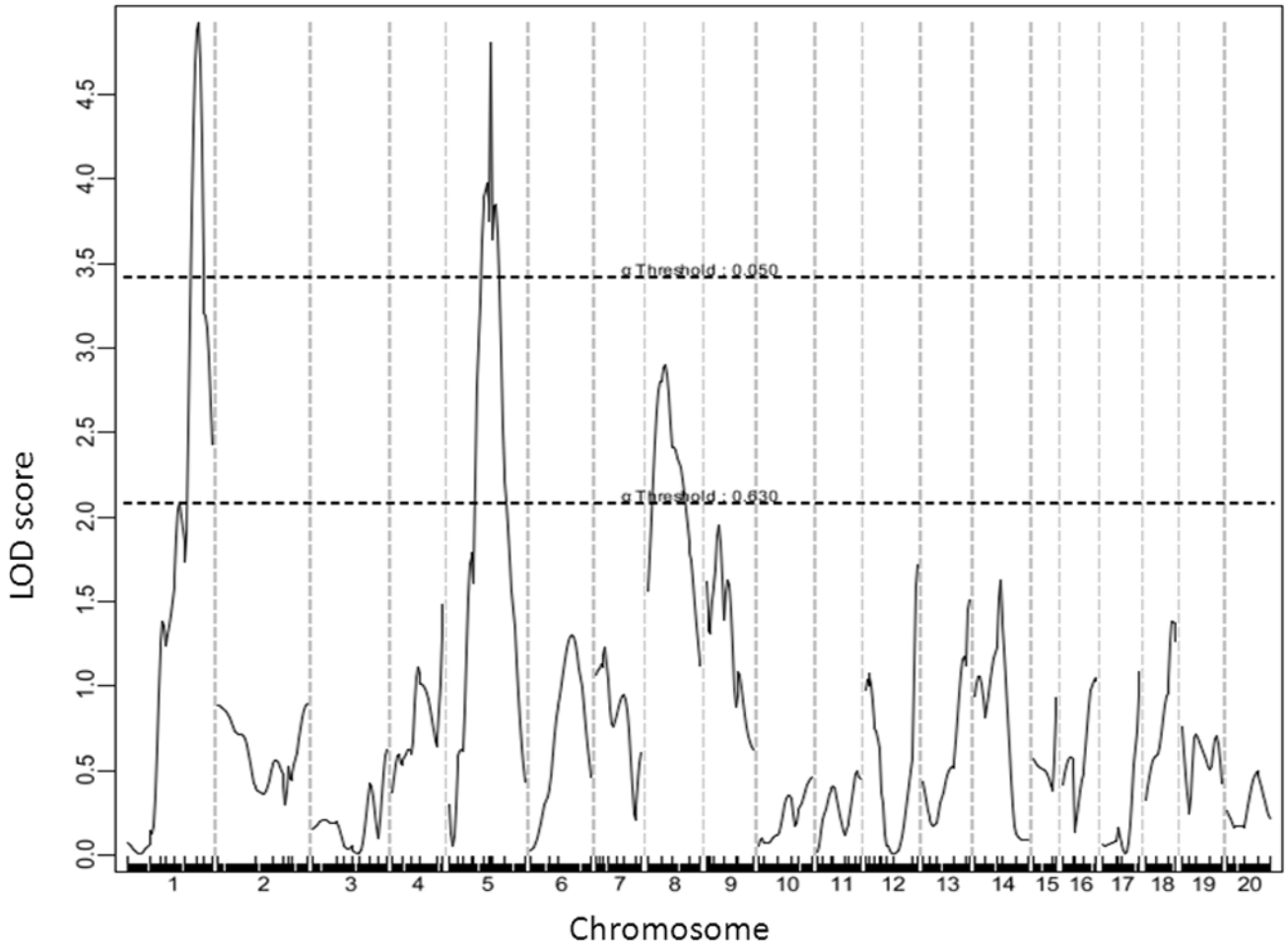

Figure 4.

A genome-wide scan to search for loci for fasting plasma glucose levels on the Western diet. Mice were fed the high-fat diet for 12 weeks. Blood was collected from overnight fasted F_2 mice the day before being euthanized.

