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Variation in Bone Biomechanical Properties, Microstructure, and Density in BXH Recombinant Inbred Mice

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ABSTRACT

To test the hypothesis that factors associated with bone strength (i.e., volumetric bone mineral density [vBMD], geometry, and microstructure) have heritable components, we exploited the 12 BXH recombinant inbred (RI) strains of mice derived from C57BL/6J (B6; low bone mass) and C3H/HeJ (C3H; high bone mass) progenitor strains. The femurs and lumbar vertebrae from each BXH RI strain were characterized for phenotypes of vBMD, microstructural, biomechanical, and geometrical properties. Methods included bending (femur) and compression (vertebra) testing, peripheral quantitative computed tomography (pQCT), and microcomputed tomography (μCT). Segregation patterns of femoral and vertebral biomechanical properties among the BXH RI strains suggested polygenic regulation. Femoral biomechanical properties were strongly associated with femoral width in the anteroposterior (AP) direction and cortical thickness—geometric properties with complex genetic regulation. Vertebral vBMD and biomechanical properties measured in BXH RI strains showed a greater variability than either B6 or C3H progenitors, suggesting both progenitor strains have independent subsets of genes that yield similar vBMD and strength. The μCT and pQCT data suggested that the distribution of vertebral mineral into cortical and trabecular compartments is regulated genetically. Although the B6 and C3H progenitors had similar vertebral strength, their vertebral structures were markedly different: B6 had good trabecular bone structure and modest cortical bone mineral content (BMC), whereas C3H had high cortical BMC combined with a deficiency in trabecular structure. These structural traits segregated independently in the BXH RI strains. Finally, vertebral strength was not correlated consistently with femoral strength among the BXH RI strains, suggesting genetic regulation of bone strength is site specific. (J Bone Miner Res 2001;16:206–213)

Key words: biomechanics, bone density, osteoporosis, genetics

INTRODUCTION

OSTEOPOROSIS IS primarily a disease of bone fragility resulting from decreased bone mass, altered microarchitecture, and possibly impaired bone quality. Key among the factors contributing to the prevalence of osteoporosis is reduced skeletal density.[1] Studies in twins have determined that approximately 70% of the variability in bone density is genetically based.[2–4] This variation in bone density probably is influenced by multiple genes, which have not yet been identi-

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fied.[5] Beamer et al.[6] showed a large difference in the femoral volumetric bone mineral density (vBMD) and bone mineral content (BMC) between the inbred mouse strains C3H/HeJ (C3H) and C57BL/6J (B6). Thus, these strains appear to be very good models for the analyses of high and low bone mass, respectively. However, the genetic influence on skeletal bone microstructure and strength in these mice is site specific and complex. For example, C3H mice have thicker femoral cortices and stronger femoral shafts when compared with B6 but are deficient in trabecular bone structure in the proximal femur and lumbar spine.[7] Although C3H mice have significantly stronger femurs compared with B6, their lumbar vertebrae are not stronger, but instead are more brittle,[7] suggesting that the genes contributing to improved femoral strength have no effect or even a negative effect on trabecular bone structure in the spine. These findings led us to hypothesize that multiple genes contribute independently to cortical and trabecular bone microstructures and bone fragility in the C3H and B6 strains.

In the current study, we evaluated the bone microstructure, vBMD, and biomechanical properties of femurs and vertebrae in BXH recombinant inbred (RI) mouse strains. RI strains were created by first crossbreeding two different inbred strains, intercrossing the resulting F1 hybrids and then inbreeding pairs of F2 progeny for 20 subsequent generations.[8] This breeding strategy creates a set of RI strains. The mice within each RI strain are "twins" and thus contain the same genetic alleles, half of these alleles come from each of the two progenitor strains. A set of 12 RI strains (denoted BXH) were previously created from B6 and C3H progenitor strains. Each of the BXH RI strains contains a unique combination of genetic alleles from the B6 and C3H strains that can be phenotyped for genetically based polymorphic traits that differ between the B6 and C3H progenitors. Comparison among strains of the distribution pattern for a quantitative trait can be used to gain chromosomal location of genes responsible for the trait of interest.[9] Originally, RI strains were developed for mapping single genes with major effects; mapping precision rapidly degenerates with increasing genetic complexity of a trait.[10] Nevertheless, such strains can be exploited to discriminate between single versus polygenic inheritance, as well as to gain clues about subsets of regulatory genes and possible locations of major genes.[11]

We hypothesized that factors associated with bone strength (i.e., BMD, geometry, and microstructure) have heritable components. To test this hypothesis, we measured femoral and vertebral bone strength in the 12 BXH RI strains and the progenitor B6 and C3H strains. In addition, we measured femoral and vertebral geometry, vBMD, and trabecular microstructure in the lumbar spine. The resultant phenotypic segregation patterns differed from each other, indicating distinct inheritance patterns and a complexity of genetic regulation for these traits.

