Quantitative Trait Loci That Determine BMD in C57BL/6J and 129S1/SvImJ Inbred Mice

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ABSTRACT: BMD is highly heritable; however, little is known about the genes. To identify loci controlling BMD, we conducted a QTL analysis in a (B6 × 129) F2 population of mice. We report on additional QTLs and also narrow one QTL by combining the data from multiple crosses and through haplotype analysis.

Introduction: Previous studies have identified quantitative trait loci (QTL) that determine BMD in mice; however, identification of genes underlying QTLs is impeded by the large size of QTL regions.

Materials and Methods: To identify loci controlling BMD, we performed a QTL analysis of 291 (B6 × 129) F2 females. Total body and vertebral areal BMD (aBMD) were determined by peripheral DXA when mice were 20 weeks old and had consumed a high-fat diet for 14 weeks.

Results and Conclusions: Two QTLs were common for both total body and vertebral aBMD: Bmd20 on chromosome (Chr) 6 (total aBMD; peak cM 26, logarithm of odds [LOD] 3.8, and vertebral aBMD; cM 32, LOD 3.6) and Bmd22 on Chr 1 (total aBMD; cM 104, LOD 2.5, and vertebral aBMD; cM 98, LOD 2.6). A QTL on Chr 10 (Bmd21, cM 68, LOD 3.0) affected total body aBMD and a QTL on Chr 7 (Bmd9, cM 44, LOD 2.7) affected vertebral aBMD. A pairwise genome-wide search did not reveal significant gene–gene interactions. Collectively, the QTLs accounted for 21.6% of total aBMD and 17.3% of vertebral aBMD of the F2 population variances.

Bmd9 was previously identified in a cross between C57BL/6J and C3H/HeJ mice, and we narrowed this QTL from 34 to 22 cM by combining the data from these crosses. By examining the Bmd9 region for conservation of ancestral alleles among the low allele strains (129S1/SvImJ and C3H/HeJ) that differed from the high allele strain (C57BL/6J), we further narrowed the region to ∼9.9 cM, where the low allele strains share a common haplotype. Identifying the genes for these QTLs will enhance our understanding of skeletal biology.

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Key words: BMD, haplotype, mice, peripheral DXA, quantitative trait loci

INTRODUCTION

OSTEOPOROSIS is a polygenic disease common in the elderly population, and its prevalence is increasing. BMD is an important component of human bone strength and can affect the risk of osteoporosis along with other environmental factors. Although we know a great deal about the effects of environmental factors on osteoporosis risk, we know very little about the genes, whose influence is likely to be substantial, that regulate BMD. (1) Knowing the genes will enhance our understanding of osteoporosis and may provide novel molecular targets for either preventing or treating it.

Quantitative trait loci (QTL) analysis is a powerful technique for identifying genomic regions that harbor genes with allelic variation contributing to complex traits such as BMD. (2–4) Phenotypic variation in BMD can be caused by environmental factors that are independent of genotype or through gene–environment interactions, which are common and sometimes provide novel insights to dissect the phenotype. Epidemiological studies link osteoporosis with hyperlipidemia and cardiovascular disease, which often coincide in the elderly population. This suggests that lipids themselves or a high-fat, high-cholesterol diet, which leads to elevated plasma lipids, play a pivotal role in osteoporosis as an environmental factor. (5–8) Mice of different inbred strains exhibit great BMD variation either on chow or high-fat diets; female C57BL/6J (B6) mice have low levels of BMD, whereas female 129S1/SvImJ (129) mice have relatively high levels of BMD. (9,10) We conducted a QTL analysis of 291 (B6 × 129) F2 females fed a high-fat diet for 14 weeks. Previously, we reported QTLs detected with this
intercross for plasma high-density lipoprotein (HDL)-cholesterol levels and atherosclerosis susceptibility and for obesity and plasma non–HDL-cholesterol and triglyceride levels. We report here the QTLs for total and vertebral aBMD (aBMD) in this cross, and narrow one QTL, Bmd9, found in this cross and previous crosses, by using combining the data from multiple crosses and by haplotype analysis.

