Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57BLKS/J

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Abstract **Mice from the inbred strain C57BLKS/J (BKS) exhibit increased susceptibility to both diabetes and atherosclerosis compared to C57BL/6J (B6) mice. To determine whether the differences in diabetes and atherosclerosis are related, we carried out a cross between B6-*db/db* and BKS. We selected 99 female F_2-*db/db* progeny, tested the progeny for plasma lipids, plasma glucose, and fatty-streak lesions, and used quantitative trait loci (QTL) analysis to identify the chromosomal regions associated with these phenotypes. No major QTL were found for total cholesterol, VLDL-cholesterol, or triglycerides. Two suggestive QTL were found for HDL-cholesterol (LOD scores of 2.7 and 2.8), and two suggestive loci were found for plasma glucose (LOD scores of 2.3 and 2.0). Lesion size was not correlated with plasma lipid levels or glucose. Lesion size was determined by a locus at *D12Mit49* with a LOD score of 2.5 and a significant likelihood ratio statistic. The gene for apolipoprotein apoB lies within the region, but apoB levels were similar in strains B6 and BKS. The QTL on Chr 12 was confirmed by constructing a congenic strain with BKS alleles in the QTL region on a B6 genetic background. We conclude that susceptibilities to diabetes and atherosclerosis are not conferred by the same genes in these strains and that a major gene on Chr 12, which we name *Ath6*, determines the difference in atherosclerosis susceptibility.**—Mu, J-L., J. K. Naggert, K. L. Svenson, G. B. Collin, J. H. Kim, C. McFarland, P. M. Nishina, D. M. Levine, K. J. Williams, and B. Paigen. **Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57 BLKS/J. *J. Lipid Res.* 1999.** 40: **1328–1335.**

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While mice from both inbred strains C57BL/6J (B6) and C57BLKS/J (BKS) develop aortic fatty-streak lesions when fed an atherogenic diet, the lesions in BKS mice are significantly larger than those in B6. Similarly, fatty-streak lesions are larger in mice carrying the diabetes mutation (*db*) in the BKS background than in mice carrying the same mutation in the B6 background (1). This suggests that the genetic background of the BKS strain is more atherogenic than that of the closely related B6 strain. Interestingly, the BKS genetic background is also more diabetogenic than that of B6 (2). This raises the question of whether there is a relationship between the factors that cause diabetes and those that cause increased atherosclerosis in BKS. Evidence for a link between diabetes and atherosclerosis has previously been provided by studies demonstrating that individuals with diabetes are at increased risk for developing cardiovascular disease (3).

The strain BKS arose through an accidental genetic contamination of B6 in the 1940s, followed by inbreeding (4). Recently we showed (4) and others confirmed (5) that the contaminating strain was DBA and that the BKS strain is a mixture of 84% B6-like genes and 16% DBA-like genes. The chromosomal regions containing the genetic contribution from DBA have been described (4, 5). The goal of the present study is to determine whether the genetic determinants of atherosclerosis and of diabetes are related to one another and to ascertain the chromosomal location of these traits. To achieve this goal, we carried out a cross between the B6-*db/db* and BKS strains. The F_1 mice heterozygous for the *diabetes* (*db*) mutation were mated, and F_2 *db/db* progeny were tested for plasma lipids, plasma glucose, and fatty-streak lesions in the aorta. We then car-

Abbreviations: QTL, quantitative trait loci; B6, C57BL/6; BKS, C57BLKS; LOD, log of odds.

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ried out a scan of the genomic regions that differed between the strains, searching for an association of alleles with these phenotypes. This report describes *Ath6*, a major gene affecting lesion size, which is independent of plasma lipids or plasma glucose.

MATERIALS AND METHODS

Animals

Mice of strains B6, BKS, B6-*db*/*db*, B6-*db*/+, BKS-*db*/*db*, and BKS-*db*/+ were obtained from the production department of The Jackson Laboratory, Bar Harbor, ME. Because homozygous *db*/*db* mice do not breed, we transplanted ovarian grafts from B6-*db*/*db* females into immune-deficient mice of a different coat color, NOD/LtSz-*scid*/*scid* females. These females were mated with BKS males to produce the F_1(B6-*db*/*db* × BKS) progeny; these F_1 were mated together to produce the F_2 progeny. We selected only the *db*/*db* F_2 progeny, which represented about 25% of the total. As male B6 mice do not develop fatty-streak lesions (6), only female *db*/*db* mice were used. We obtained 99 female *db*/*db* mice from the slightly more than 800 F_2 progeny. Mice were maintained in a temperature- and humidity-controlled environment with a 14-h:10-h light–dark cycle. Experiments were approved by the Institutional Animal Care and Use Committee.

