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Quantitative Trait Loci Analysis of Structural and Material Skeletal Phenotypes in C57BL/6J and DBA/2 Second-Generation and Recombinant Inbred Mice

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Abstract

QTL analyses identified several chromosomal regions influencing skeletal phenotypes of the femur and tibia in BXD F₂ and BXD RI populations of mice. QTLs for skeletal traits co-located with each other and with correlated traits such as body weight and length, adipose mass, and serum alkaline phosphatase.

Introduction— Past research has shown substantial genetic influence on bone quality, and the impact of reduced bone mass on our aging population has heightened the interest in skeletal genetic research.

Materials and Methods— Quantitative trait loci (QTL) analyses were performed on morphologic measures and structural and material properties of the femur and tibia in 200-day-old C57BL/6J × DBA/2 (BXD) F₂ (second filial generation; $n = 400$) and BXD recombinant inbred (RI; $n = 23$ strains) populations of mice. Body weight, body length, adipose mass, and serum alkaline phosphatase were correlated phenotypes included in the analyses.

Results— Skeletal QTLs for morphologic bone measures such as length, width, cortical thickness, and cross-sectional area mapped to nearly every chromosome. QTLs for both structural properties (ultimate load, yield load, or stiffness) and material properties (stress and strain characteristics and elastic modulus) mapped to chromosomes 4, 6, 9, 12, 13, 15, and 18. QTLs that were specific to structural properties were identified on chromosomes 1, 2, 3, 7, 8, and 17, and QTLs that were specific to skeletal material properties were identified on chromosomes 5, 11, 16, and 19. QTLs for body size (body weight, body length, and adipose mass) often mapped to the same chromosomal regions as those identified for skeletal traits, suggesting that several QTLs identified as influencing bone could be mediated through body size.

Conclusion— New QTLs, not previously reported in the literature, were identified for structural and material properties and morphological measures of the mouse femur and tibia. Body weight and length, adipose mass, and serum alkaline phosphatase were correlated phenotypes that mapped in

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close proximity of skeletal chromosomal loci. The more specific measures of bone quality included in this investigation enhance our understanding of the functional significance of previously identified QTLs.

Keywords

quantitative trait loci; bone mechanics; adipose mass; body weight; alkaline phosphatase

INTRODUCTION

THE IMPACT OF reduced bone mass on our aging population has heightened the interest in research on the genetic influence of bone quality. Past research on twins has shown that genes may influence as much as 75% of the variance in BMD.⁽¹⁾ Until quite recently, genetic research focused on candidate genes with identifiable polymorphisms. Such an approach requires a prior hypothesis for candidate genes and often produces inconsistent results for continuously distributed phenotypes, including skeletal measures. The problem stems from complex polygenic control and the confounding effects of environment. Extrinsic factors such as nutrition, lifestyle, and skeletal loading are impossible to control over substantial time periods in human populations.

Recombinant inbred (RI) strains and (second filial F₂ generation) mice are commonly used in quantitative trait loci (QTL) analysis to detect genetic loci associated with phenotypic traits that show continuous distribution patterns. The use of experimental mice in environmentally controlled, pathogen-free facilities can reduce the phenotypic variance associated with environment, nutrition, and disease, thereby increasing statistical power.

QTL analysis typically employs F₂ generation and recombinant inbred mice. An F₂ population is produced by first mating two highly inbred progenitor strains such as C57BL/6 and the DBA/2 strains. The inbred progenitor strains are homozygous (in like allelic state) for nearly all genes. The two progenitor strains will be in different allelic state for some genes and in like allelic state for others. The resulting offspring from the mating of the highly inbred progenitor strains are the F₁ or first filial generation. At each locus, a single allele is inherited from each parent or each progenitor strain. The F₁ generation is a hybrid and will be heterozygous (having different alleles at a locus) for all loci where the progenitor strains had different allelic states (from each other). This means that the genome is known for the F₁ generation to the extent that the progenitor strain genomes are known, and they are heterozygous at every locus where the two progenitor strains differ. The F₁ generation is crossed, and the resulting offspring are the F₂ or second filial generation. One-quarter of the mice in the F₂ generation will be homozygous in one state, one-quarter will be homozygous in the other state, and one-half will be heterozygous. Animals from the F₂ generation are inbred under a strict regimen of mating, and the new inbred strains resulting after 20 generations of brother and sister inbreeding are the RI strains. The alleles for the RI strains will be the same as one of the progenitor strains and will have become fixed at each locus because of the re-inbreeding. Within the RI series, several RI strains are produced, and these strains can vary from each other as to which homozygous progenitor alleles are present along the genome. The resulting inbred strains from the two progenitor strains (C57BL/6 and DBA/2) are classified as the BXD series. The resulting BXD series is a mixture of the two progenitor strain genomes or a “recombination” of the progenitor genomes.^(2,3)

QTL analysis allows one to examine a phenotype within the context of the system. The limitation of this is that, when a QTL is found, we cannot state for certainty where in the complex pathways of the system it is exerting its influence. A frequent criticism of QTL analysis is that it is unlikely to lead to discovery at the gene level. However, significant

information can be extracted on the basis of QTL results without identifying specific genes. Responses to treatment or varying environments can be investigated based on the QTL effect. In this regard, congenic strains provide the opportunity to investigate a QTL with allele genotypes of one parental strain on the background of the contrasting parental strain, and genotypically selected mice allow for the investigation of high and low QTL genotypes on a heterogeneous background. The potential for extracting useful information about how a complex phenotype responds given the identity of a QTL and the accompanying genetic background is valuable in and of itself. There are also multiple strategies to narrow the QTL location and thereby locate specific genes. One approach is to generate an advanced intercross line, which is an extension past the F₂ to the F₃ and F₄ generations and so on. With each generation, the number of recombinations is doubled, thereby reducing the informative marker intervals, which allow QTLs to be more precisely mapped.

Several investigators have used genome-wide scans to search for QTLs for skeletal phenotypes.^(4–8) Many QTLs with similar locations have been identified across studies, lending credibility to the technique. In several such studies, similar phenotypes were often used, but the method of measurement varied (e.g., pQCT versus DXA for BMD measures). Other methods of quantifying skeletal quality include mechanical testing of the midshaft of long bones in bending and torsion, shear testing of the femoral neck, and compression testing of vertebrae. Each of these methods for measuring skeletal phenotypes can offer distinct insight into bone quality and often identify QTLs specific to that skeletal measure. However, it is also the case that some QTL positions for different skeletal phenotypes overlap and provide replication across studies for the same QTL.

Many QTLs identified for skeletal phenotypes including total BMD, structural parameters from mechanical testing, and morphologic measures may be related to body size and overall geometric differences. Studies have reported strong correlations among muscle, bone, and body size phenotypes, and these correlations add to the difficulty in identifying genetic influence on skeletal traits independent of these correlated phenotypes.^(7,9–12) Such relationships could be because of an overall scaling effect, where larger animals have bigger muscles and bones. Skeletal differences could also be the result of bone adaptation to differences in the loads imposed by differences in body weight, the increased mechanical demand produced by larger muscles, or differences in bone's response to mechanical loading. An equally plausible alternative hypothesis is that bone growth and development are primary and therefore dictate muscle mass and body size.

The intent of this study was to identify skeletal QTLs that result from differences associated with body size, as well as QTLs that function irrespective of body size. To identify skeletal QTLs independent of body size, skeletal measures were normalized for body size to remove the correlation with body weight and body length. Material properties of bone, as well as morphologic and structural properties, were assessed.