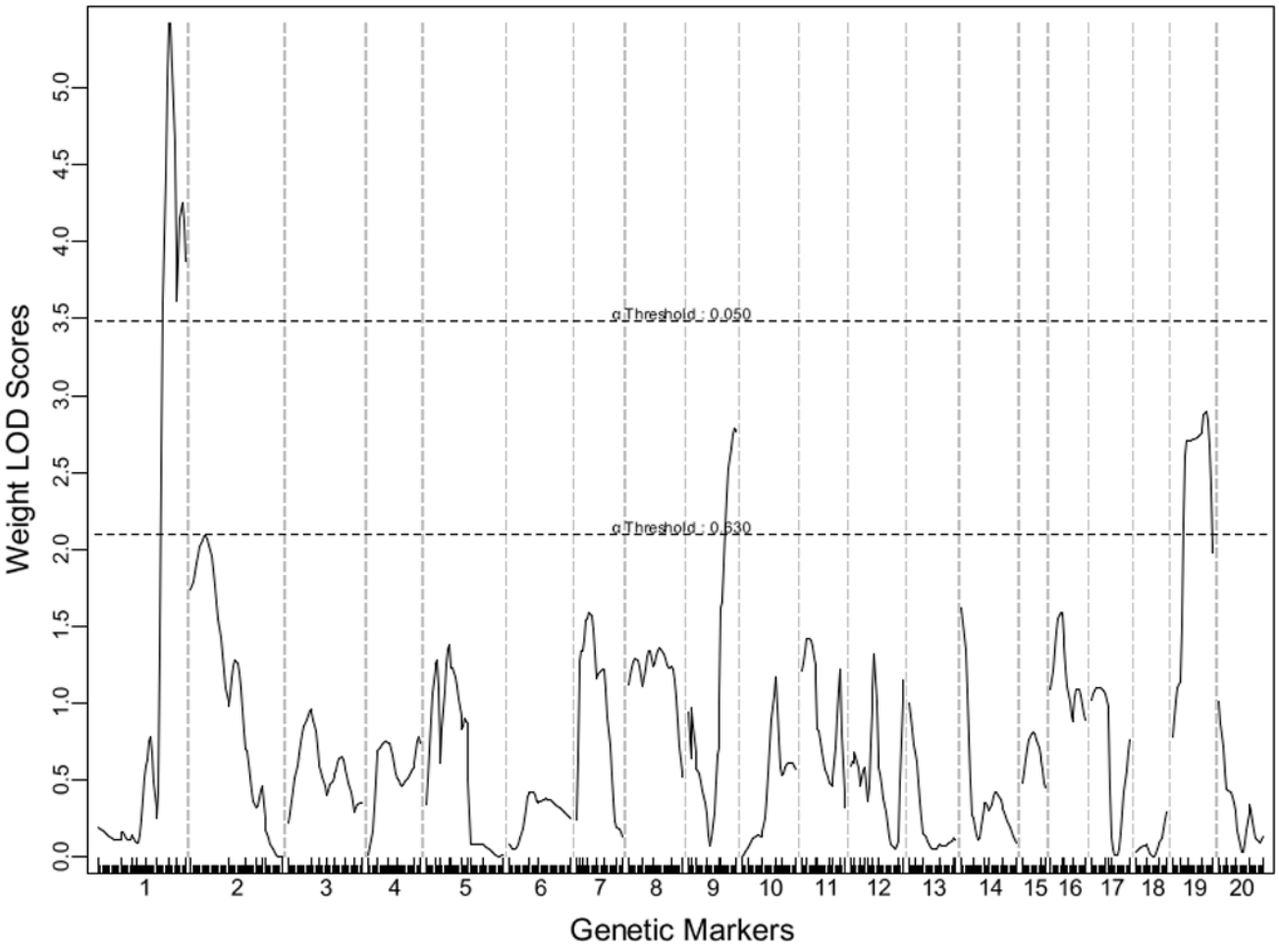

Figure 5.
A genome-wide scan to search for loci for body weight. Mice were weighed the day being euthanized.