Diet and evaluation of aortic lesions

When 2 to 4 months of age, female *db*/*db* F_2 progeny were provided with an atherogenic diet, described in detail elsewhere (7), containing 15% fat (cocoa butter), 1.25% cholesterol, and 0.5% cholic acid (wt/wt). Mice were maintained on this diet for 18 weeks and killed by cervical dislocation. The hearts and upper section of the aorta were removed, fixed, and evaluated for lesions as described previously (8). Briefly, aortas were sectioned on a cryostat, and cross sections from the root of the aorta were stained with Oil Red O and hematoxylin and counterstained with light green. For each mouse, the lesion size was measured in five cross sections and the lesion size/cross section was averaged to provide the mean lesion size/mouse ± SEM.

Phenotypes

Plasma glucose was measured using an enzymatic assay (Sigma #510 DA) after 10, 12, 14, and 16 weeks of atherogenic diet consumption. Blood samples were obtained from unfasted mice between 8:00–10:00 am. Plasma glucose levels were averaged over four measurements. The mean coefficient of variation (c.v.) among these four samples was 19%; 68% of mice had a c.v. less than 20% and 92% had a c.v. less than 40%. The mean standard deviation of non-diabetic mice (plasma glucose <300 mg/dl) was ±73 mg/dl and the mean standard deviation for diabetic mice (plasma glucose > 300 mg/dl) was ±83 mg/dl. Immunoreactive plasma apolipoprotein B (apoB) was measured as previously described (9). Plasma total cholesterol, HDL-cholesterol, and triglycerides were measured as described previously (10). In the mouse most non-HDL cholesterol is in the VLDL fraction; the value for this fraction was obtained by subtracting HDL-cholesterol from total cholesterol.

Genotyping the progeny

DNA from spleen or tail-tip tissue was isolated using standard procedures of phenol/chloroform extraction, ethanol precipitation, resuspension in 500 μl TE (10 mm Tris–1 mm Na_2 EDTA, pH 7.4), and storage at 4°C (11). Oligonucleotide primers were obtained from Research Genetics (Huntsville, AL). PCR amplifications were carried out in a 10-μl reaction volume containing 50–100 ng genomic DNA; 10 mm Tris-HCl (pH 8.3); 50 mm KCl; 1.5 mm $MgCl_2$; 0.01% (w/v) gelatin; 50 μm of dATP, dCTP, dGTP, and dTTP; 4.0 pmol of each of the primers; and 0.25 units of Taq DNA Polymerase (Perkin-Elmer/Cetus, Norwalk, CN). For PCR products with base-pair differences less than 8 between the two strains, the forward primer was end labeled with γ-^{33}P ATP (>1000–3000 Ci/mmol, NEN, Boston, MA) and used in the PCR reaction described above. Initial denaturation at 95°C for 5 min was followed by 35 cycles with denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min. The final extension step was 72°C for 10 min. The PCR products with more than an 8-base-pair difference between the two strains were electrophoresed on 4% NuSieve agarose gels (FMC Corporation, Rockland, ME), and those with less than an 8-base-pair difference were electrophoresed on 6% denaturing polyacrylamide gels.

We used the phenotypic pooling method to scan the genome. DNA from individual mice were standardized to 0.5 mg/ml in TE and stored at 4°C. Five-μl aliquots of individual DNA from the 20 mice with no lesions were pooled, as was DNA from the 20 mice with the largest lesions, followed by a 5-fold dilution with TE to yield a final concentration of 100 μg/ml. Pools were mixed thoroughly by repeated pipetting and gentle vortexing. They were stored frozen and vortexed again before PCR amplification. Loci that differed in intensity between the high and low pools were used to type individual mice in the high and low pools. All 99 mice were typed for those loci with significant differences between the high and low pools; no evidence for segregation distortion was found.

Statistics

The association between phenotypes and genetic markers in the F_2 population was determined by MapManager QTb27 (13), a program based on interval mapping, using the free regression model. Significance was determined by 10,000 permutations to provide likelihood ratio statistics (LRS) that are suggestive, significant, or highly significant. The LOD score can be approximated by dividing the LRS by 4.6. MAPMAKER/EXP (14, 15) was also used. The log of lesions size + 1 was used for analysis because lesion size did not have normal distribution and because there were many mice with no lesions.