MATERIALS AND METHODS

Animal care

The study involved 12 BXH RI strains of mice (n = 7–13) and the B6 and C3H progenitor strains. All mice used in the study were female and 8 months of age. Adult femoral density is achieved at about 4 months of age in mice and maintained until about 8–12 months of age.[6] Consequently, the mice in this study are mature adults with peak bone mass. These mice were group-housed in polycarbonate cages at The Jackson Laboratory (Bar Harbor, ME, USA). Water was acidified with HCl to achieve a pH of 2.8–3.2 for suppression of bacteria and was freely available. The diet used for all mice was pasteurized National Institutes of Health (NIH) 31 (6% fat diet, vitamin and mineral fortified; PMI, Richmond, IN, USA) and was freely available. Use of mice in this research project was reviewed and approved by the Institutional Animal Care and Use Committee of The Jackson Laboratory.

BMD measurements

Isolated femurs and vertebrae were assessed using peripheral quantitative computed tomography (pQCT; Stratec XCT 960M; Norland Medical Systems, Ft. Atkinson, WI, USA) as described previously.[6] Briefly, bones were isolated and stored in 95% EtOH until assessed by pQCT for vBMD. Thresholds of 1.300 attenuation units differentiated mineralized bone from water, adipose tissue, muscle, and tendon; a threshold of 2.000 differentiated high-density cortical bone from bone of lower density. Precision of the pQCT for femoral, tibial, and vertebral vBMD were 1.2, 1.5, and 1.4%, respectively. Isolated femurs were scanned at 2-mm intervals over their entire lengths. Total vBMD was calculated by dividing the total mineral content by the total bone volume. Femoral cortical thickness was calculated at the midpoint of the diaphysis. Isolated lumbar vertebrae (L5) were scanned at their measured midpoints and vBMD was calculated as described previously. Cortical BMC values also are presented for the L5 vertebrae.

Bone microstructure measurements

The L5 vertebra from BXH RI strains with the highest and lowest bone strength were analyzed using a desktop micro-CT (μCT 20; Scanco Medical AG, Bassersdorf, Switzerland). A microfocus X-ray tube with a focal spot of 10 μm was used as a source. To perform a measurement, the specimen was mounted on a turntable that could be shifted automatically in the axial direction. Six hundred projections were taken over 216° (180° plus half the fan angle on either side). A standard convolution-backprojection procedure with a Shepp and Logan filter was used to reconstruct the CT images in 1024 pixel $\times$ 1024 pixel matrices. For each sample, a total of 100–200 microtomographic slices, with a slice increment of 17 μm, were acquired. Measurements were stored in three-dimensional (3D) image arrays with an isotropic voxel size of 17 μm. A constrained 3D Gaussian filter was used to partly suppress the noise in the volumes. The bone tissue was segmented from marrow using a global thresholding procedure.[12] In addition to the visual assessment of structural images, morphometric indices were determined from the microtomographic data sets. Cortical and trabecular bone were separated using a semiautomated contour tracking algorithm to detect the outer and inner boundaries of the cortex. In trabecular bone, basic structural metrics were measured using direct 3D morphometry.[13]

TABLE 1. BODY WEIGHT AND FEMUR GEOMETRY, INCLUDING LENGTH, WIDTH IN AP AND ML DIRECTIONS, AND MIDDIAPHYSEAL THICKNESS, FOR B6, C3H, AND BXH RI STRAINS