MATERIALS AND METHODS

Animals and diet

C57BL/6J (B6) and 129S1/SvImJ (129) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and mated to produce the (B6 × 129) F1 progeny, which were intercrossed to produce an F2 population, of which 291 females were used in this study. Mice were maintained in a temperature- and humidity-controlled environment with a 14-h light:10-h dark cycle and given unrestricted access to food and acidified water. The cages were covered with polyester filters and contained a bedding of pine shavings. Six-week-old mice were fed a high-fat diet, containing 15% dairy fat, 1% cholesterol, and 0.5% cholic acid, for 14 weeks, after which they were killed by cervical dislocation. Experiments were reviewed and approved by the Institutional Animal Care and Use Committee of The Jackson Laboratory.

Quantitative phenotype measurements

When mice were 20 weeks old, after consuming the high-fat diet for 14 weeks, their total body aBMD (total aBMD) and lumbar vertebrae aBMD (vertebral aBMD) were measured using peripheral DXA (pDXA; PIXImus, GE-Lunar, Madison, WI, USA), a method whose accuracy has been validated in mice. PIXImus measurements of total and site-specific aBMD correlate well with volumetric density data from pQCT. Moreover, PIXImus measurements of mineral content are very highly correlated with mineral content of hydroxyapatite standard of known density ($r^2 = 0.997$).

Genotyping

Initially, the 291 F2 female progeny were genotyped with 88 simple sequence length polymorphic (SSLP) markers (Research Genetics, Huntsville, AL, USA) spaced ~20 cM apart. Later, they were genotyped with 23 additional SSLP markers in the QTL regions as previously described. The average spacing between these markers was 14 ± 12 (SD) cM. Methods used for DNA isolation, PCR amplifications, and subsequent gel electrophoreses have been described previously. Reported genetic map positions were retrieved from the Mouse Genome Informatics database (http://www.informatics.jax.org).

QTL analyses

As described previously, a three-step QTL analysis was conducted to search for main effects and pairwise gene interactions and to integrate all the main and interacting QTL-phenotype associations into a multiple regression. In the regression analysis, we combined all significant and suggestive QTL and interactions in a multiple regression model. Terms that did not meet the nominal 0.02 level in the regression were eliminated in backward stepwise fashion, with the exception that main effect terms involved in a significant interaction were retained. Final models were reported for each trait. QTLs were deemed significant if they either met or exceeded the 95% genome-wide adjusted threshold, which was assessed by permutation analysis for each trait (LOD $> 3.0$ for total aBMD and LOD $> 3.3$ for vertebral aBMD); they were deemed suggestive if they either met or exceeded the 37% genome-wide adjusted threshold (LOD $> 1.8$ for total aBMD and LOD $> 1.9$ for vertebral aBMD) but were not significant. QTL CIs were calculated according to the posterior probability density of QTL locations, as described previously. Variance (%) indicates the percentage of the total F2 phenotypic variance associated with each marker. Analyses were carried out using Pseudomarker 1.06 software.

Statistically combining crosses

Finding repetitive QTLs in crosses with different strains suggests that they may have arisen from shared ancestral alleles. To narrow the QTL region, we combined data from multiple crosses. Because QTL localization is dependent on the locations of recombination breakpoints, combining crosses can effectively narrow the CI by providing more recombination within the region. We combined the raw data for Chr 7 from the cross between B6 and 129 with that from the cross using B6 and C3H mice by recording a B6 genotype as a high vertebral BMD allele and 129 and C3H genotypes as a single low vertebral BMD allele. A LOD score was computed at 2-cM intervals across the QTL interval for each cross separately and then for both crosses combined. The combined data were analyzed with the “vertebral BMD phenotype” as standardized and “cross” as an additive covariate.

Haplotype analysis

Recent evidence showed that inbred mouse strains are genetic mosaics of alleles principally derived from Mus musculus domesticus ancestors, and QTLs found in multiple crosses using different strains can be effectively narrowed by comparing haplotypes from the parental strains throughout the QTL region. Single nucleotide polymorphisms (SNPs) for use in the haplotype analysis were obtained from data sets generated by Fletcher et al. and Petkov et al. The data sets contained 183 SNPs in Bmd9, spanning 68.6–110.2 Mb, and the average spacing of the SNPs was ~0.23 Mb.