Mapping

Seventeen loci in the *Ath6* region were genotyped using The Jackson Laboratory BSS backcross panel, which contains DNA from 94 animals from a (C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei backcross (16). The typed loci were *D12Mit37*, *D12Mit44*, *D12Mit82*, *D12Mit103*, *D12Mit104*, *D12Mit135*, *D12Mit136*, *D12Mit169*, *D12Mit182*, *D12Mit183*, *D12Mit185*, *D12Mit197*, *D12Mit215*, *D12Mit264*, *D12Mit269*, *D12Nds11* and *ApoB*. To map *ApoB* in this cross, we used primers that span a $(CA)_n$ region as previously described (17). The two primers were PCR1 (5′ TTCATG CATTTCTATGCGTGCATG) and PCR2 (5′ GGAGTG GAAAT GGGTCGTAGCCT). Data are available from The Jackson Laboratory's site on the World Wide Web at http://www.jax.org/resources/documents/cmdata.

RESULTS

Comparison of B6 and BKS parental strains

As we previously reported (1), both the B6 and BKS inbred strains of mice develop fatty-streak lesions in the aorta when fed the atherogenic diet; however BKS mice develop much larger lesions than B6. Furthermore,

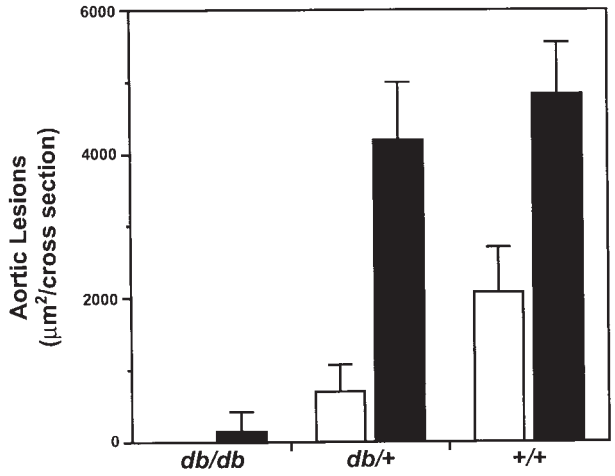

Fig. 1. Lesions in B6 and BKS mice with and without the *db* mutation. White bars depict lesion size in B6 mice; black bars depict lesion size in BKS mice. For B6 mice carrying the *db/db* mutation, there were no lesions. Figure redrawn using data from reference 1.

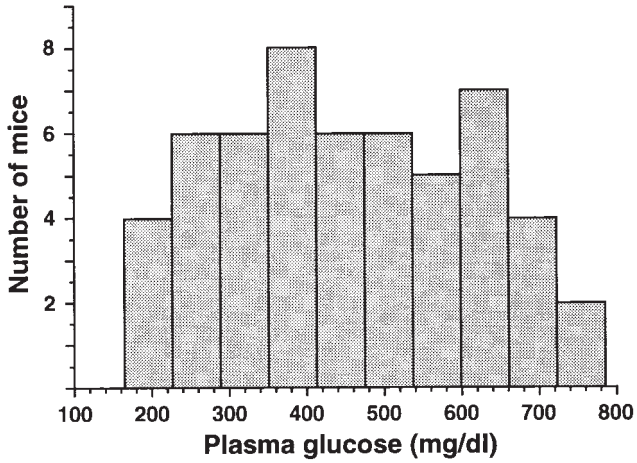

Fig. 2. Distribution of plasma glucose levels among F_2 progeny. The plasma glucose levels are the mean of measurements from mice fed an atherogenic diet for 10, 12, 14, and 16 weeks. Mean plasma glucose (±SD) for the parental strains are 210 ± 65 for B6-*db/db* and 594 ± 177 for BKS-*db/db*.

mouse mutations that confer obesity, such as *diabetes* (*db*) and *obese* (*ob*), decrease lesion size when compared to wild type (1). The mechanism is unknown but may related to the observation that these mutations also cause HDL-cholesterol to increase. The strain difference in lesion size between B6 and BKS is illustrated in **Fig. 1**, which compares the two inbred strain with or without the *db* mutation. If mice carry *db/db*, BKS mice have small lesions and B6 mice have no lesions. If mice are heterozygous *db/+*, BKS have lesions that are several fold larger than B6. If mice are wild-type, BKS mice have lesions that are twice as large as B6. The data in Fig. 1 are from an experiment described previously (1); a repeat comparison of lesion development carried out during the experiments described in this report showed BKS *db/db* mice have a mean lesion size of 6000 ± 2000 μm^2 (n = 5) and B6 *db/db* mice have a mean lesion size of 60 ± 60 μm^2 (n = 5).