MATERIALS AND METHODS

Animal husbandry

Ten male and 10 female mice from 23 BXD RI strains, as well as 200 male and 200 female F₂ animals derived from C57BL/6J and DBA/2 progenitor strains, were examined at 200 days of age (adults). Animal breeding and maintenance were conducted in a barrier facility maintained by The Center for Developmental and Health Genetics at The Pennsylvania State University. Mice were weaned into like-sex sibling groups at about 25 days of age, with four animals per cage. They were fed a diet of autoclaved Purina Mouse Chow 5010 (content: 1.0% calcium, 0.67% phosphorus, 0.22% magnesium, and 4.4 IU/g vitamin D) ad libitum, designed to be equivalent to Purina 5001 (content: 0.95% calcium, 0.67% phosphorus, 0.21%

magnesium, and 4.5 IU/g vitamin D) after autoclaving. The barrier facility was maintained under positive air pressure with a temperature- and humidity-controlled environment and a 12-h light/dark cycle. All procedures complied with and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Phenotypic measures

Serum alkaline phosphatase—Serum samples were taken from a ventral tail incision at 150 and 178 ± 14 days of age. Blood samples were centrifuged, frozen, and sent to the Clinical Blood Chemistry Laboratory at Wake Forest Medical Center for analysis. Serum from the two samples was analyzed for alkaline phosphatase using a Chem 1 Blood Analyzer (Bayer, Tarrytown, NY, USA), and results were averaged for use in QTL analyses.

Tissue harvest and gross dimensional measurements—Animal weight was recorded before euthanasia. Nose-to-anus length was recorded immediately after death by cervical dislocation. Epididymal fat pads (in males) and uterine fat pads (in females) were extracted and weighed to 0.1 g accuracy on an electronic balance. The right hind limb of each animal was harvested, and the femur and tibia were cleaned and stored at -20°C until mechanically tested.

At the time of testing, the bones were thawed at ambient temperature. A digital caliper accurate to 0.01 mm was used to measure femoral length and femoral width at the center of the diaphysis in both the sagittal and coronal planes and epiphyseal width in the coronal plane. Femoral head and neck diameter were also measured. The tibia was measured similarly, except that the proximal, rather than distal, epiphyseal width was measured.

Flexural testing of the femur and tibia—Femora and tibias were tested to failure in three-point bending in an MTS MiniBionix 858 testing apparatus (MTS Systems, Eden Prairie, MN, USA) using support spans of 8 (femur) and 10 mm (tibia) and a displacement rate of 1 mm/minute. Femurs were consistently oriented so that the nosetip was posteriorly directed in respect to the diaphysis. A small section of the anterior flare of the proximal tibia was carefully removed to stabilize the bone on the support span where it was loaded with the anterior cortex in tension. All testing was executed with the bones wet and at ambient temperature. Yield load, yield displacement, energy absorbed at yield, failure load, failure displacement, energy absorbed at failure, and stiffness were determined.

Shear testing of the femoral neck—The proximal fragment of the femur was used to measure the functional strength of the intertrochanteric region and femoral neck. The proximal femur was embedded vertically to 3 mm below the top of the femoral head in a mounting pot containing low melting point alloy and secured in the MTS materials testing machine. The femoral head was loaded, parallel to the femoral shaft, at a rate of 1 mm/minute, and structural properties were derived.

Compositional analysis—After mechanically testing the femoral shaft and neck, the femoral fragments were wiped clean by visual inspection to ensure that the bone fragments were free of any remaining metal particles from the shear test of the femoral neck. Femoral bone fragments were ashed in a muffled furnace at 800°C for 24 h to determine femoral ash mass. After flexural testing of the tibia, the distal fragment was dried in a vacuum oven at 100°C for 24 h and ashed at 800°C for 24 h. Percentage water, organic, ash, and mineralization were obtained based on the wet, dry, and ash mass of the tibia.

Tissue processing and histomorphometry—The proximal tibia and previously ashed distal femur were embedded in methyl methacrylate using a three-step three-solution approach.

(13) A diamond wire saw (Delaware Diamond Knives, Wilmington, DE, USA) was used to cut 150-mm diaphyseal cross-sections. Digital images of each cross-section were collected using a light microscope equipped with a 4× objective and a high-resolution CCD video camera interfaced to a personal computer. Images were captured using NIH IMAGE software (version 1.61; NIH, Bethesda, MD, USA). Total area within the periosteal surface, medullary area within the endosteal surface, cortical area, centroid of the cross-section, cross-sectional moment of inertia (CSMI), average cortical width, and distance from the centroid to the tensile periosteal surface were calculated using a MATLAB program (version 6.5 release 13; Math-Works).

Material properties—Cross-sectional data, together with data from the flexural tests, were used to calculate the yield and failure stress ($\sigma = FLc/4I$), strain ($\epsilon = 12cd/L^2$), and elastic modulus ($E = FL^3/d48I$) of each diaphysis. Equations for material properties were derived using beam theory, where σ is the bending stress, F is yield or failure load, L is unsupported span length, c is the distance from the cross-section centroid to the tensile periosteal surface, I is the cross-sectional moment of inertia, and d is the machine displacement.

Genotyping—Genotypes for the RI lines, consisting of 672 microsatellite markers in each BXD strain, were obtained from Williams et al. for use in the subsequent QTL analyses.⁽¹⁴⁾ F₂ animals were genotyped using 96 microsatellite markers distributed throughout the genome (not including the Y chromosome) with an average spacing of 15–20 cM. Marker analyses were conducted on purified DNA samples procured from tail snips using an automated, fluorescence-based detection system described in detail in Vandenberg et al.⁽¹⁵⁾

QTL analyses—Separate sex-specific QTL analyses were performed on both the RI and F₂ cohorts to locate chromosomal regions influencing phenotypic variables. QTL analyses were also performed on male and female data combined, correcting for sex differences. RI QTL analyses were performed using strain means. All phenotypes were screened for normality, and when necessary, a log or square root transformation was used. All phenotypic measures were adjusted for body size through multiple regression of body weight and body length onto each phenotype, with the residuals constituting the adjusted phenotype. Subsequent QTL analyses were performed on the adjusted as well as the nonadjusted raw measures.

All analyses were conducted using QTL Cartographer.^(16,17) Both interval mapping and composite interval mapping were executed. Composite interval mapping allows for the control of genetic variance outside a window surrounding each test site along the genome, whereas standard interval mapping does not. Five markers were included as co-factors in the composite interval model. These markers were selected as the top five most significant sites outside a window of 10 cM of each test site. The significance rank was based on forward step-wise regression.

QTL significance levels are reported as LOD scores or base 10 logarithm of the odds in favor of linkage. The F₂ population was used to nominate QTLs, and the RI cohort was used for confirmation. QTLs that were nominated in the F₂ analyses at a LOD ≥ 4.3 and confirmed in the RI at a LOD ≥ 1.5 were considered confirmed significant linkage,⁽¹⁸⁾ whereas QTLs nominated in the F₂ analyses using a less stringent LOD score (2.8–4.2) and confirmed in the RI (LOD ≥ 1.5) were considered confirmed suggestive linkage. In addition, unconfirmed significant QTLs were reported that showed significant LOD scores in either the F₂ (LOD ≥ 4.3) or RI (LOD ≥ 3.3) analysis, but did not replicate in the complementary analysis. QTLs that did not meet any of the three criteria outlined above, but were nevertheless interesting because of their genomic location, are reported as “potential QTLs.” These sites either met the suggestive criterion in the F₂ analysis or were nominated in the F₂ cohort at LOD scores of 1.9–2.7 and subsequently confirmed in the RI (LOD ≥ 1.5).

RESULTS

QTL results from both F₂ and RI analyses are presented in Tables 1–4. Within each table, F₂ results are presented on the left, and RI results are presented on the right, indicating potential QTL confirmation between the two populations. QTLs identified within 30 cM between the F₂ and RI analyses are paired together in the tables.