Statistical analysis

Values are given as means ± SE. One-way ANOVAs with Tukey’s correction for multiple pairwise comparisons were used to determine statistically significant differences in total aBMD and vertebral aBMD between mouse groups. The aBMD phenotypes and phenotypes that have been reported in the previous report, body weight, BMI,
and non–HDL-cholesterol (non-HDL) (12) were examined using Pearson’s correlation. Data were analyzed using Graphpad Prism (Windows v4.00; GraphPad Software, San Diego, CA, USA).

Naming QTLs

In accordance with the International Committee on Standardized Genetic Nomenclature for Mice (http://www.informatics.jax.org/mgihome/nomen) and the Complex Traits Consortium (4), we named QTLs if they were significant or if they were suggestive but confirmed QTLs reported previously. They were given the same name if the crosses identifying them shared at least one common strain and a new name if the crosses identifying them involved no common strains.

RESULTS

Inheritance of total aBMD and vertebral aBMD

Total aBMD and vertebral aBMD were measured after female mice had been fed the high-fat diet for 14 weeks (Table 1). Total and vertebral aBMDs of B6 mice (n = 10) were significantly lower (p < 0.001) than those of 129 mice (n = 10). Although aBMDs of the F1 mice (n = 8) were intermediate to those of the parental strains, they were significantly higher (p < 0.001) than those of B6 mice but not significantly different from those of 129 mice. Figure 1 shows the distributions of total and vertebral aBMDs of the F2 mice (n = 291) with mean values for parental progeny.

![Figure 1](image1.png)

**Figure 1.** Distributions for (A) total aBMD and (B) vertebral aBMD in 291 female (B6 × 129) F2 progeny fed a high-fat diet for 14 weeks. Positions of mean aBMD for the parental strains, B6 and 129, and F1 progeny are indicated by arrows, whereas the bell-shaped line depicts the theoretical normal distribution.

**Table 1. Total aBMD and Vertebral aBMD of Female B6, 129, F1, and (B6 × 129) F2 Progeny**

<table>
<thead>
<tr>
<th>Mice</th>
<th>n</th>
<th>Total aBMD (mg/mm²)</th>
<th>Vertebral aBMD (mg/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>10</td>
<td>0.461 ± 0.009¹ ²</td>
<td>0.670 ± 0.019¹ ²</td>
</tr>
<tr>
<td>129</td>
<td>10</td>
<td>0.569 ± 0.007</td>
<td>0.926 ± 0.040</td>
</tr>
<tr>
<td>F1</td>
<td>8</td>
<td>0.532 ± 0.015</td>
<td>0.861 ± 0.026</td>
</tr>
<tr>
<td>F2</td>
<td>291</td>
<td>0.537 ± 0.003</td>
<td>0.837 ± 0.008</td>
</tr>
</tbody>
</table>

* Data are presented as the mean ± SE.
¹ Significant difference (p < 0.001, by ANOVA) vs. 129.
² Significant difference (p < 0.001, by ANOVA) vs. F1.
³ Because it is the distribution and not the mean among the F2 population that is most important for detecting genetic linkage to a phenotype, we did not test for significant differences between F2 progeny and the other groups.

![Figure 2](image2.png)

**Figure 2.** Genome-wide scans for (A) total aBMD and (B) vertebral aBMD in 291 female (B6 × 129) F2 progeny fed a high-fat diet for 14 weeks. Chromosomes 1 through X are represented numerically on the ordinate. The relative width of the space allotted for each chromosome reflects the relative length of each chromosome. The abscissa represents the LOD score, the traditional metric of genetic linkage. The significant (p < 0.05) and suggestive (p < 0.63) levels of linkage were determined by permutation testing. (4,18,20)