Relationship between aortic lesions, plasma glucose, and plasma lipids in female *db/db* progeny

The distribution of plasma glucose levels, which were averaged from four separate measurements for each animal, range from 170 mg/dl to 720 mg/dl (**Fig. 2**). The distribution is similar to a bell-shaped curve, suggesting that multiple genes determine the difference in plasma glucose. However, there is a suggestion of a peak just above 600 mg/dl, which would be typical of the BKS *db/db* parental measurements.

Fatty-streak lesions in the 99 *db/db* F_2 progeny were distributed into two separate groups (**Fig. 3**) that resembled either the B6 *db/db* parental strain with no or very small lesions or the BKS *db/db* parental strain with large lesions. Such a bimodal distribution suggests that a single gene determines the difference in lesion size between B6 and BKS. The distribution of small lesions compared to large lesions was 77:22, which is consistent with a 3:1 distribution. This distribution suggests a dominant resistant allele for lesion size or two genes with one of them epistatic to the other.

A central question of this study was whether the diabetogenic and atherogenic factors in the BKS strain were related to one another. A comparison of plasma glucose and lesion size for each F_2 *db/db* progeny shows no correlation between the two traits (**Fig. 4**), suggesting that separate genes determine the atherogenic and diabetogenic phenotypes. Comparisons of lesion size to total cholesterol, HDL-cholesterol, VLDL-cholesterol, or triglycerides also showed no significant correlations, suggesting that the genetic determinants of lesion size act independently of plasma lipids.

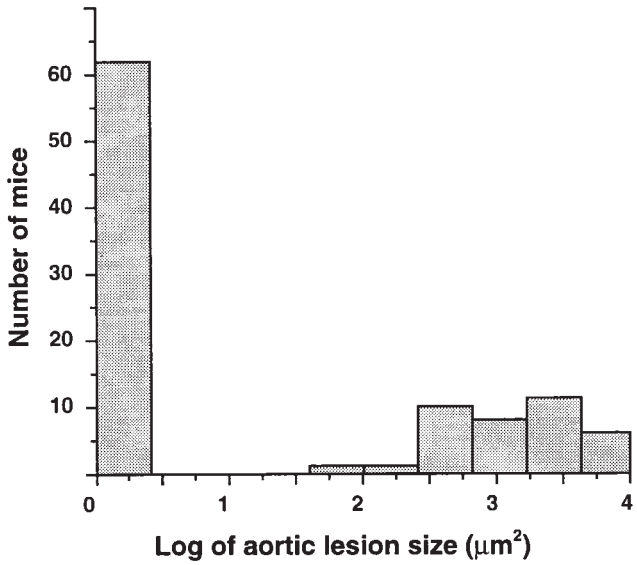

Fig. 3. Log of lesion size in aortas. Lesion size in aortas of 99 *db/db* F_2 female mice was determined by the average of five aortic cross sections near the root of the aorta.

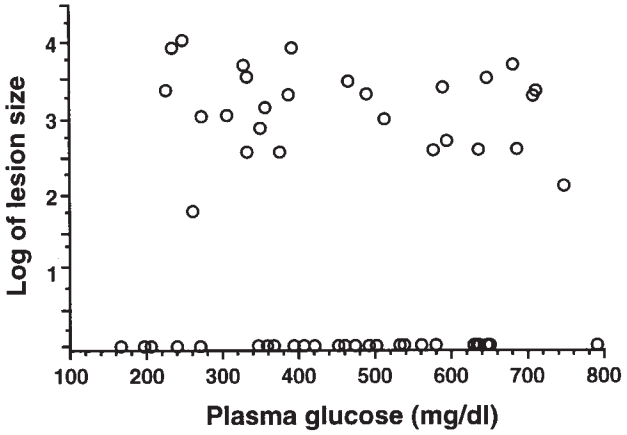

Fig. 4. Lack of correlation of lesion size and plasma glucose. Lesion size and plasma glucose levels are depicted for the F_2 mice. Plasma glucose levels were obtained for the first 57 mice. Values were obtained as described in Figs. 1 and 2. The actual values plotted are the log (lesion size + 1) so the mice at the bottom of the figure are those with no lesions.