Skeletal QTLs for structural size and shape measures such as length, width, cortical thickness, and area mapped to every chromosome (Tables 1–4; suggestive QTLs identified on chromosome 10 or chromosome X did not meet the criteria for inclusion in Tables 1–4). The majority of the QTLs identified in this study were found using combined data from both sexes, although many sex-specific QTLs, not found using combined data, were identified when data were analyzed for each sex separately. QTLs for both structural properties (load, displacement, or stiffness) and material properties (stress, strain, or elastic modulus) mapped to chromosomes 4, 6, 9, 12, 13, 15, and 18. QTLs specific for structural properties, but not material properties, were identified on chromosomes 1, 2, 3, 7, 8, and 17, whereas QTLs specific for skeletal material properties were identified on chromosomes 5, 11, 16, and 19. Many of the QTLs identified in this study correspond to QTL positions previously reported to be linked to BMD, thereby confirming and expanding on the functional significance of these sites.

Some skeletal QTLs mapped to positions close to QTLs for nonskeletal phenotypes, including body length, body weight, BMI, visceral adipose mass, and serum alkaline phosphatase. The co-localization of skeletal and body size QTLs could be an indication of a genetic effect mediated through body size. In many instances, after adjusting for body weight and length, the adjusted phenotype yielded a decreased LOD score, relative to the unadjusted counterpart. For example, in the unadjusted analyses, a QTL for body weight (LOD = 6.6) was found on chromosome 13 at 24 cM (Table 3), and a skeletal QTL for femur stiffness was found on the chromosome at 16 cM. When body weight and length were regressed onto femur stiffness, the adjusted LOD score became insignificant. In contrast, many highly significant skeletal QTLs were found using the adjusted phenotype, but were not found for the raw unadjusted phenotype. An example is found on chromosome 7 between 15 and 30 cM (Table 2). Initially, femur yield and ultimate load did not map to this region using interval mapping. However, after adjusting for body size, femur ultimate load mapped to 21.4 cM (LOD = 5.4), and femur yield load mapped to 30.5 cM (LOD = 4.5).

DISCUSSION

QTL analysis is essentially a genome-wide search for regions of chromosomes that influence the phenotype under investigation. Whereas QTL positions are often reported in centimorgans, indicating the position of the peak LOD score, the chromosomal region could include many genes, and the exact locations of the gene or genes influencing the quantitative trait are unknown. In this study, correlated phenotypes often mapped in close proximity to each other, indicating potential pleiotropic gene effects. However, the co-localization of correlated phenotypes to the same locus or chromosomal region is not definitive evidence that there is one gene controlling all the correlated phenotypes. Because of the substantial number of genes that could be present within the chromosomal region encompassing the QTL, the same region could include several tightly linked genes that influence the correlated phenotypes independently.

One of the strengths of this study is that it used two populations (F₂ and RI) of genetically manipulated mice, with the intent to identify QTLs in one population and confirm their presence in the second. QTL localization within 30 cM between the F₂ and RI analyses were listed on the same line in the result tables, indicating QTL confirmation under our criteria. Of the QTLs

that were confirmed, 31% were within 5 cM or less between the F₂ and RI QTL analyses and 68% were within 15 cM or less. However, it should be acknowledged that differences in marker density between the F₂ and RI analyses, as well as differences in map length because of map expansion of the RI genome, complicates localization comparisons between the two analyses.

The majority of skeletal QTL studies have focused on skeletal and/or whole body BMD, as measured by DXA or pQCT, as their phenotype. QTLs for BMD (whole body, femoral, or vertebral) have been mapped to every chromosome in the mouse genome, except for chromosome 10, and most of these BMD sites correspond to QTLs identified in this study for differing skeletal phenotypes.^(5–8,11,19–25) The proximal region of chromosome 6 was the only region identified in this study that has not previously been identified for BMD. Novel QTLs for tibial stiffness, tibial length, and femoral ultimate stress were mapped to this region and are unique to this study.

Many highly significant QTLs for skeletal morphology were found in this study, but identified sites of greatest functional interest were those for skeletal structural and material properties and nonskeletal phenotypes that co-localize to these sites. In many cases, QTLs influencing the mechanical performance of long bones were in close proximity to QTLs previously identified for BMD, showing that these genetic loci are important modulators of skeletal integrity at specific sites. QTLs identified in this study but not previously associated with a given functional phenotype are indicated with an *. QTLs for skeletal phenotypes that co-localize with QTLs reported in the literature for the same functional phenotype (e.g., cross-sectional area, moment of inertia, strength, or stiffness) but at a unique skeletal site (e.g., tibia versus femur or humerus) are indicated with a ^.

Skeletal QTLs often co-localized with QTLs for body weight, body length, and adipose mass. Bone, muscle, and fat are three primary contributors to overall body weight and are highly correlated, making it difficult to determine the function of these genetic loci. This co-localization could be caused by pleiotropic gene effects where the same gene is influencing these correlated phenotypes independent of each other or through causal pathways. Such interrelationships may be representative of genetic regulation of bone quality and additional insight can be gained by observing the difference in LOD score between the adjusted and raw phenotypes. A reduction in the LOD score of a particular skeletal phenotype after adjustment could be indicative of the genetic effect modulated through body size, whereas an increase in LOD score may suggest that a confounding effect was eliminated.

A potential limitation of the body length measurement used in adjusting the phenotypic data to body size is that length was measured after cervical dislocation. Cervical dislocation was performed to preserve brain tissue for subsequent neurophysiologic assays. As a reliability check of the nose to anus length measurement, equal numbers of animals within an RI strain were assigned either an A or B. Subsequent strain means for each A and B group were determined for each strain and the correlation of the strain means was examined as a measure of reliability. RI males and females yielded good correlations of $r = 0.92$ and $r = 0.82$ for males and females, respectively ($p < 0.001$). The high correlations suggest that the body length measurement was reliable as a within-study relational measure, despite its limitations imposed by cervical dislocation.

QTLs specific to skeletal structural properties

QTLs on chromosomes 1, 2, 3, 7, 8, and 17 were specific for structural properties in this study. The distal region of chromosome 1 from 60 to 100 cM has often been implicated in QTL studies of bone health. This study identified the same region, but the majority of mapped phenotypes were measures of bone size or the geometric distribution of its material.

A potential QTL for structural tibia stiffness, a parameter dependent on both geometry and material behavior, was identified on chromosome 1 at 89 cM. This QTL was confirmed “marginally” in the RI analysis within 1 cM of the F₂ analyses, although the LOD score did not meet the suggestive criterion. Femur stiffness was identified at 84.6 cM in the RI analyses with a LOD score of 4.3; however, it was not nominated in the analysis. This site was also F₂ linked to several morphologic measures including tibia CSMI and tibia medullary area (LOD = 4.5), but no linkages to material properties (i.e., elastic modulus, ultimate stress) were found. QTLs in this region of chromosome 1 have been reported for BMD of the femur at 95.8⁽¹¹⁾ and 81.6⁽⁵⁾ cM, whole body BMD at 74.1⁽²²⁾ and 95.0⁽⁶⁾ cM, and femur BMD and breaking strength at 82–103.8 cM.⁽²¹⁾ These data suggest that this site modulates architectural features rather than material behavior or mineralization.

Interestingly, in the F₂ analyses, adipose mass mapped in close proximity to skeletal QTLs at 91 cM on chromosome 1 with a LOD score of 6.1, although this finding was not confirmed in the RI analysis. For the adipose mass QTL, the *DBA/2* allele was associated with increasing adiposity, and the *C57BL/6* allele was associated with increasing femur width, and increasing stiffness, CSMI, medullary area, and sagittal width for the tibia. These results are consistent with a model of cortical expansion compensating for end-osteal resorption in the B6-like mice compared with the D2-like mice having increased adipose mass and decreased bone resorption, perhaps resulting from the protective effects of adipose. Reid et al.⁽²⁶⁾ reported a positive correlation between adipose mass and BMD, independent of body weight. The protective effects of fat mass on BMD may involve leptin, a hormone expressed by adipocytes. Leptin has been shown to be positively correlated with body fat, and in vitro studies have shown that leptin can stimulate osteoblast and inhibit osteoclast differentiation.⁽²⁷⁾

The results from Shultz et al.⁽²⁰⁾ for femur BMD and circumference in congenic sublines indicated two QTLs at 36.9–49.7 and 73.2–100.0 cM on chromosome 1. Our study identified a “potential” QTL for femur cortical area (LOD = 7.5) at 47.6 cM in the RI analyses. Tibia cortical thickness and femur sagittal width also mapped to this general region.