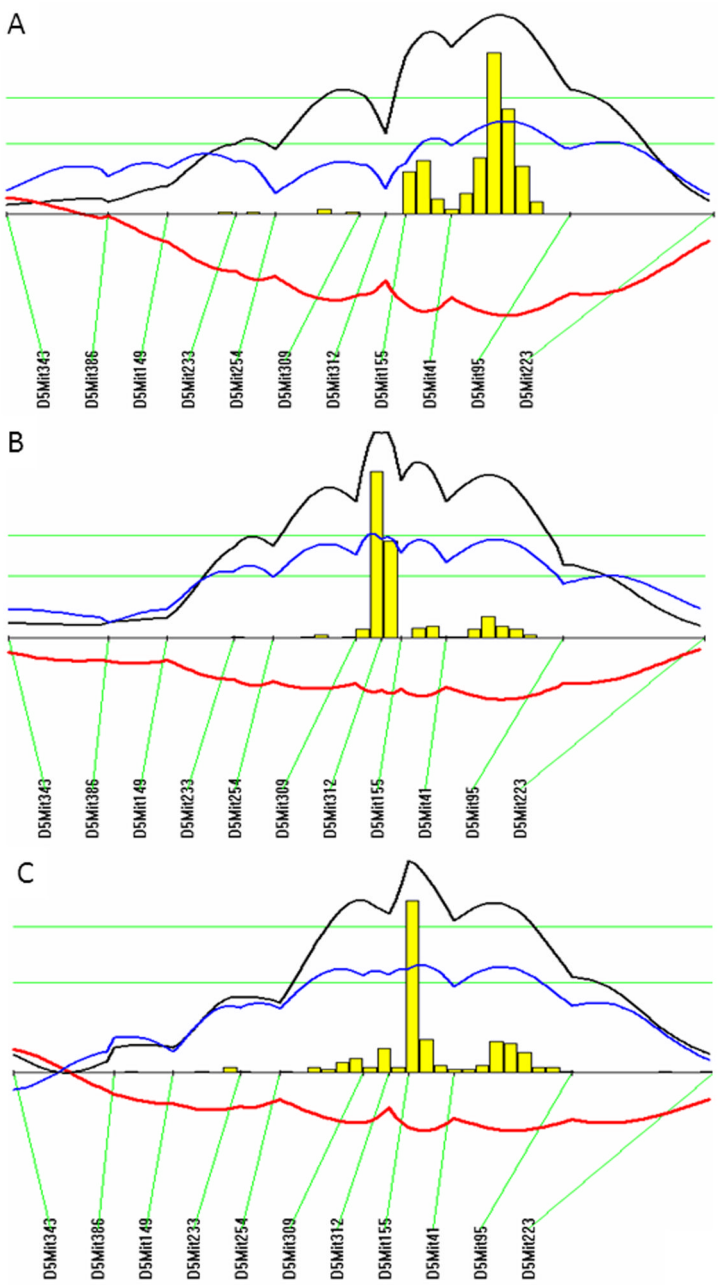

Figure 6.

LOD score plots for atherosclerotic lesion size (A), fasting plasma glucose levels on the chow diet (B), and fasting plasma glucose levels on the Western diet (C) on chromosome 5. Plots were created with the interval mapping function of Map Manager QTX, including a bootstrap test shown as a histogram estimating the confidence interval for the QTL. Two green horizontal lines represent genome-wide significance thresholds for suggestive or significant peaks (P=0.63 and P=0.05, respectively). Black plots reflect the LOD calculated at 1-cM intervals. The blue plot represents the effect of the B6 allele, and the red plot represents the effect of the BALB allele.