**Table 2. Pearson Correlation Coefficients Among Total aBMD, Vertebral aBMD, Body Weight, BMI, and Non-HDL in 291 (B6 × 129) F2 Females Fed a High-Fat Diet for 14 Weeks**

<table>
<thead>
<tr>
<th>Total aBMD</th>
<th>Body weight</th>
<th>BMI</th>
<th>Non-HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aBMD</td>
<td>0.59*</td>
<td>0.28*</td>
<td>-0.48*</td>
</tr>
<tr>
<td>Vertebral aBMD</td>
<td>0.83*</td>
<td>0.39*</td>
<td>0.15¹</td>
</tr>
</tbody>
</table>

* p < 0.001.
¹ p < 0.05.

Naming QTLs

In accordance with the International Committee on Standardized Genetic Nomenclature for Mice (http://www.informatics.jax.org/mgihome/nomen) and the Complex Traits Consortium (4), we named QTLs if they were significant or if they were suggestive but confirmed QTLs reported previously. They were given the same name if the crosses identifying them shared at least one common strain and a new name if the crosses identifying them involved no common strains.

Inheritance of total aBMD and vertebral aBMD

Total aBMD and vertebral aBMD were measured after female mice had been fed the high-fat diet for 14 weeks (Table 1). Total and vertebral aBMDs of B6 mice (n = 10) were significantly lower (p < 0.001) than those of 129 mice (n = 10). Although aBMDs of the F1 mice (n = 8) were intermediate to those of the parental strains, they were significantly higher (p < 0.001) than those of B6 mice but not significantly different from those of 129 mice. Figure 1 shows the distributions of total and vertebral aBMDs of the F2 mice (n = 291) with mean values for parental progeni-
tors and F1 mice indicated by arrows. Regression analyses of F2 progeny revealed significant positive correlations between aBMDs, body weight, and BMI, and a negative but significant correlation with non-HDL (Table 2), indicating that aBMDs increased as body weight or BMI increased and as non-HDL decreased.

Identification of genetic loci affecting total aBMD and vertebral aBMD

The genome-wide scans for aBMD QTLs are presented in Fig. 2. A summary of the scan results is shown in Table 3. We identified a significant QTL on each of Chrs 6 and 10 and one suggestive QTL on Chr 1 for total aBMD (Fig. 2A) and one significant QTL on Chr 6 and one suggestive QTL on each of Chrs 1 and 7 for vertebral aBMD (Fig. 2B). We detected no significant interacting QTL in the pairwise genome scan.

For the Chr 6 QTL for total aBMD (Fig. 3A; peak LOD 3.8 at cM 26), named Bmd20, the 129 allele conferred increased total aBMD (Fig. 3B). We also identified a Chr 6 QTL for vertebral aBMD (Fig. 3C; peak LOD 3.6 at cM 32); the 129 allele also conferred increased vertebral aBMD (Fig. 3D). Because it co-localized with Bmd20, we gave it the same name. For the Chr 10 QTL (Fig. 3E; peak LOD 3.0 at cM 68), named Bmd21, the B6 allele conferred increased total aBMD in a dominant fashion (Fig. 3F). For the suggestive Chr 7 QTL for vertebral aBMD (Fig. 3G; peak LOD 2.7 at cM 44), the B6 allele conferred increased Bmd20 score (Fig. 3H). The D7Mit300 locus confirmed a QTL, Bmd9, identified earlier using strains B6 and C3H. We named this locus Bmd9, because it co-localized with the B6 allele also conferred increased vertebral aBMD (Fig. 3I; peak LOD 3.6 at cM 98, Chr 1). We gave it the suggestive Chr 1 QTL for total aBMD (Fig. 3J; peak LOD 2.7 at cM 40), named Bmd22 (this study). Table 3 summarizes the chromosomal locations, 95% CIs, LOD scores, and nearest markers for BMD QTLs identified in the single gene scan and revealed two additional ones on Chrs 4 and 17 (Table 3). Taken together, these QTLs explained 21.6% of the total F2 phenotypic variance, Bmd20 and Bmd22 each accounting for about 6%, and the others each accounting for 2–3%.