Our results (Table 1) and the foregoing discussion suggest that two distinct regions on chromosome 1 link to skeletal traits. Both of these regions overlap with QTLs identified for hematopoietic stem cell frequency that map near the genes *Adprp* (98.6 cM) and *Acrp* (52.3 cM).⁽²⁸⁾ Marrow isolates from DBA/2 mice have been found to have an 11-fold higher frequency of hematopoietic stem cells compared with C57BL/6 mice.⁽²⁸⁾ Leptin has been shown to induce hemopoietic cell proliferation, differentiation, and activation.⁽²⁹⁾ The localization of skeletal measures, adipose mass, and stem cell frequency to the same region on chromosome 1 suggests that these three phenotypic measures are causally related, perhaps through the actions of leptin.

The region on chromosome 2 from 80 to 108 cM was also specific for structural properties and contained QTLs unique to this study for femur and tibia cortical thickness and ultimate load and femur yield load (LOD = 5.5). While this region has been linked to BMD,^(5,7,22) this study is the first to identify QTLs for bone strength and cortical thickness in the region of 80–108 cM. Li et al.⁽³⁰⁾ reported a QTL for femur breaking strength and periosteal circumference at 55 cM in an MRL × SJL F₂ cross. Several other studies have also reported skeletal QTLs in the region from 40 to 60 cM; thus, it seems that there may be two separate regions on chromosome 2 influencing skeletal measures.

The site on chromosome 3 at 2–30 cM contains QTLs for tibia ultimate load and serum alkaline phosphatase. This region was previously used in the construction of a congenic strain by Shultz et al.⁽²⁰⁾ and was significant for femur BMD and periosteal circumference. Although Li et al.⁽³⁰⁾ identified an epistatic effect between chromosomes 3 and 12 for femoral breaking strength,

our study is the first to identify the direct effect of QTLs for femoral neck shear load (LOD = 4.2), and femur shaft ultimate load at 57–68 cM.

Unique QTLs for structural measures on chromosome 7 were identified at 15–30 cM for femur yield (LOD = 5.5) and ultimate load. Interval mapping results from the combined analyses identified QTLs for femur ultimate load (LOD = 5.4) and visceral adipose mass (LOD = 7.6), both at 21.4 cM. This region has been previously linked to skeletal measures, including BMD of the spine,⁽¹⁹⁾ femoral BMD, cortical thickness,⁽⁷⁾ cross-sectional area,⁽³¹⁾ and whole body BMD.⁽²⁵⁾ Our study is the first to localize a QTL for femoral shaft strength to this region, confirming the functional importance of this site.

This study identified the region from 30 to 60 cM on chromosome 8 to be associated with skeletal phenotypes. This region has been previously identified for femoral strength and structural properties such as cross-sectional area and moment of inertia.^(21,30–32) Our study has additionally identified QTLs for femoral work to yield, cortical thickness, and tibia ultimate load and displacement.

Numerous structural properties mapped to chromosome 17 in both the F₂ and RI analyses. However, the centimorgan position between the two analyses did not agree. In general, the RI analyses identified the region from 3 to 52 cM, with the majority of skeletal traits mapping between 3 and 30 cM, and the F₂ analyses identified the region from 18 to 56 cM, with the majority of traits mapping between 44 and 56 cM. Corva et al.⁽³³⁾ reported QTLs for carcass ash mass and femur length at 48 cM on chromosome 17, and several studies have reported skeletal measures including femur BMD^(21,23,25) and femur breaking strength,⁽²¹⁾ all mapping between 4 and 12 cM.

QTLs specific to skeletal material properties

The LOD scores resulting from analyses on material properties were less pronounced than those for structural measures. This outcome could be caused by variability in the diaphyseal cross-sections. Femur and tibia diaphyses were sectioned to obtain a complete cross-section as close as possible to the bending failure site. However, this location varied by necessity, depending on damage through the section. In several instances, complete cross-sections were unavailable, which decreased the statistical power even further. In addition, femoral shafts were embedded and sectioned after the bone was ashed, which may have altered the geometry. However, any effect from ashing the bone should be systematic across individuals and should not appreciably affect QTL analysis, which is based on variability across F₂ individuals or RI strains. Nevertheless the material properties derived using these data should be interpreted with caution.

Despite these limitations, QTLs specific to skeletal material properties were identified on chromosomes 5, 11, 16, and 19. Although structural mechanical properties did not map to these chromosomes, many QTLs for morphologic phenotypes often overlapped. Interestingly, this study is the first to identify several QTLs for skeletal phenotypes other than BMD on chromosomes 5, 16, and 19.

Novel QTLs for femoral cortical thickness, area, and CSMI were localized to chromosome 5. QTLs for femur modulus and yield stress mapped to chromosome 5 at 70–72 cM in the F₂ (LOD = 2.1) and may have been detected in the RI at 49–54 cM, but did not meet the F₂ suggestive criteria of LOD = 2.8. Adipose mass also mapped to this area at 44–58 cM with a LOD score of 3.4, as did percent mineralization of the tibia. This region has been linked to circulating levels of insulin-like growth factor (IGF)-1.⁽³⁴⁾ IGF-1 is known to be an important factor in longitudinal growth as well as skeletal remodeling.

The region from 31 to 74 cM on chromosome 11 has been identified in many studies focused on BMD.^(5,6,19–23,25) Femur cortical thickness index⁽⁸⁾ and periosteal circumference⁽²¹⁾ have also been mapped to this region. This study confirmed the localization of tibia ultimate strain at 52 and 49 cM. Although it did not meet the suggestive criteria, the co-localization with adipose fat (LOD = 5.0) at 46–55 cM is intriguing. Body length, tibia length, and femur coronal width also mapped to this region in our study. A striking number of studies have identified linkage at this region to body weight,^(21,35–41) growth,^(33,40) and fat.^(21,39) Moreover, Haan et al.⁽⁴²⁾ identified a region on chromosome 11 at 47 cM that showed linkage to stem cell proliferation in BXD RI strains. The DBA/2 mice were found to have more rapid hematopoietic stem and progenitor cell cycling compared with C57BL/6 mice, which had much slower cell proliferation. These same investigators performed microarray analysis on stem cells from the parental strains and the largest cluster of differentially expressed genes mapped to the same location on chromosome 11.⁽⁴²⁾

QTLs specific for tibial material properties were identified on chromosome 16 at 4–12 cM. Although these QTLs did not meet suggestive criteria, they were confirmed in both the F₂ and RI analyses. The percentage of organic material in the tibia also mapped close to this region at 20–23 cM, as did several femur morphologic measures. Several investigators have mapped BMD to a region from 25–30 cM on chromosome 16.^(5,19,22,25) The additional insight provided by our results suggest that this site is linked to material behavior and composition as well as tissue distribution.

Tibia and femur material properties mapped to a region from 16 to 53 cM on chromosome 19. Femur ultimate stress and tibia yield and ultimate stress mapped to the same region as tibia cortical area, average thickness, and femur sagittal width. Two studies have reported skeletal QTLs for this region. Klein et al.⁽²²⁾ mapped whole body BMD to 53 cM, and Li et al.⁽²¹⁾ mapped femur BMD to 32.8 cM.