TABLE 1. Polymorphic SSLP primers used for genome-wide scan

D1Mit5, 67, 70	*D2Mit1, 56*
D3Mit5,10, 14, 49, D3Nds2	*D4Mit13, 16, 27, 101, 172, 186, 312*
D5Mit11, 15, 25, 61, 193	*D6Mit15, 55, 102, 329*
D7Mit8, 12, 178	*D8Mit25, 31, 95, 102*
D9Mit8, 18, 64	*D10Mit15, 83, 179*
D11Mit4, 23, 35, 41, 61, 78	*D12Mit1, 2, 5, 11, 12, 182, 185*
D13Mit13	*D14Mit1, 5, 75*
D15Mit2, 33, 41	*D16Mit6, 9, 106, 182*
D17Mit10, 16, 24, 43, 57, 133, 143	*D18Mit9, 31*
D19Mit16	*DXMit1, 16, 19, 22*

QTL analysis of plasma glucose, lipids, and aortic lesions

A genome-wide scan is usually carried out by selecting polymorphic markers at 20-cM intervals throughout the 1500 cM of the mouse genome. However, because B6 and BKS are 84% identical across the genome, only those regions contributed primarily by DBA were screened. We selected markers at the ends of each region known to differ between the strains (4, 5) and at 10-cM intervals within those regions. However, this approach does not provide complete coverage of the genomic regions that differ between the strains. Although the *db* mutation arose on the BKS strain, it was intercrossed with B6-*m/m*. *Misty* (*m*) arose on the DBA strain and was backcrossed for 5 generations followed thereafter by intercrossing. During the process of constructing the double congenic carrying both *db* and *m*, there were opportunities for the loss or addition of regions of DBA genes. Thus, the DBA genes that differ between B6-*db/db* and BKS-*db/db* are not exactly the same as those that differ between B6 and BKS. Similarly, when *db* was moved into B6 by five generations of backcrossing, there were opportunities for DBA-like genomic regions to be added to the resulting B6-*db/db* congenic strain. Therefore, we needed additional information about polymorphic markers between B6-*db/db* and BKS-*db/db* to ensure that we covered the genomic areas that differed between the strains. To obtain this information, we tested DNA from B6-*db/db* and BKS-*db/db* in a systematic search for polymorphic markers that was carried out simultaneously with a test for differences between inbred strains SWR and SJL (18, 19). We screened about 3000 SSLP markers during this search and found several additional loci that differed between B6-*db/db* and BKS-*db/db*. If these mapped to genomic regions not identified in the previous comparison of the parental strains (4), they were added to the present screen. The polymorphic markers used in this study are listed in **Table 1**.

The QTL analysis of plasma glucose was carried out on individual mice from the upper and lower 20% of the F2 distribution. The analysis showed two putative loci at *D8Mit195* and *D17Mit24* with LRS of 10.4 and 9.1, respectively (equivalent to LOD scores of 2.3 and 2.0). Permutation tests showed an LRS of 7.8 to be suggestive and 14.9 to be significant, so both loci were in the suggestive range. For both loci, the BKS allele was responsible for elevated plasma glucose levels.

The QTL analysis of plasma lipids was carried out on individual mice from the upper and lower 20% of the F2 distribution. The analyses showed no QTL that reached a significance level of suggestive for total cholesterol, triglycerides, or VLDL cholesterol. However, HDL-cholesterol showed two suggestive loci at *D5Mit193* and *D17Mit24* that had a likelihood ratio statistic of 12.5 and 12.7, respectively (approximately equal to LOD scores of 2.7 and 2.8). Permutation tests indicated an LRS of 7.7 for suggestive and 14.9 for significant, so both loci were in the suggestive range.

For the QTL analysis of log of lesion size, a pool of DNA from mice from the upper and lower 20% of the distribution of lesion size were tested. The pools were constructed from 20 mice with the largest lesions and 20 mice with no lesions, selected randomly from the total of 62 mice with no lesions. If the BKS and B6 alleles in the high and low pools appeared to differ in intensity, these markers were typed in the individual animals from the high and low pools. All 99 F2 progeny were genotyped for those markers that appeared to be associated with lesion size, but no evidence of segregation distortion was found. The only QTL found was near the proximal end of Chr 12 at *D12Mit49* (**Fig. 5**) with an LRS of 11.4 (approximately equal to LOD of 2.5). A test with 10,000 permutations using MapManager indicated that a LRS of 7.0 is significant and 19.9 is highly significant, so this locus was significant. Analysis with MAPMAKER indicated a LOD of 2.6, with 25% of the variance accounted for by the region on Chr 12.