The regression analysis confirmed the three vertebral aBMD QTLs identified in the single gene scan and revealed an additional one on Chr 4. Taken together, these QTLs explained 17.3% of the total F2 phenotypic variance.

Combining crosses to narrow Bmd9

A vertebral aBMD QTL in this analysis, Bmd9, has been identified previously in other QTL crosses between B6 and C3H and B6 and DBA/2. We combined the raw data for Bmd9 from both the present (B6 × 129) F2 intercross and the previous (B6 × C3H) F2 intercross by recoding a B6 genotype as a high BMD allele and 129 and C3H genotypes as a single low BMD allele (data from B6 × DBA/2 cross was not available to us). Reanalysis of the combined Chr 7 data reduced the CI from 34 to 22 cM, spanning cM 38–60, and increased the LOD score for Bmd9 to 7.2 at cM 46 (Fig. 4).

Haplotype analysis of Bmd9

We carried out a haplotype analysis to further narrow Bmd9. We compared haplotypes from all four parental strains throughout the QTL interval to identify genomic regions shared among strains 129, C3H, and DBA but differing from strain B6. We defined a common haplotype block to be three or more consecutive shared alleles shown as an outlined box, although this definition may miss small haplotypes that would be evident with more densely spaced SNPs. Figure 5 shows the SNPs assayed in the region, highlighting the nine haplotype blocks where the SNPs from the strains with the alleles for low BMD are shared, and also differ from B6; these shared SNPs are likely to represent a DNA region that is identical by descent. The intervals in the haplotype blocks vary from 1.4 to 3.6 Mb and total 9.9 cM.

DISCUSSION

In this study, we described two inbred mouse strains, B6 and 129, which displayed different total and vertebral aBMD when fed a high-fat diet. Three-step QTL analyses on 291 (B6 × 129) F2 females resulted in the localization of four QTLs for total and/or vertebral aBMDs: Bmd9, Bmd20, Bmd21, and Bmd22. The use of combined data from multiple crosses and haplotype analysis to narrow the interval of the initial QTL, Bmd9, to ~9.9 cM shows the power of finding repetitive QTLs.
TABLE 4. MULTIPLE REGRESSION ANOVAS FOR TOTAL aBMD AND VERTEBRAL aBMD IN 291 (B6 × 129) F2 FEMALES