QTLs for both structural and material properties

QTLs were identified for both structural and material properties on chromosomes 4, 6, 9, 12, 13, 15, and 18. Chromosome 4 had two regions of interest; the first region from 13 to 26 cM has been previously linked to whole body BMD.⁽²³⁾ Our study expands the actions of this QTL to include femur medullary area (LOD = 3.9), CSMI, femur ultimate stress, and tibia ultimate load. The second region, at 30–48 cM, was linked to tibia ultimate load (LOD = 4.5) and stiffness. This region overlapped with a QTL for alkaline phosphatase in male, female, and combined analyses at 53–59 cM. Several skeletal studies have reported QTLs for BMD in the same region (57–62 cM),^(5,21,23) whereas Robling et al.⁽⁴³⁾ identified a QTL for skeletal mechanosensitivity.

Novel QTLs on chromosome 15 for femur stiffness, femur modulus of elasticity, femur ultimate load, and femur medullary area were confirmed in both F₂ and RI analyses. These QTLs were tightly clustered between 45 and 62 cM. The QTL for femur ultimate load at 52 cM (LOD = 4.3) found in the F₂ analysis was confirmed to within 3 cM in the RI animals. Previous skeletal studies have only reported QTLs in this region for femur BMD,⁽¹¹⁾ whole body BMD,⁽²²⁾ and periosteal circumference.⁽⁴⁴⁾

The same region of chromosome 15 has also been linked to IGF-1.^(38,45) Rosen et al.⁽⁴⁵⁾ reported a second QTL for serum IGF-1 at 51 cM on chromosome 6, which overlaps with several of the QTLs for material properties identified in this study. Brockman and Bevova⁽³⁸⁾ found a QTL for IGF-1 at 39 cM on chromosome 18 that also overlaps with QTLs identified in this study for femur sagittal width, ultimate strain, and elastic modulus, as well as tibia coronal width.

Two regions of significance were identified on chromosome 9. The first region was at 7–23 cM and included novel QTLs for material properties of the femur (modulus of elasticity and ultimate stress), shear ultimate load (LOD = 3.6), and tibia cortical area. The second region was at 36–65 cM, with novel QTLs for tibia stiffness and tibia ultimate load (LOD = 3.6). Both of these regions have been linked to other skeletal traits in previous studies.^(5,21–23,25,33)

Candidate genes

Whereas QTL analyses is limited in the precision with which a locus is mapped, identifying potential candidate genes within the region of a QTL is often the first step taken to elucidate the underlying mechanisms and genes responsible for the association. Although there are potentially hundreds of genes that could reside within an identified chromosomal region, occasionally candidate genes have been previously mapped in close proximity and are worthy of discussion. Table 5 summarizes candidate genes for select chromosomal regions containing novel QTLs identified in this study.⁽⁴⁶⁾

Of particular interest is the region identified on chromosome 2 from 68 to 108 cM. Several candidate genes have been identified within this region, including genes for matrix metalloproteinase 9, an osteoclastic enzyme, growth differentiation factor 5, and of most interest, bone morphogenetic protein 2, known to induce bone formation. Bone morphogenetic protein 2 has recently been implicated in a human osteoporosis linkage study.⁽⁴⁷⁾

The proximal and distal portions of chromosome 5 contained QTLs novel to this study. The gene for fibroblast growth factor receptor 3, which is involved in tyrosine kinase activation and is known to inhibit osteogenesis, resides within the proximal region. The gene for interleukin 6, a cytokine that stimulates bone resorption, also resides within this locus. The distal region of chromosome 5 includes candidate genes for bone morphogenetic protein 3 and the secreted phosphoprotein osteopontin, which is involved in osteoclast formation.

In summary, multiple QTLs for skeletal phenotypes have been identified that meet the strict criteria for nomination and confirmation at the suggestive and significant levels. Significant QTLs for correlated skeletal phenotypes and for more diverse phenotypes such as serum alkaline phosphatase, visceral fat mass, and body size often mapped to the same general chromosomal locations, many of which have been identified previously as being linked to BMD. The majority of the significant QTLs were linked to skeletal structural properties and/or morphologic measures related to size and shape. However, several suggestive QTLs were identified for material properties as well. Adjusting skeletal measures for body size gives additional insight into genetic influence mediated through body size-related co-variates. In many instances, removing the variance caused by body weight and length allowed for the identification of skeletal QTLs that were not previously identified for the unadjusted phenotypes. Investigating sex-specific QTL analyses identified many skeletal QTLs that would not have been found in the combined analyses alone.

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Table 1
F₂ and RI QTL Results Presented in Order of F₂ Chromosomal Location

Map	Phenotype	Sex _F	Chromosome 1 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Tibia Cort Thick *	M _A	25 (13–38)	3.0	45 (43–47)	1.6	
CIM	Femur Cort Area *	F _R	21 (12–32)	1.9	48 (46–48)	7.5	
CIM	Tibia % Water *	M _A	51 (30–61)	3.5	62 (60–65)	2.0	
IM	Femur Sag Width	C _R	53 (36–80)	4.8			
IM	Tibia Medul Area ^	M _R	70 (45–86)	4.0	64 (63–64)	1.5	M _A
IM	Tibia Length	C _A	74 (64–86)	9.9	89 (85–92)	3.1	M _{A+R} , C _R
CIM	Tibia Length	C _A	74 (64–86)	10	90 (85–91)	6.9	M _{R+A} , C _R
IM	Femur Length	M _R	74 (45–86)	7.3	90 (85–92)	3.3	M _A , C _R , C _A F _{R+A}
CIM	Tibia CSMI ^	M _A	86 (72–93)	5.6	69 (66–70)	5.5	
IM	Body Weight	C _R	78 (66–97)	3.4			
CIM	Body Length	C _R	80 (64–93)	3.2	71 (69–77)	2.2	
CIM	Femur Length	M _A	80 (64–95)	6.9	90 (87–91)	15	C _A , F _R
IM	Body Length	C _R	80 (66–91)	3.5			M _R
IM	Femur Stiffness ^	F _A			85 (82–89)	4.3	
IM	Tiba CSMI ^	M _A	87 (76–95)	4.8			
IM	Tibia Stiffness ^	M _A	89 (65–99)	2.2	89 (85–92)	1.6	F _R , C _{R+A} M _R , F _A
IM	Tibia Total Area ^	C _A	89 (72–97)	3.9			C _R , M _R
IM	Tiba CSMI ^	C _A	89 (76–97)	3.8	92 (83–96)	1.6	C _R
CIM	Tibia Sag Width ^	M _R	91 (70–99)	2.3	87 (85–91)	2.6	
IM	Femur Sag Width ^	M _A	91 (64–99)	3.3			
IM	Adipose Mass	M _a	91 (80–97)	6.1			M _R , C _A
IM	Body Mass Index ^	C _R	91 (74–99)	3.7			
IM	Tibia Medul Area ^	C _A	93 (72–101)	4.5	64 (61–78)	1.6	C _R
			Chromosome 2 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _F	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Femur Ult Load	F _A	68 (54–79)	3.2	75 (70–81)	1.5	
IM	Adipose Mass	F _A	73 (39–81)	3.2			
CIM	Femur Yld Work *	F _A	79 (73–83)	3.4	75 (71–76)	10	
CIM	Femur Yld Load *	C _R	81 (77–102)	5.5	76 (69–80)	2.3	C _A
CIM	Femur Length	C _A	81 (77–100)	6.1	76 (69–80)	2.2	
IM	Femur Yld Load *	C _A	81 (75–102)	4.8	78 (70–80)	2.1	C _R , F _{A+R}
CIM	Tibia Length	C _A	83 (73–98)	3.3	69 (67–73)	7.1	M _R
CIM	Tibia Cort Thick *	C _A	83 (75–104)	2.9	69 (67–80)	1.8	
IM	Femur Length	C _A	83 (75–104)	4.5			
IM	Tibia Ult Load *	C _A	86 (77–100)	3.6			C _R
IM	Fem Cort Thick *	F _R	108 (94–108)	2.9			
			Chromosome 3 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _F	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Tibia Ult Load *	F _R	4 (0–12)	3.4			F _A
CIM	Body Weight	C _R	4 (3–13)	2.1	2 (2–12)	2.1	
IM	Adipose Mass	M _R	4 (3–40)	2.2	19 (15–21)	1.8	M _A , C _R F _R , C _A C _A , M _{A+R}
IM	Body Length	M _R	12 (4–20)	3.7			
IM	Alk Phos	C _R	14 (6–24)	4.2			
IM	Tibia Length *	C _R	14 (4–22)	4.4			
CIM	Neck Ult Load *	M _A	57 (47–65)	4.2	27 (22–31)	5.5	
CIM	Alkaline Phosph	F _A	63 (49–74)	3.0	35 (34–41)	3.4	
CIM	Alkaline Phosph	F _R	67 (46–74)	2.2	71 (70–75)	3.2	
IM	Femur Ult Load *	F _A	68 (51–78)	2.8			F _R
IM	Adipose Mass	F _A	76 (63–81)	2.8			