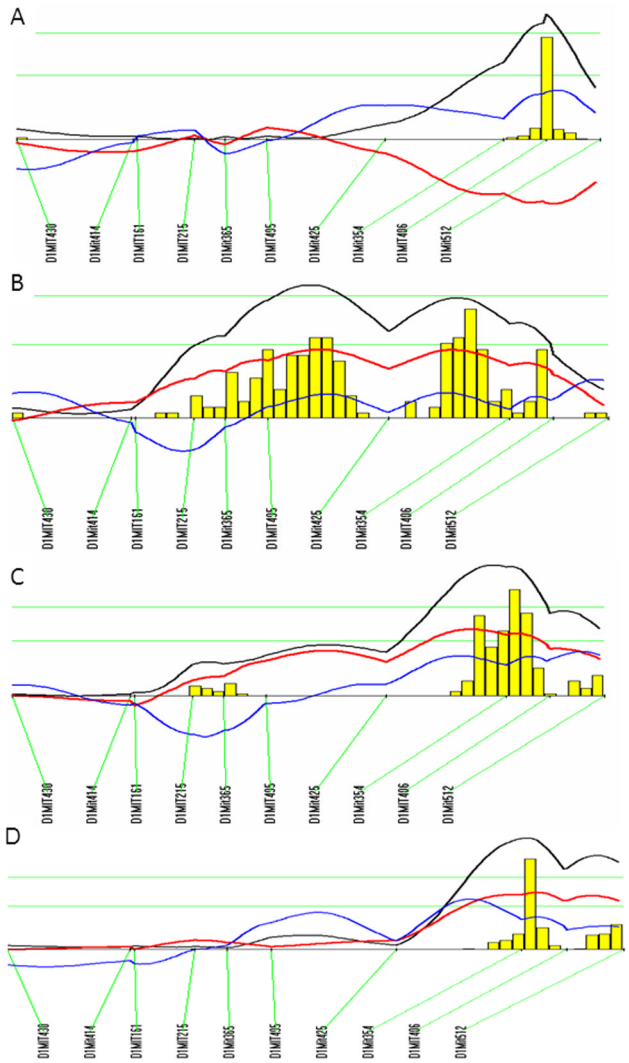

Figure 7.

LOD score plots for atherosclerotic lesion size (A), fasting plasma glucose levels on the chow diet (B), fasting plasma glucose levels on the Western diet (C), and body weight (D) on chromosome 1. Plots were created with the interval mapping function of Map Manager QTXb20, as stated above. The histogram denotes the confidence interval of the QTL.

Table 1

Significant and suggestive QTLs for atherosclerotic lesion size and fasting plasma glucose levels l in F_2 mice derived from B6. *Apoe*$^{-/-}$ and BALB. *Apoe*$^{-/-}$ mice.

Locus name[a]	Chr	Trait	LOD score[b]	95% CI[c]	Peak[d]	P value[e]	High allele	Mode of inheritance[f]
Ath1	1	Atherosclerosis	**3.82**	82.3–92.3	89	0.019	B6	Additive
Ath41	2	Atherosclerosis	**3.77**	26.2–64.2	52.2	0.026	B6	Additive
Athsq1	4	Atherosclerosis	2.8	55.6–69.1	63.3	0.191	BALB	Additive
Ath42	5	Atherosclerosis	**5.69**	48.5–60.7	54.7	0.001	B6	Dominant
Ath29	9	Atherosclerosis	**3.88**	17.8–31.8	19.8	0.016	B6	Additive
Bglu12	1	Glucose, chow	**3.94**	48.3–78.3	60.3	0.022	BALB	Additive
Bglu3	1	Glucose, chow	**3.45**	64.3–84.3	74.3	<0.05	BALB	Additive
Bglu13	5	Glucose, chow	**6.72**	42.7–56.7	47.3	0.001	B6	Dominant
Bglu14	9	Glucose, chow	3.06	17.8–61.8	47.8	0.11	BALB	Recessive
Bglu15	12	Glucose, chow	**3.43**	5.5–21.5	10.0	0.05	B6	Additive
Fbg-2	15	Glucose, chow	2.14	18.5–33.4	26.5	0.579	B6	Heterosis
Bglu3	1	Glucose, West	**4.93**	76.3–86.3	82.3	0.001	BALB	Additive
Bgl13	5	Glucose, West	**4.81**	38.7–58.7	48.7	0.001	B6	Dominant
Giq1	8	Glucose, West	2.90	17.7–57.7	33.7	0.16	B6	Additive
BW8q1	1	Body weight	**5.42**	76.3–94.3	80.3	0.0001	BALB	Dominant
W10q13	9	Body weight	2.785	55.8–69.8	67.8	0.188	B6	Recessive
Wtmq9	19	Body weight	2.898	18.3–46.3	40.3	0.154	BALB	Dominant

Plasma glucose levels were measured before (chow) mice were started on the Western diet and at the end of the 12 weeks' Western diet (West) feeding period. Blood was drawn from overnight-fasted mice.

[a] QTLs were named if they were significant or if they overlapped with previously reported suggestive ones. The nomenclature of *Ath* was for atherosclerosis QTLs, and *Bglu* for blood glucose QTLs. The newly identified QTLs were underlined to easily distinguish them from known ones.

[b] LOD scores were obtained from QTL analysis. The significant LOD scores were highlighted in bold. The suggestive and significant LOD score thresholds were determined by 1,000 permutation tests for each trait. Suggestive and significant LOD scores were 2.087 and 3.458, respectively, for atherosclerosis; 2.071 and 3.428 for plasma glucose on the chow diet; 2.084 and 3.423 for glucose on the Western diet, and 2.099 and 3.486 for body weight.