Confirmation of the Chr 12 QTL

To confirm the QTL for lesion size, we constructed a congenic strain by moving the Chr 12 BKS region into the B6 strain. In each generation, mice that were heterozygous for *D12Mit182* and *D12Mit185* were mated to B6 and the offspring were tested for heterozygosity at the same markers for the next generation. The DBA-like region on the proximal end of Chr 12 extends from the centromere to about cM 18; these two markers are at cM 2 and 11, re-

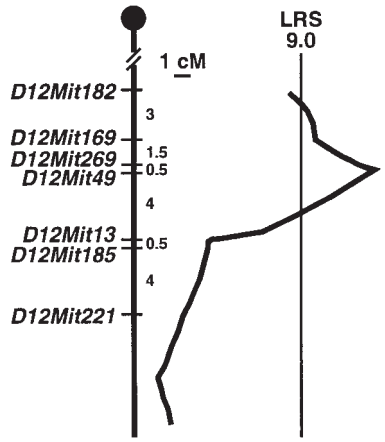

Fig. 5. Chr 12 interval map for log of lesion size. Interval map of Chr 12 with free regression model. Analysis using 10,000 permutations showed that an LRS value of 9.0 was significant. The marker order and genetic distance were calculated using MapManager and based on the 99 mice in this cross.

spectively. At N_3 we inbred the heterozygotes and selected females that were homozygous BKS at these markers. An incipient congenic is generally not intercrossed to homozygosity until N_5, but the BKS strain already comprises 84% B6 alleles, and half of the remaining 16% of DBA-like alleles are lost at each generation. We estimated that the N_3F_3 mice had only 2% of the BKS genes other than the selected region on Chr 12. We also inbred to homozygosity after N_5 (BKS would contribute about 0.5% of its genome at N5). Female mice from both incipient congenic strains were fed the atherogenic diet for 8 weeks, as were B6 and BKS controls. After 8 weeks the lesions of the congenic strains were significantly larger than those of B6 (<0.01) but not significantly different from those of BKS (**Table 2**). These results confirm the presence of a gene on Chr 12 that affects the size of atherosclerotic lesions. We name this gene *Ath6*.

Relationship of apoB to Ath6

The gene *ApoB*, which encodes apolipoprotein B (apoB), maps to the same region of Chr 12 as the *Ath6* gene. Because apoB is the major protein component of atherogenic lipoproteins, *ApoB* is a candidate gene for *Ath6*. To determine whether *ApoB* was a viable candidate gene for *Ath6*, we attempted to map *ApoB* in the same cross that provided the *Ath6* map location. Primers that amplified the 5′ $(CA)_n$ hypervariable region of the mouse *ApoB* gene were constructed (21), but no polymorphic difference was observed between strains B6 and BKS. However, because *ApoB* was polymorphic between B6 and *Mus spretus*, we were able to use The Jackson Laboratory BSS backcross DNA panel to map *ApoB* with respect to the SSLP markers that flanked the *Ath6* region in the mapping cross. These results indicate that *ApoB* maps about 1 cM proximal to *D12Mit182* (**Fig. 6**). *D12Mit182* is the most proximal marker used to construct the congenic, and *ApoB* maps centromeric to this marker. However, the congenic strain could contain BKS genes both centromeric and distal to the flanking markers. Thus, the map position of *ApoB* does not exclude it as a candidate gene for *Ath6*.

To determine whether *Ath6* determines a difference in apoB levels, we measured apoB concentrations in B6 and BKS mice that were fed the atherogenic diet for 3 weeks. No differences in immunoreactive apoB concentrations were found between the two strains (**Table 3**). We also examined the lipoprotein fraction on SDS-PAGE and found no differences in apoB levels in BKS and B6 mice that were fed chow or atherogenic diet (data not shown). These data indicate that the *Ath6* gene does not determine a difference in the quantity of apoB. However, the possibility remains that *Ath6* could determine a mutation in *ApoB* that affects its function.

TABLE 2. Lesion size among *Ath6* congenic mice (N_3F_3 and N_5F_3), B6, and BKS mice

Strain	Lesion Size	n
	μm^2	
Ath6 congenic N_3F_3	1681 ± 669	10
Ath6 congenic N_5F_3	1552 ± 298	10
C57BL/6J	495 ± 302	7
C57BKS/J	2815 ± 867	8

All mice were fed atherogenic diet for 8 weeks. There was no significant difference between the congenic strains and C57BKS. Lesion sizes in the two congenic strains are significantly larger than B6 ($P < 0.01$) but do not differ significantly from BKS. Values given as mean ± SE; n, number of mice.