Inbred strain	n	*Body weight (g)*	*Femur length (mm)*	*Width ML (mm)*	*Width AP (mm)*	*Cortical thickness (mm)*
B6	8	28.4 ± 1.5	16.1 ± 0.11	1.80 ± 0.05[a]	1.23 ± 0.02[a]	0.40 ± 0.01[a]
C3H	10	27.7 ± 1.0	16.0 ± 0.08	1.60 ± 0.01[b]	1.31 ± 0.02[b]	0.56 ± 0.01[b]
BXH-2[c]						
BXH-3	10	25.6 ± 1.0	16.0 ± 0.09	1.54 ± 0.04[b]	1.11 ± 0.02[a,b]	0.36 ± 0.01[a]
BXH-4	11	31.4 ± 1.3	**17.2 ± 0.07**[a,b]	1.81 ± 0.02[a]	1.28 ± 0.02[b]	0.49 ± 0.01[a,b]
BXH-6	13	25.7 ± 1.3	16.1 ± 0.04	1.73 ± 0.03[a]	1.02 ± 0.01[a,b]	0.45 ± 0.01[a,b]
BXH-7	8	26.9 ± 2.3	15.9 ± 0.08	**1.90 ± 0.05**[a,b]	1.16 ± 0.03[a,b]	0.37 ± 0.01[a]
BXH-8	10	24.3 ± 1.3	16.1 ± 0.09	1.84 ± 0.03[a]	1.20 ± 0.01[a]	0.44 ± 0.02[a,b]
BXH-9	8	31.9 ± 1.9	**16.4 ± 0.05**[a,b]	1.82 ± 0.04[a]	1.26 ± 0.02[a]	0.46 ± 0.02[a,b]
BXH-10	10	26.6 ± 0.7	15.1 ± 0.06[a,b]	1.52 ± 0.02[b]	1.07 ± 0.01[a,b]	0.37 ± 0.01[a]
BXH-11	7	24.5 ± 1.8	16.3 ± 0.14[a]	1.84 ± 0.02[a]	1.27 ± 0.03	0.41 ± 0.01[a]
BXH-12	10	28.9 ± 2.5	16.0 ± 0.09	1.60 ± 0.02[b]	1.15 ± 0.02[a,b]	0.44 ± 0.01[a,b]
BXH-14	9	26.2 ± 1.4	**16.6 ± 0.10**[a,b]	1.66 ± 0.03[b]	1.10 ± 0.01[a,b]	0.49 ± 0.02[a,b]
BXH-19	8	21.7 ± 0.4[a,b]	16.2 ± 0.07	1.72 ± 0.03[a]	1.08 ± 0.01[a,b]	0.46 ± 0.02[a,b]

Data presented are mean ± SEM.
Boldface indicates significantly greater than both C3H and B6; underline indicates significantly less than both C3H and B6.
[a] Significantly different from C3H.
[b] Significantly different from B6.
[c] BXH-2 developed early onset leukemia and were excluded from the study.

The measurements included bone volume density (BV/TV), bone surface density (BS/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp). Previous studies have shown that trabecular structural metrics measured using μCT closely correlate with those measured using standard histomorphometry.[14,15] Average height and width of the L5 vertebrae were measured directly on midcoronal μCT images.

Biomechanical tests

We measured bone strength of the femur and the L5 lumbar vertebrae. Femurs were tested at the mid-shaft by three-point bending at room temperature. Load was applied in the anteroposterior (AP) direction midway between two supports that were 5 mm apart. Load-displacement curves were recorded at a crosshead speed of 0.5 mm/s using a microforce materials testing machine (Vitrodyne 2000). Data were stored on a microcomputer. Ultimate force (F_u), stiffness (S), and work to failure (U) were calculated from the load-displacement curve as described elsewhere.[16] F_u reflects the strength of the bone, while S reflects the rigidity and U is the energy necessary to cause a fracture. Widths of the cortical midshaft in the mediolateral (ML) and anteroposterior (AP; a.k.a. cranial-caudal) directions were measured using digital calipers accurate to 0.01 mm, with a precision of ±0.005 mm (Mitutoyo, Aurora, IL, USA).

The L5 vertebrae were dissected free and the posterior elements were removed using a small clipper. The endplates of the vertebral body were cut parallel using a diamond wafering saw (Isomet, Buehler, Lake Bluff, IL, USA). Mechanical tests were performed in compression using a servohydraulic materials testing machine (810; MTS Corp., Minneapolis, MN, USA). All tests were done with the specimen submerged in 37°C saline using a displacement rate of 1 mm/s. The standard parameters of F_u, S, and U were calculated from the resulting load-displacement curves. Variation was noted in the specimen height resulting from both genetic differences and effects of making the endplates parallel. Because stiffness and work to failure are affected by specimen height, these parameters were normalized by dividing U by specimen height and by multiplying S by specimen height.

Statistical tests

Comparisons among the BXH RI strains and the B6 and C3H progenitors were made using analysis of variance (ANOVA) implemented by StatView software (Abacus Concepts, Berkeley, CA, USA). Post hoc comparisons between groups were accomplished using a Fisher's protected least significant difference test. Statistical significance was assumed at $p < 0.05$.

RESULTS

The average body weights for BXH RI strains were not significantly different from the B6 and C3H progenitor strains, with the exception of BXH-19 mice, which were significantly lighter than both progenitor strains (Table 1). The C3H mice had greater femoral strength (F_u), stiffness (S), and work to failure (U) compared with B6, as previously reported.[7] For most RI strains, femoral biomechanical properties fell between those of the progenitor strains (Fig. 1, note that mice from the BXH-2 RI strain developed early onset leukemia and were excluded from the study). However, five RI strains (BXH-3, -6, -7, -10, and -11) had