[c] 95% Confidence interval in cM obtained from a whole genome QTL scan.

[d] QTL peak position in cM.

[e] The p-values reported represent the level of genome-wide significance as they were generated based on genome-wide permutation tests

[f] Mode of inheritance was defined according to allelic effect at the nearest marker of a QTL.

Table 2

Effects of B6 (B) and BALB (C) alleles in different QTLs on atherosclerosis, plasma lipids, and body weight in the B6. *Apoe*$^{-/-}$ and BALB. *Apoe*$^{-/-}$ intercross

Locus name	Chr	Trait	Peak Marker	BB	CC	BC	P-value
Ath1	1	Atherosclerosis	*D1mit406*	249571±123560 (55)	167588±83413 (64)	226485±106846 (137)	5.51E-05
Ath41	2	Atherosclerosis	*D2mit328*	243816±109941 (61)	186641±116201 (59)	223779±110268 (135)	1.76E-02
Athsq1	4	Atherosclerosis	*D4mit203*	189830±108061 (60)	253744±115878 (72)	212235±109735 (126)	3.37E-03
Ath42	5	Atherosclerosis	*D5mit41*	243106±106054 (68)	165375±99213 (55)	231499±115933 (127)	1.68E-04
Ath29	9	Atherosclerosis	*D9mit247*	253591±126554 (68)	173685±87719 (54)	217985±109284 (137)	4.59E-04
Bglu12	1	Glucose, chow	*D1mit495*	149.2 ± 25.8 (60)	170.7 ± 28.8 (55)	161.6 ± 29.1 (111)	2.91E-04
Bglu3	1	Glucose, chow	*D1mit354*	149.7 ± 25.1 (53)	169.1 ± 29.7 (61)	160.7 ± 29.7 (112)	1.78E-3
Bglu13	5	Glucose, chow	*D5mit312*	160.5 ± 28.6 (62)	141.6 ± 19.5 (49)	168.4 ± 29.6 (119)	2.12E-07
Bglu14	9	Glucose, chow	*D9mit274*	159.0 ± 29.9 (60)	172.8 ± 23.7 (57)	154.3 ± 28.0 (115)	2.27E-04
Bglu15	12	Glucose, chow	*D12mit84*	171.7 ± 34.2 (47)	149.5 ± 26.0 (60)	160.9 ± 26.8 (125)	3.41E-04
Fbg-2	15	Glucose, chow	*D15mit123*	166.9 ± 27.4 (45)	166.7 ± 32.0 (64)	154.6 ± 27.4 (123)	6.51E-03
Bglu3	1	Glucose, West	*D1mit354*	252.0 ± 54.2 (57)	301.5 ± 70.8 (68)	288.6 ± 61.5 (128)	4.44E-05
Bgl13 *Giq1*	5 8	Glucose, West Glucose, West	*D5mit155* *D8mit68*	284.3 ± 56.1 (71) 305.0 ± 60.7 (73)	249.6 ± 71.3 (51) 265.3 ± 54.9 (63)	299.5 ± 64.0 (135) 283.4 ± 70.3 (126)	1.74E-05 1.69E-03
BW8q1	1	Body weight	*D1mit354*	22.1 ± 2.3 (57)	25.0 ± 4.1 (68)	24.3 ± 3.4 (132)	5.45E-06
W10q13	9	Body weight	*D9mit279*	25.3 ± 3.5 (62)	23.2 ± 3.4 (59)	23.7 ± 3.5 (142)	2.48E-03
Wtmq9	19	Body weight	*D19mit90*	22.7 ± 3.2 (64)	24.8 ± 4.1 (69)	24.2 ± 3.3 (131)	1.94E-03

Measurements are expressed as means ± SD. The units for these measurements are: μm2/section for atherosclerotic lesions; mg/dl for glucose; and g for body weight. BB, homozygous for B6 alleles at the linked peak marker; CC, homozygous for BALB alleles; BC, heterozygous for B6 and BALB alleles at the peak marker. The number in the bracket represents the number of progeny with a specific genotype at a specific marker. ANOVA was used to determine the significance level (p value) of differences for a specific phenotype among progeny with three different genotypes at a specific marker.