Map: QTL mapping method; interval mapping (IM), composite interval mapping (CIM).

* QTL results unique to this study for specific functional phenotype.

^ QTL results that co-localize with QTLs in the literature for the same functional phenotype but at a different skeletal site (e.g. tibia versus femur or humerus).

Sex†: QTL cohort; males (M), females (F), combined males and females (C).

‡ Subscripts indicate raw unadjusted data (R) and body weight and length adjusted residuals (A).

cM: Peak cM position for interval.

1 LOD SI: Centimorgan range of the 1 LOD support interval.

LOD: Base 10 logarithm of the odds favoring linkage.

§ Other Cohorts: Additional analyses that identified QTLs in the same region (Bold indicates cohorts that met the confirmed significant criteria).

Shading: QTLs satisfying the confirmed significant criteria. (F2-LOD = 4.3 and RI-LOD = 1.5).

Table 2
F₂ and RI QTL Results Presented in Order of F₂ Chromosomal Location

Map	Phenotype	Sex†	Chromosome 4 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Adipose Mass	C _R	2 (2–14)	3.2			
CIM	Body Mass Index	F _R	2 (2–11)	2.4	24 (21–26)	6.4	
IM	Body Weight	F _R	2 (2–10)	2.5	24 (18–26)	1.7	
IM	Adipose Mass	F _R	4 (2–12)	3.3	24 (17–27)	1.6	
CIM	Tibia CSMI *	M _R	6 (2–16)	2.9	2 (0–3)	8.1	
CIM	Body Weight	C _R	8 (2–16)	2.8	19 (15–24)	4.6	
CIM	Fem Medul Area *	F _A	14 (6–30)	3.9	13 (10–21)	2.1	C _A
CIM	Femur Ult Stress *	M _R	18 (8–36)	3.0	5 (2–7)	3.2	
IM	Femur Total Area *	C _A	18 (10–40)	3.3	26 (25–27)	1.7	M _R
CIM	Femur CSMI *	C _A	18 (10–36)	3.5	28 (26–30)	5.1	C _R
CIM	Femur Total Area *	C _R	18 (10–38)	3.4	29 (25–33)	2.0	
CIM	Femur Yld Stress*	C _A	20 (10–38)	3.2	28 (21–32)	2.2	
IM	Femur CSMI *	C _A	22 (12–48)	3.2	26 (26–31)	1.8	
CIM	Body Length	C _R	24 (2–42)	3.1	8 (5–12)	1.9	
CIM	Tibia Ult Load *	C _R	26 (14–40)	3.4	2 (0–3)	7.2	
IM	Body Length	M _R	28 (10–44)	3.4	24 (16–27)	1.9	
IM	Tibia Ult Load *	C _R	30 (16–49)	3.1	36 (33–41)	1.9	
IM	Tibia Stiff *	C _R	32 (11–61)	2.1	37 (27–40)	1.7	F _R , F _A
IM	Tibia Ult Load *	M _R	32 (20–42)	4.5			M _A
CIM	Femur CSMI *	C _A	32 (12–49)	3.5	26 (24–27)	5.6	C _R
CIM	Tibia Yld Load *	M _R	32 (18–48)	3.9	36 (33–37)	6.8	
CIM	Tibia % Mineral	C _A	34 (15–51)	2.0	48 (44–53)	1.7	
IM	Tibia Yld Load *	M _R	34 (20–49)	4.0	36 (35–39)	2.5	
IM	Alkaline Phosph	C _A	59 (55–60)	19	53 (51–58)	3.5	
CIM	Alkaline Phosph	F _R	74 (62–76)	11	56 (54–58)	13	C _R , M _A M _R , F _{R+A} F _A
Map	Phenotype	Sex†	Chromosome 5 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Femur CSMI *	C _R	13 (9–27)	3.1	37 (34–43)	2.3	
IM	Femur Cort Thick *	C _A	13 (9–27)	3.5			C _R
CIM	Femur Cort Area *	C _R	15 (9–27)	3.2	2 (0–2)	3.4	
CIM	Tibia % Mineral	F _A	44 (30–52)	3.7	73 (70–75)	5.0	
IM	Femur Epi Width *	F _R			44 (44–46)	3.5	
IM	Adipose Mass	F _A	58 (45–67)	3.4	44 (44–46)	2.7	
IM	Femur Modulus *	C _R	70 (59–76)	2.1	50 (44–55)	1.8	F _{R+A} , C _A
IM	Femur Yld Stress*	C _R	72 (61–76)	2.1	54 (48–57)	1.7	
Map	Phenotype	Sex†	Chromosome 6 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Body Weight	F _R	6 (6–22)	2.7	10 (10–13)	3.7	
CIM	Femur Ult Stress *	M _A	6 (6–18)	2.8	35 (32–38)	1.9	
IM	Tibia Length ^	C _A	6 (0–20)	4.2			
IM	Adipose Mass	F _R	6 (6–19)	3.7			C _R
IM	Body Weight	F _R	6 (6–22)	3.0			M _R , C _R
CIM	Tibia Stiffness *	C _A	12 (6–28)	4.2	8 (7–10)	2.4	
IM	Neck Yld Load	M _A	41 (22–50)	4.7			
IM	Neck Ult Work *	M _R	46 (37–58)	3.5	19 (17–22)	1.9	M _A
IM	Neck Ult Displ *	M _R	46 (37–54)	5.1	20 (17–27)	1.6	
CIM	Neck Ult Displ *	M _A	46 (41–56)	5.6	25 (23–30)	3.7	F _A
IM	Neck Ult Displ *	M _A	46 (39–56)	5.1			M _R , C _A
IM	Femur Cor Width	C _A	50 (35–58)	6.7			C _R , M _A
CIM	Tibia Ult Strain *	M _A	54 (35–63)	2.3	44 (44–46)	2.5	
CIM	Femur Sag Wid	C _A	62 (50–70)	4.7	44 (42–44)	6.1	C _R
IM	Tibia Ult Load ^	C _R	64 (52–72)	4.5			C _A
CIM	Tibia Ult Strain *	M _R	72 (64–74)	2.4	53 (51–61)	3.3	