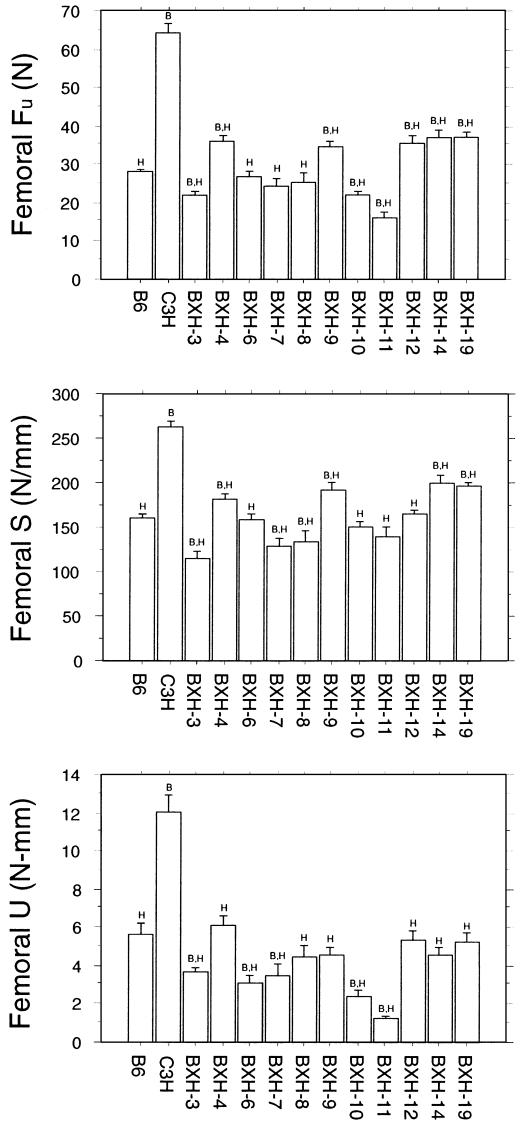

FIG. 1. Femoral biomechanical properties for B6, C3H, and BXH RI inbred strains of mice. Data presented are mean ± SEM (n = 7–13). B indicates a significant difference from B6 and H indicates a significant difference from C3H; F_u is ultimate force, S is stiffness, and U is work to failure.

significantly reduced U compared with either of the progenitor strains. Three of these strains (BXH-3, -10, and -11) also had significantly reduced F_u compared with progenitors. This indicates that these strains had femurs that are more fragile. For the most part, the increased femoral fragility was caused by a combination of reduced cortical thickness and reduced femoral width in the AP direction (Table 1). A remarkable exception was BXH-11, where cortical thickness and AP femoral width were not different from B6, yet F_u and U were only 60% and 25% of B6, respectively. These results suggest that BXH-11 mice had diminished femoral bone quality. We explored this issue further by calculating femoral toughness, which is a measure of the bone tissue integrity corrected for the size and shape of the bone.[16] Femoral toughness in BXH-11 mice (2.0 ± 0.2 mJ/mm^3) was significantly lower than all other RI strains ($p < 0.001$) and considerably less than the toughness of B6 (8.1 ± 0.5 mJ/mm^3; $p < 0.0001$) or C3H (17.2 ± 0.6 mJ/mm^3; $p < 0.0001$) progenitors.

There was variation among the strains in femoral geometry. Femoral length was not significantly different between C3H and B6 progenitor strains but varied greatly among BXH RI strains (Table 1). In particular, mice from BXH-4, -9, and -14 strains had femoral lengths that exceeded those from either progenitor strain, whereas mice from the BXH-10 strain had significantly shorter femurs. Femoral width varied greatly among the progenitor and RI strains, particularly in the ML direction as presented in Table 1. Femurs from the B6 progenitor mice were 15% wider in the ML dimension compared with C3H measurements. Among the RI strains, ML width values distributed rather uniformly as intermediate, B6-like, or C3H-like.

Total vBMD values for femurs were higher in C3H mice, compared with B6 (Table 2). The total femoral vBMD values for BXH RI strains fell between those of the progenitor strains and in all instances were significantly different from both progenitors. The femoral cortical BMC values for the RI strains tended to segregate into low B6-like levels (BXH-3, -7, and -10), intermediate levels (BXH-6, -8, -11, and -12), or high C3H-like levels (BXH-4, -9, -14, and -19).

There were no differences in vertebral bone strength (F_u) between these 8-month-old C3H and B6 mice, yet there was considerable variation in bone strength among the BXH RI strains. Four RI strains (BXH-3, -7, -10, and -12) had significantly weaker vertebrae than either B6 or C3H progenitors, while one RI strain—BXH-4—had significantly greater vertebral bone strength than both progenitors (Fig. 2). Normalized stiffness ($S*h$) and normalized work to failure (U/h) tended to distribute more normally with values similar to, as well as between, those of the B6 and C3H progenitors.