<table>
<thead>
<tr>
<th>Traits</th>
<th>Location Chr (cM)</th>
<th>Nearest marker</th>
<th>df*</th>
<th>Type III SS†</th>
<th>Variance (%)‡</th>
<th>F value</th>
<th>p</th>
<th>Locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aBMD</td>
<td>Chr 6 (26)</td>
<td>D6Mit209</td>
<td>2</td>
<td>0.000</td>
<td>6.1</td>
<td>10.76</td>
<td>3.14 × 10⁻⁵</td>
<td>Bmd20</td>
</tr>
<tr>
<td></td>
<td>Chr 10 (70)</td>
<td>D10Mit35</td>
<td>2</td>
<td>0.000</td>
<td>3.5</td>
<td>6.14</td>
<td>0.0025</td>
<td>Bmd21</td>
</tr>
<tr>
<td></td>
<td>Chr 1 (104)</td>
<td>D1Mit406</td>
<td>2</td>
<td>0.000</td>
<td>6.0</td>
<td>10.55</td>
<td>3.82 × 10⁻⁵</td>
<td>Bmd22</td>
</tr>
<tr>
<td></td>
<td>Chr 4 (80)</td>
<td>D4Mit42</td>
<td>2</td>
<td>0.000</td>
<td>3.5</td>
<td>6.12</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chr 17 (20)</td>
<td>D17Mit180</td>
<td>2</td>
<td>0.000</td>
<td>2.5</td>
<td>4.43</td>
<td>0.0128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>290</td>
<td>0.010</td>
<td>21.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral aBMD</td>
<td>Chr 6 (32)</td>
<td>D6Mit209</td>
<td>2</td>
<td>0.258</td>
<td>5.0</td>
<td>8.44</td>
<td>0.0003</td>
<td>Bmd20</td>
</tr>
<tr>
<td></td>
<td>Chr 7 (44)</td>
<td>D7Mit300</td>
<td>6</td>
<td>0.165</td>
<td>3.2</td>
<td>5.40</td>
<td>0.0050</td>
<td>Bmd9</td>
</tr>
<tr>
<td></td>
<td>Chr 1 (98)</td>
<td>D1Mit115</td>
<td>2</td>
<td>0.282</td>
<td>5.5</td>
<td>9.25</td>
<td>0.0001</td>
<td>Bmd22</td>
</tr>
<tr>
<td></td>
<td>Chr 4 (76)</td>
<td>D4Mit42</td>
<td>6</td>
<td>0.188</td>
<td>3.6</td>
<td>6.14</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>290</td>
<td>5.170</td>
<td>17.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* df indicates degrees of freedom and includes main effect and any interactions.
† SS, sums of squares.
‡ Variance indicates the percentage of the total F₂ phenotypic variance associated with each marker.
Previously, Klein et al. \(^{(28)}\) used recombinant inbred (RI) mouse strains derived from a cross between B6 and DBA progenitors (RI-BXD) to identify a whole body aBMD QTL on Chr 7 (cM 44). Beamer et al. \(^{(27)}\) found a vertebral vBMD QTL on Chr 7 (cM 65.6) using a \((B6 \times C3H)\) F\(_2\) intercross and named it \(Bmd9\). We confirmed the QTL and named it \(Bmd9\). Potential candidate genes for the initial interval of \(Bmd9\) based on known function are \(Pth\) (cM 52.5), coding for PTH, \(Calca\) (cM 54), coding for a calcitonin, and \(Sox6\) (cM 55), coding for a chondrocyte-specific Sry-related HMG box transcriptional factor. Haplotype analysis highlighted nine haplotype blocks where 129, C3H, and DBA shared alleles that differed from B6 mice; the

**FIG. 4.** Narrowing \(Bmd9\) by combining crosses. Data for \(Bmd9\) from both the present \((B6 \times 129)\) F\(_2\) intercross and the previous \((B6 \times C3H)\) F\(_2\) intercross were combined using a novel statistical method to narrow the QTL. \(^{(13)}\) and the 95\% CI was reduced from 34 to 22 cM. Genome-wide scan and posterior probability density were represented by a solid line and a broken line, respectively.

**FIG. 5.** Haplotype map of middle chromosome 7. The \(Bmd9\) interval from C57BL/6J (B6), 129S1/SvImJ (129), C3H/HeJ (C3H), and DBA/2J (DBA) were compared with identify genomic regions shared among strains 129, C3H, and DBA and that differed from strain B6. SNP ID numbers are listed in the left column. The physical position column indicates the distance, in megabases, from the centromere according to the Ensembl database (Build 33). The mouse strains are indicated at the top of the figure. The SNPs are shown as observed nucleotides, A, T, G, and C, at each position among the strains. B6 nucleotides at each SNP position are highlighted yellow. 129, C3H, and DBA nucleotides at each SNP position are highlighted yellow if equal to B6 or blue if different. The common haplotype block was defined as to be three or more consecutive shared alleles; common SNPs are shown as an outlined box according to the co-inheritance hypothesis.
blocks excluded the candidate genes Pth and Calca. One haplotype block, spanning 102.1–103.7 Mb, contained Sox6. Sox6 is expressed with other transcription factors, Sox5 and Sox9, in all chondroprogenitors and all differentiated chondrocytes, and mediates differentiation and proliferation of chondrocytes. These differentiated chondrocytes sustain a series of sequential changes that include conversion to hypertrophic chondrocytes, ability to calcify the extracellular matrix, cell death, and replacement by bone. These facts make Sox6 an excellent candidate for genes underlying Bmd9. Alternatively, genes underlying these QTLs are entirely novel genes that might otherwise not have been considered.