Map	Phenotype	Sex _†	Chromosome 4 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Tibia Ult Strain *	C _R	72 (64–74)	2.9			
CIM	Femur Cor Width *	F _A	74 (66–74)	3.3	72 (69–72)	7.0	
IM	Femur Cor Width *	F _R	74 (68–74)	4.3			
Map	Phenotype	Sex _†	Chromosome 7 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Femur Yld Load *	C _A	15 (5–29)	5.5	0 (0–2)	2.3	
CIM	Femur Ult Load *	C _R	15 (5–31)	4.3	30 (20–41)	1.6	
CIM	Body Mass Index	C _R	19 (5–39)	3.4	36 (28–40)	1.6	M _R
IM	Adipose Mass	C _R	21 (14–34)	7.6			M _R
IM	Femur Ult Load *	C _A	21 (11–35)	5.4			M _A
IM	Body Mass Index	C _R	21 (9–39)	3.4			
IM	Body Weight	C _R	23 (11–39)	3.2			
CIM	Femur Ult Work	C _A	25 (15–35)	3.8	10 (6–15)	1.8	M _A
CIM	Femur Yld Work	C _R	25 (15–35)	3.0	22 (13–27)	2.0	C _A
IM	Femur Yld Load *	M _A	31 (17–41)	4.5			C _A
CIM	Femur Ash Mass	C _A	33 (15–45)	3.8	39 (28–40)	2.9	M _A
CIM	Femur Cort Area	M _A	43 (29–53)	3.4	15 (12–19)	2.3	
CIM	Fem Head Diam *	M _R	65 (57–65)	3.6	44 (41–46)	3.9	

Data are presented in the same style as Table 1

Table 3
F₂ and RI QTL Results Presented in Order of F₂ Chromosomal Location

Map	Phenotype	Sex _F	Chromosome 8 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Femur Ult Load	C _R	29 (19–45)	2.8	54 (49–61)	20	C _A
CIM	Femur Ult Load	F _R	43 (29–47)	2.6	54 (52–59)	5.2	
CIM	Femur Cort Thick *	M _R	45 (38–57)	2.4	52 (50–59)	2.1	M _R
IM	Tibia Ult Load ^	M _A	49 (31–57)	2.1	40 (38–42)	1.7	
IM	Femur Yld Load	C _A	53 (27–57)	4.1	54 (52–57)	2.5	C _R
IM	Femur Yld Work *	C _A	55 (37–57)	2.9	54 (52–57)	2.5	C _R
CIM	Tibia % Mineral	C _A	57 (51–57)	5.3	40 (31–54)	1.5	C _{A+R}
CIM	Tibia Ult Displ *	F _R	57 (51–57)	2.8	46 (43–50)	5.9	
IM	Tibia % Mineral	M _R	57 (49–57)	4.6			
Map	Phenotype	Sex _F	Chromosome 9 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Tibia Cort Area *	F _R	7 (7–23)	3.5			F _A
IM	Femur Yld Stress *	F _R	7 (7–23)	3.3			F _A
IM	Femur Ult Stress *	F _R	7 (7–23)	3.0			F _A
IM	Femur Modulus *	F _R	1 (7–25)	2.8			F _A
CIM	Neck Ult Load *	C _A	7 (7–27)	3.6	23 (23–27)	2.7	F _R
CIM	Tibia % Ash	C _R	17 (7–27)	2.2	23 (23–27)	4.5	
IM	Femur Length	C _R			21 (13–23)	2.1	F _R
IM	Femur Stiffness *	F _A			21 (22–21)	4.5	
IM	Tibia Length ^	F _A			21 (19–21)	3.3	C _R , F _{A+R}
IM	Adipose Mass	M _A	27 (7–36)	2.9	12 (9–17)	1.6	
IM	Alkaline Phosph	C _A	28 (14–40)	2.2	21 (12–33)	1.6	C _A
CIM	Femur Epi Width	C _R	36 (11–46)	3.0	52 (46–54)	2.6	
IM	Femur Ash Mass	C _R	48 (32–56)	4.0			C _A
IM	Tibia Epi Width ^	F _A	48 (34–69)	2.8	36 (32–44)	1.5	
CIM	Tibia % Mineral	C _R	50 (40–60)	3.0	52 (49–54)	1.5	C _A
IM	Tibia % Mineral	C _R	50 (40–61)	2.7	52 (49–56)	4.0	
IM	Femur Length	C _R	52 (40–67)	3.2			F _A , C _{R+A}
IM	Tibia Stiffness *	F _R	54 (40–71)	2.9			
IM	Tibia Length ^	F _R	54 (34–65)	3.3			F _A , C _{R+A}
IM	Tito Ult Load ^	F _R	65 (42–71)	3.6			
Map	Phenotype	Sex _F	Chromosome 14 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Femur Cor Width *	C _R	22 (11–28)	4.6	19 (18–23)	5.6	C _A , M _A
IM	Femur Cor Width *	C _R	22 (11–28)	4.6	19 (0–23)	1.7	
Map	Phenotype	Sex _F	Chromosome 11 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Adipose Mass	C _A	32 (15–45)	2.4	55 (51–56)	2.1	C _R , F _A
IM	Body Length	F _R	34 (14–46)	4.7			
CIM	Femur Cor Width	C _R	38 (20–46)	3.5	63 (61–67)	2.4	C _{R+A}
CIM	Tibia Length *	F _R	42 (32–58)	4.1	28 (27–30)	3.8	
IM	Tibia Length *	F _R	42 (32–62)	3.9	41 (38–50)	1.9	M _R
IM	Adipose Mass	M _A	46 (37–54)	5.0	55 (51–61)	1.5	
CIM	Tibia Ult Strain *	M _R	52 (37–66)	2.0	49 (43–55)	2.9	
Map	Phenotype	Sex _F	Chromosome 12 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Tibia Ult Stress *	M _A	28 (28–38)	3.0			M _R
IM	Tibia CSMI ^	M _A	32 (28–40)	3.5			
CIM	Tibia Cort Area ^	M _R	32 (28–42)	3.3	16 (14–20)	3.1	

			Chromosome 8 F ₂ Analyses		RI Analyses		
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	§ Other Cohorts
CIM	Tibia Medul Area ^	C _R	38 (28–44)	3.1	44 (41–44)	2.1	
CIM	Fem Head Diam *	C _R	53 (47–57)	5.6	50 (50–52)	2.6	
IM	Fem Head Diam *	C _R	53 (47–57)	4.9			F _{R+A} , C _A
IM	Tibia % Ash	M _R	55 (45–57)	3.0	31 (19–34)	1.6	M _A
IM	Neck Yld Work *	C _R	57 (49–57)	3.2			C _A , F _A
CIM	Tibia % Ash	C _R	57 (49–57)	3.5	53 (49–55)	3.5	C _A
			Chromosome 13 F ₂ Analyses		RI Analyses		
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	§ Other Cohorts
IM	Femur Stiffness *	C _R	16 (16–30)	3.5			
IM	Body Mass Index	C _R	22 (16–36)	4.6			
CIM	Body Mass Index	C _R	24 (16–36)	4.8	5 (4–11)	1.6	
IM	Body Weight	C _R	24 (16–34)	6.6			M _R
IM	Adipose Mass	C _R	24 (16–37)	4.8			
IM	Tibia Length *	C _R	26 (16–40)	4.4			
CIM	Body Weight	M _R	28 (16–38)	5.5	9 (4–12)	1.9	
IM	Body Length	C _R	28 (16–50)	3.1			
CIM	Femur Ult Load *	C _R	30 (16–56)	2.4	36 (24–40)	2.2	
CIM	Body Length	M _R	34 (16–58)	2.7	26 (26–30)	3.5	
CIM	Femur Ult Strain *	M _R	68 (56–74)	2.5	45 (43–49)	3.0	
IM	Femur Yld Work	M _R			68 (66–70)	3.8	M _A
IM	Femur Yld Load	M _A			70 (66–70)	3.4	M _R