The total vBMD values for L5 vertebrae distributed differently from vBMD in femurs: five of the BXH RI strains (BXH-3, -7, -8, -10, and -11) were statistically below both progenitor strains, whereas BXH-4, -6, -9, and -19 were like the B6 and C3H progenitors, and only BXH-14 exceeded the high-density C3H value. C3H mice had substantially more vertebral cortical BMC when compared with B6 values. The vertebral cortical BMC (Table 2) showed that six of the RI strains (BXH-3, -6, -7, -8, 10, and -11) had significantly lower BMC values than both progenitor strains and two RI strains (BXH-9 and -12) were statistically identical with B6 progenitors, whereas three RI strains (BXH-4, -14, and -19) showed BMC values like that of the C3H progenitors. None of the BXH RI strains had vertebral BMC values statistically greater than C3H mice.

μCT analyses revealed marked differences between the vertebrae with highest (BXH-4) and lowest (BXH-3) bone strength (Fig. 3). BXH-4 had superior bone microstructure compared with BXH-3 and progenitor strains, including significantly higher trabecular bone volume and thickness (Table 3). Both BXH-3 and C3H mice had poor trabecular bone structure with reduced Tb.N and increased Tb.Sp compared with B6 or BXH-4 mice. There were no significant differences in trabecular structure between BXH-3 and C3H; however, the vertebral bone strength for BXH-3 was

TABLE 2. BONE PARAMETERS DETERMINED BY pQCT FOR THE FEMUR AND LUMBAR VERTEBRAE FOR B6, C3H, AND BXH RI STRAINS OF MICE

Strain	n	*Femoral total vBMD (mg/mm³)*	*Femoral cortical BMC (mg)*	*Midvertebral total vBMD (mg/mm³)*	*Midvertebral cortical BMC (mg)*
B6	8	0.459 ± 0.009[a]	7.77 ± 0.25[a]	0.228 ± 0.007[a]	0.45 ± 0.07[a]
C3H	10	0.680 ± 0.010[b]	11.9 ± 0.83[b]	0.240 ± 0.007[b]	0.69 ± 0.06[b]
BXH-2[c]					
BXH-3	10	0.511 ± 0.013[a,b]	6.78 ± 0.43[a]	0.189 ± 0.008[a,b]	0.09 ± 0.02[a,b]
BXH-4	11	0.601 ± 0.006[a,b]	12.7 ± 0.16[b]	0.246 ± 0.008	0.57 ± 0.07
BXH-6	13	0.602 ± 0.012[a,b]	9.69 ± 0.41[a,b]	0.220 ± 0.006	0.29 ± 0.05[a,b]
BXH-7	8	0.524 ± 0.012[a,b]	7.61 ± 0.38[a]	0.200 ± 0.009[a,b]	0.19 ± 0.03[a,b]
BXH-8	10	0.548 ± 0.016[a,b]	9.95 ± 0.58[a,b]	0.213 ± 0.007[a,b]	0.20 ± 0.04[a,b]
BXH-9	8	0.585 ± 0.007[a,b]	11.0 ± 0.46[b]	0.229 ± 0.006	0.39 ± 0.05[a]
BXH-10	10	0.551 ± 0.004[a,b]	7.01 ± 0.11[a]	0.188 ± 0.004[a,b]	0.10 ± 0.01[a,b]
BXH-11	7	0.522 ± 0.008[a,b]	9.66 ± 0.46[a,b]	0.189 ± 0.005[a,b]	0.20 ± 0.04[a,b]
BXH-12	10	0.594 ± 0.008[a,b]	9.50 ± 0.36[a,b]	0.226 ± 0.007	0.32 ± 0.05[a]
BXH-14	9	0.613 ± 0.013[a,b]	11.2 ± 0.40[b]	**0.279 ± 0.010**[a,b]	0.82 ± 0.11[b]
BXH-19	8	0.604 ± 0.008[a,b]	10.9 ± 0.33[b]	0.256 ± 0.010[b]	0.67 ± 0.07[b]

Data are presented as mean ± SEM.
Boldface indicates significantly greater than both C3H and B6; underline indicates significantly less than both C3H and B6.
[a] Significantly different from C3H.
[b] Significantly different from B6.
[c] BXH-2 developed early onset leukemia and were excluded from the study.

only 54% of C3H. These results apparently are caused by the very low vertebral cortical BMC of BXH-3, compared with C3H (Table 2). B6 vertebrae were shorter than those from C3H, whereas C3H vertebrae were more narrow. The selected BXH RI strains tended to retain the height of C3H and the width of B6 (Table 3).