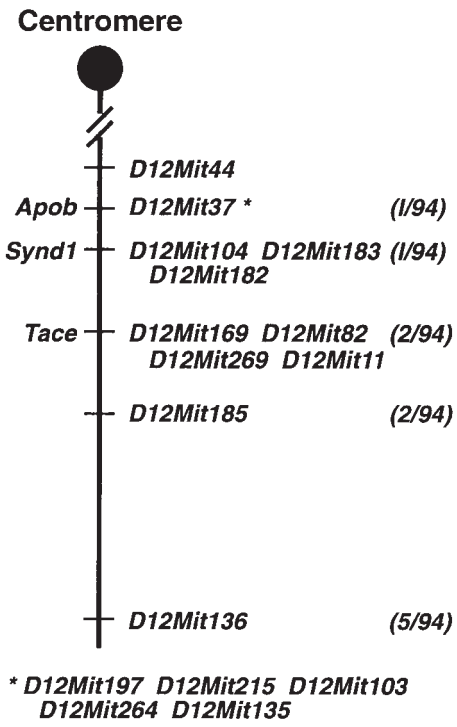

Fig. 6. Map position of *Ath6* and candidate genes. Map of mouse Chr 12 between *D12Mit44* and *D12Mit136* as determined in Jax BSS cross. The number of mice recombinant for a locus and the locus immediately above it are shown in parentheses on the right side of the map. These markers were typed in our laboratory except for *Tace* (28) and syndecan1 (29).

TABLE 3. Plasma apolipoprotein B (apoB) levels in strains B6 and BKS fed an atherogenic diet

Strain	Apolipoprotein B (n = 5)
	μg/ml
B6	314 ± 16
BKS	307 ± 17

Five mice of B6 and 5 mice of BKS at age 8 weeks were fed atherogenic diet for 3 weeks. Plasma was obtained by centrifugation of the whole blood for 5 min at 12,000 *g* at 4°C. The plasma was frozen at −20°C until measurement of apoB. There is no significant difference in apoB levels between the two strains. Values given as mean ± SE.

DISCUSSION

One of the major reasons for this study is our interest in finding an animal model to examine the relationship of diabetes and heart disease. While diabetes is a major risk factor for heart disease, the reasons are not well understood. An animal model in which diabetes increased the risk for atherosclerosis would provide an experimental system that permitted direct testing of hypotheses regarding this relationship. Hummel, Coleman, and Lane (2) and Coleman and Hummel (22) first demonstrated that, in the presence of an obesity mutation, the BKS genetic background carried factors predisposing to diabetes while the B6 genetic background did not. We previously examined the development of aortic lesions in the mutants known to exhibit an insulin-resistant and/or diabetic phenotype, such as *obese*, *diabetes*, *fatty*, and *tubby*, thinking that we might find an animal model in which diabetes increased the risk of atherosclerosis. Instead we found that the obesity mutations conferred an increase in HDL cholesterol levels that protected previously susceptible inbred strains from developing atherosclerosis (1). It was in that study, however, that we also noticed the increased risk of atherosclerosis in the BKS genetic background compared to the B6 background, both in the parental strains and in strains carrying the *ob* or *db* mutations (Fig. 1). This led to the hypothesis that if the BKS background conferred increased susceptibility to both diabetes and atherosclerosis, the susceptibilities might be conferred by the same genetic determinants. Mapping and identifying such determinants would potentially lead to an increased understanding of why diabetes is such an important risk factor for atherosclerosis.

Motivated by this hypothesis, we first undertook a systematic search for marker or allele differences between strains B6 and BKS. BKS arose through contamination of the B6 strain by another strain. When a fire in 1947 destroyed The Jackson Laboratory and its inbred mouse strains, scientists rebuilt their colonies by requesting mice from colleagues to whom they had previously distributed strains. Dr. Kaliss received a "C57BL/6" strain from a colleague and discovered that its histocompatibility differed from that of the original B6; he inbred this strain and it was named C57BL/6Ks (4). At first it was not known whether the BKS strain carried a new mutation at the major histocompatibility locus or was a genetic contamination, but as additional allele differences were discovered it was obvious that BKS was a contamination of B6 by another strain. As a result of our systematic search, we discovered that B6 and BKS were alike at 84% of their genome and differed in the remaining 16% (4). The genomic differences were compatible with strain DBA having been the source of contamination. This was consistent with Coleman's previous work demonstrating that DBA was also a diabetogenic strain as the *db* mutation produced severe diabetes when maintained in either the DBA or BKS background (23).

The QTL analysis of atherogenic factors differing between B6 and BKS could have been carried out either with a cross between the two parental strains or with a cross that included the *db* mutation. However, the QTL analysis of the diabetes factor could only have been carried out with the *db* mutation present. We chose to include the *db* mutation because our central question concerned the genetic relationship between diabetes and atherosclerosis. However, the original hypothesis that susceptibilities to diabetes and atherosclerosis are conferred by the same factor(s) proved to be incorrect. Two suggestive loci at *D8Mit195* and *D17Mit24* appeared to be responsible for the elevated glucose levels; these did not coincide with the one major QTL for lesion size on Chr 12.