Bmd20 maps to the region of the gene (Ghrhr, cm 26) coding for growth hormone–releasing hormone receptor (GHRHR). It has been reported that mice homozygous for a missense mutation in the extracellular domain of the gene (Ghrhrd18) have reduced GH secretion and a dwarf phenotype.\(^{(31)}\)

Previously, Beamer et al.\(^{(32)}\) using a (B6 × CAST) F2 intercross, found a femoral vBMD QTL on Chr 1 (cm 95.8) and named it Bmd1. A later (B6 × C3H) F2 intercross revealed a femoral and vertebral vBMD QTL in the vicinity of Bmd1 (cm 81.6), named Bmd5.\(^{(27)}\) However, Klein et al.\(^{(33)}\) found a whole body aBMD QTL at the distal portion of Chr 1 (cm 101.5) in a (B6 × DBA) F2 intercross. Masinde et al.\(^{(34)}\) used a (MRL × SJL) F2 intercross to identify total vBMD at the bottom of Chr 1 (cm 110.4) and named it Tbbmd1. For these three of these QTLs, the B6 alleles conferred decreased BMD. We confirmed these QTLs and named both total and vertebral aBMD QTLs Bmd22. These QTLs will need to be more finely mapped to determine whether these represent the same QTL.

Body weight is one of the strongest predictors of bone mass in human and mice, although the mechanisms that explain this relationship remain unclear.\(^{(35,36)}\) Our results are in agreement with this because aBMDs showed positive correlations between either body weight or BMI (Table 2). In fact, Bmd22 co-localized with a QTL for BMI (peak LOD 2.4 at cm 102), Obyq17, which we reported previously\(^{(12)}\). If these QTLs are determined by the same gene, the J29 allele confers both increased bone mass and increased BMI.

Increased plasma lipids are associated with osteoporosis in humans.\(^{(5–8)}\) and we found that increased non–HDL-cholesterol was correlated with reduced BMD in this cross (Table 2). Consistent with this relationship, we found that Bmd21 co-localized with a QTL for non–HDL-cholesterol (peak LOD 4.0 at cm 70), Nhdlq4.\(^{(12)}\) If these QTLs are caused by the same gene, the J29 allele confers low BMD but increased non–HDL-cholesterol.

Previous investigators found that an atherogenic diet contributes to osteoporosis in mice by blocking osteoblastic differentiation and increasing osteoclastic bone resorption in vivo.\(^{(7,27,30)}\) Oxidized lipids have been shown to induce inflammatory genes such as monocytic chemotactic protein-1 to recruit and differentiate osteoclast precursor cells.\(^{(37,39)}\) Previously, two QTL studies of BMD were carried out using parental strains B6 and DBA/2.\(^{(33,40)}\) Only one QTL was found in common; the fact that one study used high-fat diet fed mice\(^{(40)}\) and the other used chow-fed mice\(^{(33)}\) suggests that the diet affects BMD phenotypes as an environmental factor or through gene–environment interactions. In this study, we used mice after feeding them a high-fat diet for 14 weeks and identified four QTLs. Two of them, Bmd20 and Bmd21, have not been found in previous QTL crosses fed a chow diet. These facts may give clues as to the underlying pathway of the QTLs. Alternatively, they might be reflections of the statistical power, type II error, or a gender effect.\(^{(41)}\)

In summary, a QTL analysis of a (B6 × 129) F2 female cohort fed a high-fat diet for 14 weeks identified four loci that determine total and/or vertebral aBMD: Bmd20 on Chr 6, Bmd21 on Chr 10, Bmd22 on Chr 1, and Bmd9 on Chr 7. Finding repetitive QTLs in different crosses facilitates the use of comparative genomic methods, such as statistical methods to combine crosses and haplotype analysis\(^{(13,21–24)}\) to identify the underlying genetic polymorphisms. Bmd9 represents an excellent candidate QTL for these methods, because the present cross confirmed the QTLs previously found in a (B6 × C3H) F2 cross and RI-BXD strains. We effectively narrowed Bmd9, and identified nine regions where high allele strains share a common haplotype, suggesting the possibility that Sox6 is a candidate gene underlying Bmd9. Identifying the genes for Bmd9 and other aBMD QTLs will enhance our understanding of skeletal biology.

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