Because of the independent variation of cortical and trabecular bone microstructures among progenitor and RI strains, the variance in vertebral strength was not reflected consistently in the femoral strength (Fig. 4). The solid symbols in Fig. 4 denote the inbred strains that are particularly useful to illustrate the point that genetic regulation of vertebral strength is sometimes independent of regulation of femoral strength. The BXH-12 and BXH-4 strains showed a difference in vertebral strength of almost 2-fold with no corresponding difference in femoral strength. Likewise, the B6 and C3H strains showed a 2.2-fold difference in femoral strength with no difference in vertebral strength.

DISCUSSION

It is widely recognized that loss of bone strength is accompanied by a corresponding rise in risk for osteoporotic fracture. Critical insight to osteoporotic fracture will follow from a better understanding of factors regulating biomechanical properties. In this report, we have hypothesized that factors associated with bone strength (i.e., BMD, size, and microstructure) have heritable components. To test this concept, we exploited a unique genetic model system in the form of the BXH RI strains derived from B6 and C3H progenitors known to differ in many aspects of bone biology.[6,7,17–22] By phenotyping each BXH RI strain for the traits that differ between the B6 and C3H progenitors, analyses of resultant patterns yield insights about genetic regulation underlying each trait. The evidence reported here shows a remarkable complexity to the biological basis for differences in bone strength between B6 and C3H mice.

The segregation patterns of values of biomechanical properties for BXH RI femurs lead to the following conclusions. First, none of the investigated properties segregated the RI strains into subsets that simply resembled either the B6 or the C3H progenitors. In the absence of such a simple segregation pattern, we conclude that each of the phenotypes represented by F_u, S, and U of these skeletal sites is regulated by more than one gene. The modest numbers of RI strains available in this BXH set will not support linkage analyses of traits with more than one gene. Therefore, we have not compared the BXH RI strain distribution patterns recorded for any of the phenotypes reported here with published data in search of single genes. Second, several BXH RI had significantly lower femoral F_u and U values than those found in the low-strength B6 strain and none of the BXH RI strains achieved values that approached those observed in the high-strength C3H strain. This phenomenon of transgenesis suggests that (a) the C3H strain carries genetic alleles contributing to low bone strength and (b) the high bone strength of C3H may result from the interaction of specific genes in C3H but, by chance, none of the BXH RI strains contain the correct combination of C3H genes supporting high femoral strength.

Femoral geometry (overall length and ML and AP widths) also showed complex genetic regulation. Although the length of the B6 and C3H femurs were not different, half of the BXH RI strains had femurs that were either shorter or longer than the progenitor strains. Middiaphyseal ML

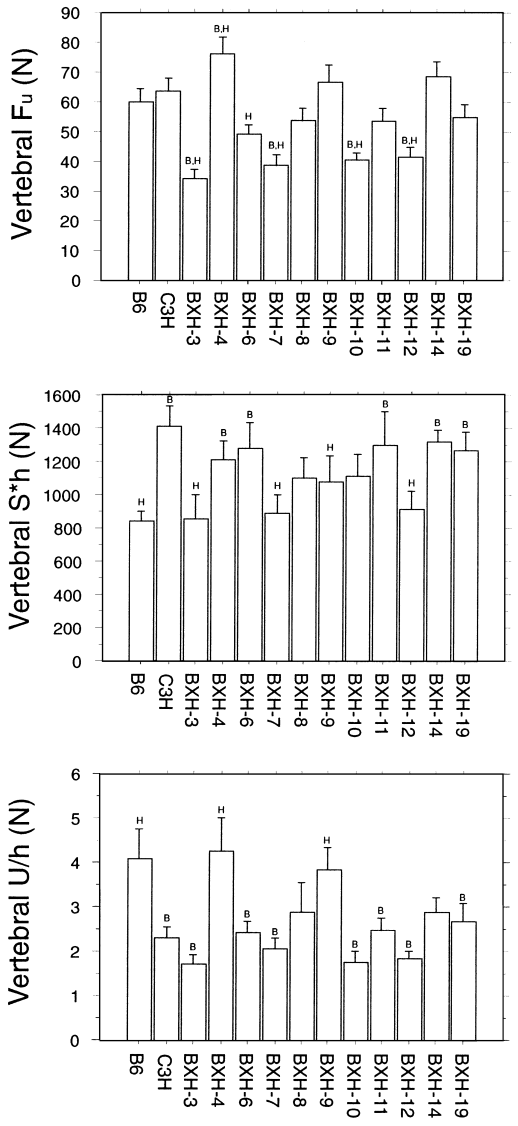

FIG. 2. Vertebral biomechanical properties for B6, C3H, and BXH RI inbred strains of mice. Data presented are mean ± SEM (n = 7–13). B indicates a significant difference from B6 and H indicates a significant difference from C3H; F_u is ultimate force, S^*h is normalized stiffness, and U/h is normalized work to failure.

