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Identification of genetic loci that regulate bone adaptive response to mechanical loading in C57BL/6J and C3H/HeJ mice intercross

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Abstract

Strain-dependent differences in bone adaptive responses to loading among inbred mouse strains suggest that genetic background contributes significantly to adaptation to exercise. To explore the genetic regulation of response to loading, we performed a genome-wide search for linkage in a cross between two strains, a good responder, C57BL6/J (B6), and a poor responder, C3H/HeJ (C3H). Using a four-point bending model, the right tibia was loaded by applying 9 N force for 36 cycles for 12 days in 10-week-old female B6×C3H F2 mice. Changes in bone density (BMD) and bone size were evaluated in vivo by pQCT. Measurements from non-loaded left tibia were used as an internal control to calculate loading-induced percent increase in BMD and bone size, thus excluding the possibility of identifying background QTL(s) due to natural allelic variation in mapping strains. A genome-wide scan was performed using 111 microsatellite markers in DNA samples collected from 329 F2 mice. Heritability of bone adaptive response to loading was between 70 and 80%. The mean increase, expressed as percent of unloaded tibia, was 5% for BMD, 9% for periosteal circumference (PC), and 14% for cortical thickness in F2 mice (n = 329). All these phenotypes showed normal distributions. Absence of significant correlation between BMD response to four-point bending and body weight or bone size suggested that the bone adaptive response was independent of bone size. Interval mapping revealed that BMD response to four-point bending was influenced by three significant loci on Chrs 1 (log-of-odds ratio score (LOD) 3.4, 91.8 cM), 3 (LOD 3.6, 50.3 cM), and 8 (LOD 4.2, 60.1 cM) and one suggestive QTL on Chr 9 (LOD 2.5, 33.9 cM). Loading-induced increases in PC and Cth were influenced by four significant loci on Chrs 8 (LOD 3.0, 68.9 cM), 9 (LOD 3.0, 13.1 cM), 17 (LOD 3.0, 39.3 cM), and 18 (LOD 3.0, 0 cM) and two suggestive loci on Chr 9 (LOD 2.2, 24 cM) and 11 (LOD 2.1, 69.9 cM). Pairwise analysis showed the presence of several significant and suggestive interactions between loci on Chrs 1, 3, 8, and 13 for BMD trait. This is the first study that provides evidence for the presence of multiple genetic loci regulating bone anabolic responses to loading in the B6×C3H intercross. Knowledge of the genes underlying these loci could provide novel approaches to improve skeletal mass.

Keywords: Mechanical loading; Four-point bending; Quantitative trait loci; Mice; vBMD

Introduction

Mechanical stimulation is one of the important factors in the development and maintenance of skeletal tissues [1–4]. Several in vivo studies have shown that increased mechanical stress on bone tissue changes the bone density and morphology, resulting in an increased bone mass and biomechanical strength, whereas lack of mechanical stress leads to a rapid bone loss as evidenced by immobilization and bed rest studies [5–14]. Thus, physical exercise has been perceived as an important therapeutic strategy in humans to maintain bone mass and prevent osteoporosis. Recent studies in humans have also shown that bone anabolic response to a given mechanical load is highly variable, with some individuals exhibiting robust bone anabolic response with others responding modestly [15–18]. A similar variation has been observed among inbred strains of mice [19–21]. We [6,19] and others [21] have shown that mouse strains such as C57BL/6J (B6) respond with a much higher increase in bone density (BMD) and bone cross-sectional area as compared to the C3H/

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HeJ (C3H) strain of mouse in response to a similar amount of in vivo loading. These data suggest that variations in skeletal response to mechanical loading in humans and mice are largely determined by genetic factors [12,21]. However, very little is known about the genetic regulation of mechanical loading and, so far, not a single gene has been identified that influences the skeletal response to mechanical loading [22].

One approach often used to perform genome-wide searches of genetic loci that contribute to differences in phenotypic variation is the quantitative trait loci (QTL) technique. This approach has been used in experimental animal models and in humans to study genetic regulation of bone density [23,24], bone metabolism [25], strength [26], quality, and size [27] and other traits [28]. By utilizing the QTL technique, one could (1) identify the regions within chromosomes that contain the functional genes of interest for a given phenotype; (2) estimate the influence of genetic variation on within-species phenotypic variation; and (3) evaluate QTL–QTL interactions for the effect on phenotypic variation. The limitations of the QTL approach include: (1) it is technically time-consuming and expensive; (2) initial QTL analysis does not allow high resolution mapping and further fine mapping is required to narrow down the loci containing possible candidate genes; and (3) many genes in a particular chromosomal interval may not be relevant for a specific trait. Despite the drawback that the relevance of QTL genes identified using mouse models to explain the phenotypic variation in humans remains to be established, the availability of genomic (sequences, SNPs) and animal resources (congenic lines, chromosomal substitution strains, recombinant inbred strains) facilitates the identification and functional testing of QTL of candidate genes and therefore has attracted attention among molecular geneticists to use mice for QTL studies. In this study, we used two inbred strains, C57BL/6J and C3H/HeJ, good and poor responder strains, respectively, to perform a genome-wide search for loci regulating bone adaptive response to mechanical loading.