During the course of this study, another laboratory did succeed in developing an animal model with both diabetes and increased atherosclerosis; this model showed that BALB/c mice exhibited accelerated atherosclerosis in response to streptozotocin-induced hyperglycemia while C57BL/6 mice did not (24). This animal model should provide valuable insights into the question of why diabetes accelerates atherosclerosis.

We also found two suggestive loci for HDL-cholesterol at *D5Mit193* and *D17Mit24*. The QTL on Chr 17 is interesting in that it affects both glucose and HDL-cholesterol. These may be controlled by separate genes that are in the same genomic region or perhaps these QTL may be related. Two previous reports have identified a QTL for HDL-cholesterol in the same region of Chr 17 using NZB/B1NJ and SM/J strains of mice (25) or C3H/HeJ and C57BL/6J strains of mice (26). Although the Chr 17 QTL had only suggestive significance in our cross, it does confirm the other studies. The second QTL for HDL-cholesterol is located at the top of the Chr 5 (cM 1.0); QTL for HDL-cholesterol have been reported previously for this chromosome but much further down near cM 40-60 (25, 26).

Although the present study did not demonstrate an association between diabetogenic and atherogenic genes, we did find one major QTL affecting atherosclerosis at the proximal end of Chr 12 that accounted for 25% of the variance of lesion size as determined by MapMaker. This QTL was statistically significant, and was confirmed by constructing a congenic strain and showing that the region on the proximal end of Chr 12 from BKS did confer increased lesion formation to the B6 strain. On the basis on this confirmation, we name this locus *Ath6*. (See reference 27 for a summary of *Ath1-Ath5*.) *Ath6* acts independently of plasma lipids since these were not correlated

with lesion size nor were any QTL found for plasma lipids that mapped to the *Ath6* region.

Ath6 may not be the only gene conferring increased lesion formation in the BKS strain, since it accounted for only 25% of the variance; moreover, the association of BKS alleles with large lesion size was not perfect. The congenic developed lesions that were significantly larger than those of B6 but not quite as large as those of BKS, although the congenic and BKS strains did not differ significantly in lesion size. This suggests that other modifying genetic factors might be influencing lesion size; if this is the case, however, none were strong enough to find in this cross. The most likely candidate gene in the *Ath6* region is *ApoB*. Both strains have similar levels of apoB protein, but it is possible that there are differences in apoB function between the strains. Two other possible candidate genes map to this region (28, 29). The first is tumor necrosis factor-alpha converting enzyme (*Tace*), an enzyme that converts the membrane-bound precursor of tumor necrosis factor-alpha to a mature secreted protein (30). Tumor necrosis factor-alpha is a proinflammatory cytokine that may participate in the atherosclerotic process. The second is syndecan1, a cell surface proteoglycan that acts as a cell adhesion molecule as well as having a role in cell signalling, growth factor interactions, and anticoagulant interactions (31). Whether any of these three candidate genes is responsible for the *Ath6* phenotype requires collecting additional recombinants in the region for positional cloning or testing the quantity or function of the proteins encoded by these genes.

Ath6 is one of a series of QTLs for lesion size that have been found (27). For only one of these has the gene underlying the QTL been tentatively identified; *Ath1* may code for a novel antioxidant protein (32). Yet each QTL that is identified has value even before the gene responsible for the phenotype is found. One reason is that making a congenic of the QTL allows one to study the physiological changes caused by the QTL and this can often provide insight into the function of the gene even before it is identified. A second reason is that knowing the map location of the major QTLs affecting a phenotype and the known genes that have been mapped often shows the coincident location of candidate genes and QTLs (20). Finally, identifying the major QTLs that affect a phenotype now will facilitate rapid utilization of the growing genomic resources for the mouse as these become available. Currently, cloning a gene starting from a QTL is a slow process compared to cloning a single gene mutation. However, the resources for positionally cloning in the mouse are improving rapidly. Sequencing the mouse genome has now begun. Other new resources being put in place are ordered BAC libraries with 15-fold coverage that will give overlapping contigs to cover each chromosome as well as full length cDNA libraries that will contain most expressed genes. While these resources are being made, it is logical to continue to find the major QTLs that affect a phenotype, so that one knows where to concentrate effort once cloning is easier. Gene-driven approaches to understanding biology, such as constructing transgenic or knockout mice, have been powerful, but the information gained by a phenotype-driven approach, such as finding and cloning QTLs, will also provide important new information that is likely to overlap but differ from a gene-driven approach. For example, a gene-driven approach can add to the understanding of known genes, but a phenotype-driven approach may lead to the discovery of new genes or pathways that affect a trait.

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