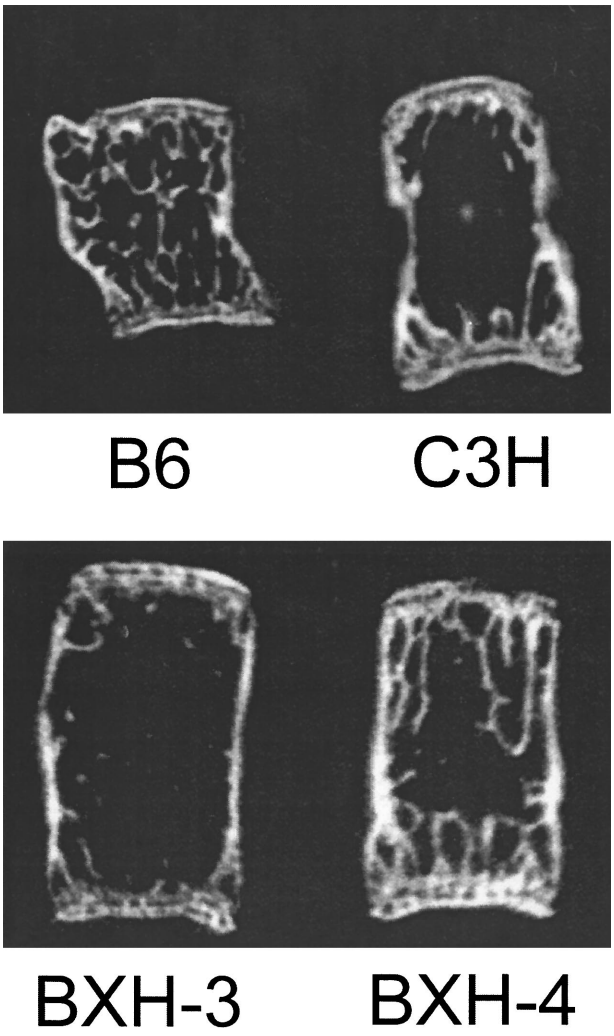

FIG. 3. μCT section of the L5 vertebral body for progenitor strains (B6 and C3H) and the BXH RI strains with maximum (BXH-4) and minimum (BXH-3) vertebral strength. The images were measured 3-D providing a 17-μm isotropic voxel size.

widths distributed into either progenitor and intermediate categories, while the majority of the RI strain AP widths were less that those of the smaller B6 progenitor. As was noted for the femoral strength properties, the thicker femoral cortex of the C3H femurs was not observed in the BXH RI strains. Collectively, these data argue cogently for complex genetic regulation of parameters for femoral size.

The RI strain BXH-11 was remarkable because apparently it had robust femoral shaft geometry, yet with weaker and more fragile femurs. The femoral toughness of the BXH-11 mice was substantially lower than other strains, indicating a deficit in bone quality. These results suggest that one of the B6 and C3H progenitor strains contains a genetic allele(s) that influences some aspect of bone quality. It is unclear, based on the present data, exactly what alteration in the BXH-11 femoral tissue caused the deficit in toughness; nevertheless, the BXH RI strains may be useful for studying genetic influences on bone quality.

As was true for biomechanical and morphology properties, segregation of total vBMD and cortical BMC for femurs was consistent with conclusions of polygenic regulation. Femoral vBMD values from the BXH RI strains were distributed between those of the progenitor strains and no BXH RI replicated either progenitor strain value, again arguing for the fact that combinations of specific genes are required to achieve the respective densities of the progenitor strains.

The biomechanical properties of the L5 vertebrae, particularly F_u, in BXH RI strains, showed a greater range of variation than either B6 or C3H progenitors, suggesting regulation by multiple genes. Likewise, patterns for vertebral vBMD values were noteworthy in that the B6 and C3H progenitors were marginally different from each other, whereas the BXH RI strains showed a much greater range of

TABLE 3. μCT ANALYSIS OF LUMBAR VERTEBRAE FOR B6, C3H, AND BXH RI STRAINS WITH THE GREATEST (BXH-4) AND LEAST (BXH-3) VERTEBRAL STRENGTH (MEAN ± SEM)

Variable	*Inbred strain*			
	B6	*C3H*	*BXH-3*	*BXH-4*
		Vertebral geometry		
Height (mm)	2.60 ± 0.05[a]	3.22 ± 0.10[b]	3.51 ± 0.11[b]	3.45 ± 0.22[b]
Width (mm)	1.95 ± 0.13[a]	1.66 ± 0.08[b]	2.03 ± 0.06[a]	1.98 ± 0.06[a]
		Trabecular structure		
BV/TV (%)	24.1 ± 1.3[a]	16.0 ± 1.0[b]	10.8 ± 1.3[a,b]	**30.0 ± 3.6**[a,b]
BS/TV (mm^{-1})	8.5 ± 0.2[a]	4.4 ± 0.2[b]	3.3 ± 0.4[a,b]	6.7 ± 0.2[a,b]
Tb.N (mm^{-1})	5.1 ± 0.2[a]	2.8 ± 0.1[b]	2.6 ± 0.2[b]	4.1 ± 0.2[a,b]
Tb.Th (μm)	61 ± 2	69 ± 4	73 ± 1	**93 ± 8**[a,b]
Tb.Sp (μm)	199 ± 9[a]	388 ± 11[b]	415 ± 38[b]	255 ± 13[a,b]