Data are presented in the same style as Table 1

Table 4
F₂ and RI QTL Results Presented in Order of F₂ Chromosomal Location

Map	Phenotype	Sex _†	Chromosome 15 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Femur Length *	C _R	11 (11–21)	3.4	33 (23–38)	1.6	
IM	Body Length	F _R	31 (11–43)	3.9			
IM	Tibia Yield Work *	M _R			41 (38–45)	3.3	M _A
CIM	Femur Stiffness *	C _R	45 (23–53)	2.1	48 (46–49)	3.5	
IM	Tibia % Ash	M _A	48 (37–52)	3.4			M _R C _{A+R}
IM	Tibia Length *	F _A			48 (43–48)	3.8	F _R M _R C _R
CIM	Femur Yld Load *	C _A	43 (23–57)	3.0	48 (41–49)	1.9	
CIM	Femur Modulus *	C _R	50 (26–53)	2.4	58 (56–62)	3.4	
CIM	Femur Med Area	C _A	50 (43–56)	2.5	62 (54–65)	1.8	F _A
IM	Femur Ult Load *	C _A	50 (11–56)	3.4	38 (37–46)	2.1	F _A
CIM	Femur Ult Load *	C _A	52 (33–59)	4.3	49 (48–53)	3.3	C _R , F _A
CIM	Body Weight	M _R	52 (33–59)	2.2	52 (49–57)	2.3	
			Chromosome 16 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Tibia Ult Strain *	C _R	4 (4–16)	2.2	8 (4–10)	1.7	M _{A+R} , C _A
CIM	Tibia Modulus *	M _R	4 (4–16)	2.2	12 (10–14)	5.0	
CIM	Fem Medul Area *	F _R	16 (6–26)	3.5	18 (14–21)	7.7	F _A
CIM	Femur CSMI *	F _R	18 (4–39)	3.2	52 (46–57)	3.5	
CIM	Tibia % Organic *	C _A	20 (4–39)	2.9	23 (21–30)	7.1	
IM	Tibia % Organic *	C _A	24 (10–39)	3.0	16 (10–28)	1.9	C _R
IM	Femur Cor Width *	C _R	33 (16–49)	5.0			C _A
CIM	Femur Cor Width *	F _R	39 (22–51)	3.9	41 (39–43)	6.5	C _R
			Chromosome 17 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Femur Length	F _A	18 (18–22)	3.4	3 (1–6)	2.3	
CIM	Femur Length	F _A	18 (18–22)	4.6	4 (1–7)	5.2	
IM	Femur Yld Displ *	C _R	24 (18–32)	2.8	23 (22–27)	2.1	
CIM	Femur Yld Disp *	C _R	24 (18–32)	2.9	43 (39–55)	2.1	
IM	Body Mass Index	C _R	34 (18–51)	3.1	41 (37–44)	1.6	
IM	Body Weight	M _R	42 (28–52)	2.2	41 (39–44)	2.0	C _R
CIM	Femur Yld Load *	C _A	45 (34–52)	2.1	21 (19–23)	1.6	C _R
CIM	Body Weight	C _R	47 (30–53)	2.4	43 (36–57)	1.5	
CIM	Neck Ult Displ *	M _A	55 (51–57)	2.9	32 (31–34)	2.2	
IM	Femur Yld Displ *	C _R	57 (52–57)	3.2	29 (27–31)	1.7	C _A
IM	Femur Stiffness *	C _A	57 (50–57)	5.1	31 (22–32)	1.6	C _R , M _{R+R}
CIM	Femur Yld Disp *	C _R	57 (53–57)	3.0	52 (46–53)	2.4	
			Chromosome 18 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Femur Stiffness	F _A			8 (6–11)	4.0	
CIM	Femur Sag Wrld *	F _R	35 (16–45)	2.8	53 (51–53)	3.7	
CIM	Femur Modulus *	F _R	37 (31–41)	2.6	49 (49–50)	3.0	
IM	Femur Ult Strain ⁴	F _R	37 (32–46)	2.2	49 (47–52)	1.7	
IM	Tibia Cor Width *	C _R	47 (39–51)	4.6			
			Chromosome 19 F ₂ Analyses		RI Analyses		§ Other Cohorts
Map	Phenotype	Sex _†	cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
CIM	Femur Ult Stress *	M _R	24 (6–37)	2.2	49 (43–50)	3.5	
CIM	Tibia Yield Stress *	F _A	33 (25–45)	2.5	16 (14–18)	10	
CIM	Tibia Ult Stress *	F _A	33 (15–53)	2.0	16 (14–18)	3.0	F _R

Map	Phenotype	Sex _†	Chromosome 15 F ₂ Analyses		RI Analyses		§ Other Cohorts
			cM (1-LOD SI)	LOD	cM (1-LOD SI)	LOD	
IM	Adipose Mass	C _R	47 (41–50)	2.3	49 (47–50)	1.9	F _A , C _A
IM	Body Weight	C _R	47 (41–49)	3.0			
CIM	Femur Sag Width *	F _R	53 (49–53)	3.7	39 (37–42)	5.8	F _A
IM	Tibia Cort Area *	M _A	53 (51–53)	2.4	47 (44–49)	3.6	M _R
IM	Tibia Cort Thick *	M _A	53 (53–53)	2.7	47 (44–48)	2.4	M _R
CIM	Tibia Cort Thick *	M _A	53 (53–53)	2.7	49 (46–50)	3.5	M _R
IM	Tibia Yield Stress*	M _R	53 (49–53)	3.7			M _A

Data are presented in the same style as Table 1

Table 5

Candidate Genes

<i>Chromosome</i>	<i>cM</i>		<i>Candidate genes</i>	<i>Function</i>
2	76.1	Bmp2	Bone morphogenetic protein 2	Induces formation of ectopic cartilage and bone in vivo.
2	89	Src	Rous sarcoma oncogene	Important in osteoclastic bone resorption.
2	90	Gdf5	Growth differentiation factor 5	GDF-5 enhances de novo bone formation capability of MSCs/HA composite.
2	96	Mmp9	Matrix metalloproteinase 9	Osteoclastic enzyme.
5	17	IL-6	Interleukin 6	Cytokine that stimulates bone resorption.
5	20	Fgfr3	Fibroblast growth factor receptor 3	Involved in tyrosine kinase activation and signal transduction. Inhibits osteogenesis.
5	55	Bmp3	Bone morphogenetic protein 3	Induce formation of ectopic cartilage and bone in vivo.
5	56	Spp1	Secreted phosphoprotein 1	Known as osteopontin-involved in osteoclast formation.
6	40	Mitf	Microphthalmia-associated transcription factor	Carry out essential functions in osteoclast development.
6	60.6	Gdf3	Growth differentiation factor 3	Upregulation of bone morphogenetic protein GDF-3/Vgr-2 expression in adipose tissue.
7	6.5	Tgfb1	Transforming growth factor beta 1	Regulates skeletal development and homeostasis of bone metabolism.
7	23.1	IL-4i1	Interleukin 4 induced 1	IL-4 regulates bone resorption.
8	46.5	Cdh11	Cadherin 11	Involved in osteoblastogenesis.
9	6	Acp5	Acid phosphatase 5, tartrate resistant	Mutants have osteopetrosis and shortening and widening of bones.
9	6	Fos	Acid phosphatase 5, tartrate resistant	Mutants have osteopetrosis and shortening and widening of bones.
12	40	Fos	FBJ osteosarcoma oncogene	Mutants have osteopetrosis.
14	13	Bmpr1a	Bone morphogenetic protein receptor, type 1A	Noggin-expressed in mature osteoblasts, inhibits osteoblast differentiation and bone formation.
14	15	Bmp4	Bone morphogenetic protein 4	Involved in bone induction and tooth development.
14	20	Mcpt5	Mast cell protease 5	Involved in IL-4 regulation: stores factors known to control bone metabolism.
15	54.5	Co12a1	Procollagen, type II, alpha 1	Type II collagen.