Boldface indicates significantly greater than both C3H and B6; underline indicates significantly less than both C3H and B6.
[a] Significantly different from C3H.
[b] Significantly different from B6.

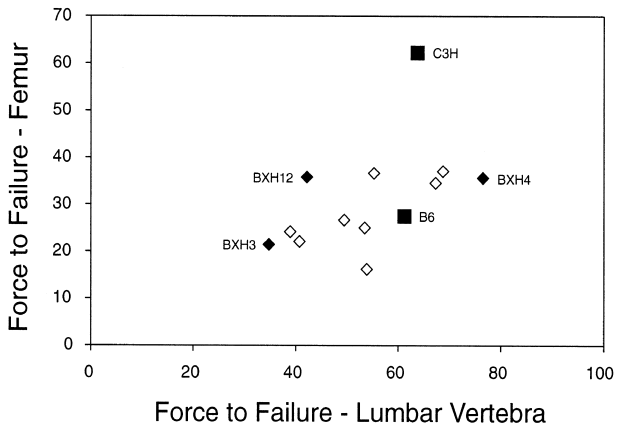

FIG. 4. Relationship between femoral strength (ultimate force) and vertebral strength for B6, C3H, and BXH RI strains. The progenitor strains (B6 and C3H) show a large variation in femoral strength with no difference in vertebral strength. Likewise, selected BXH RI strains (BXH-3, BXH-4, and BXH-12) show large variation in vertebral strength with little or no variation in femoral strength.

values. This distribution of values supports the conclusion that both progenitor strains have independent subsets of genes that yield very similar mean values. Recombination of those genes in the BXH RI strains yields a broader range of values.

Not only is vertebral vBMD under genetic control, but it appears that the distribution of vertebral mineral into cortical and trabecular compartments also is regulated genetically. Trabecular structure in the lumbar vertebra tended to vary independently of cortical BMC of the vertebra, and both trabecular and cortical bone contributed to vertebral strength. BXH-4 mice had alleles that contributed to superior vertebral strength by increasing Tb.N, reducing Tb.Sp, improving Tb.Th, and increasing cortical BMC, while BXH-3 vertebrae were osteopenic with diminished trabecular and cortical bone structure. As for the progenitor strains, B6 had good trabecular bone structure and poorer cortical BMC, whereas C3H had the opposite combination, resulting in intermediate biomechanical properties. However, the correlation between vertebral structure and biomechanical properties was not perfect because the RI strain with the highest total vertebral vBMD and cortical BMC (BXH-14) did not have the greatest vertebral strength. This observation suggests that although the measured densitometry and geometric variables explain much of the variation in vertebral strength, other important structural variables that contribute to vertebral integrity remain.

It was noteworthy that the variation in vertebral strength was not correlated consistently with femoral strength. In particular, the B6 and C3H strains indicated over a 2-fold difference in femoral strength with no difference in vertebral strength while BXH-12 and BXH-4 strains were vastly different in vertebral strength with no corresponding difference in femoral strength. These results strongly suggesting site-specific genetic regulation of bone strength.

Although the ultimate goal of our studies is to better understand the pathogenesis of human osteoporosis, the mouse model used does not mimic osteoporotic humans. We chose 8-month-old female mice, which model middle-aged, premenopausal women. Our aim was to study the variation in bone microstructure and biomechanical properties in animals with peak bone density. In women, the peak bone density achieved during childhood, adolescence, and young adulthood protects against osteoporosis in later years.[23] A further limitation of mice in the study of skeletal biology is their lack of osteonal remodeling in compact bone tissue. This key difference between mice and humans can lead to confounding results. These limitations not withstanding, mice remain one of the best animal models for studying genetic influences on human diseases and disorders, skeletal or otherwise.

In conclusion, we found that the regulation of femoral and vertebral biomechanical properties in BXH RI mice involved multiple genes and was both site specific and com-

partment specific. Independent genetic regulation of femoral geometry, cortical BMC, and vertebral trabecular microstructure contributed to the variation in biomechanical properties among the strains. The genetic control of bone strength appears to be rich and complex with many puzzles to be solved before the genetic mechanisms are understood.

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