Materials and methods

Mice

Female B6 and male C3H mice were obtained from the Jackson Laboratory (Bar Harbor, ME) to produce C3HB6 F1 mice, which were intercrossed to generate F2 mice. At 10 weeks of age, the mice were subjected to mechanical loading using a four-point bending model described previously [19,21]. All mice were housed under the standard conditions of 14-h light and 10-h darkness and had open access to food and water. The experimental protocols were in compliance with animal welfare regulation and approved by local IACUC.

In vivo loading model/regimen

The four-point bending device [Instron, Canton, MA] consists of two upper movable points covered with rubber pads, which are 4 mm (millimeter) apart, and two lower non-movable points covered with rubber pads, which are 12 mm apart. After anesthetizing the mice, the ankle of the tibia was positioned on the second lower immobile points of Instron such that the region of tibia loaded did not vary in different mice. During bending, the two upper pads touch the lateral surface of the tibia through overlaying muscle and soft tissue, while the lower pads touch the medial surface of the proximal and distal parts of the tibia. One of the limitations of this model is that force applied over soft tissue may have some local effects on blood and fluid flow. We took efforts to minimize this by changing the rubber pads frequently in the Instron mechanical tester. The loading protocol for this study consisted of a 9 N force at a frequency of 2 Hz for 36 cycles performed at the same time once a day under inhalable (5% halothane and 95% oxygen) anesthesia. The loading procedure was repeated for 6 days/week with 1 day of rest for 2 weeks. On the 15th day, in vivo pQCT measurements were performed on the loaded and non-loaded tibia of all F2 mice. Sham bending was performed as described previously [21].

Peripheral quantitative computed tomography (pQCT) measurements

To measure loading-induced changes in the bone density and geometry in loaded and non-loaded tibias, we used pQCT (Stratec XCT 960M, Norland Medical System, Ft. Atkinson, WI) as described previously [19,29]. Calibration was performed daily with a defined standard provided by the manufacturer. Mice were anesthetized, and a two-dimensional scout view was taken first, which permits the identification of landmarks and a precise selection of the appropriate site for measurement. In order to minimize the measurement errors caused by positioning of tibia for pQCT, we used the tibia–fibular junction as the reference line. We selected two slices that start 4 mm proximal from tibia–fibular junction for pQCT measurement. This region corresponds to the loading zone. Each slice is at a 1-mm interval, and the values presented in the Results section are an average of these two slices [19]. To minimize exposure time of animals to anesthesia for in vivo pQCT measurements, we choose to scan only the loading zone in the F1 or F2 mice. Based on our previous findings [19], we used two thresholds to analyze the pQCT data: a 180–730 mg/cm^3 threshold was used to measure periosteal circumference (PC) and a 730–730 mg/cm^3 threshold was used to measure total volumetric density (vBMD), cortical volumetric bone mineral density (cortical vBMD), and cortical thickness (CTh). Cortical vBMD is defined as cortical content/cortical volume excluding the marrow cavity.

PCR-based genetic analysis

Two days after the last loading, the mice were sacrificed and tissues such as liver and tibia were collected and stored at −80°C. Genomic DNA was extracted from the liver of each F2 mouse using a Maxi prep DNA extraction kit (Qiagen) and stored at −80°C. The quality and quantity of DNA were measured by Nano drop and Bio-analyzer (Agilent Technologies, Inc, CA). Polymerase chain reaction (PCR) primers were purchased from Applied Biosystems (ABIPRISM, Foster City, CA) to perform the genome-wide genotyping scan of the F2 population. PCR reaction conditions allowed 3–4 microsatellite markers to be multiplexed in a single electrophoretic lane. The pooled products were analyzed for fragments' size on the ABI 3100 Sequence Detection System, and Gene Scan software was used to detect size of the alleles. Allele calls and edits were performed using Genotyper software and in-house software and exported as text files for downstream analysis.

QTL analysis

We used parametric mapping (a mapping strategy that requires the assumption of normal distribution for the quantitative trait investigation) for

Table 1
Changes in the bone parameters in response to 12 days of sham bending at 9 N load in 10-week female B6 mice

Bone parameters	Mean ± SD		
	Non-loaded	Loaded	*P* value
Total area, mm^2	2.01 ± 0.11	2.07 ± 0.10	0.30
Total mineral content, mg/mm	1.08 ± 0.04	1.10 ± 0.04	0.46
Periosteal circumference, mm	5.02 ± 0.14	5.10 ± 0.12	0.29
Endosteal circumference, mm	4.09 ± 0.15	4.17 ± 0.12	0.31
Total vBMD, mg/cm^3	649 ± 14.64	663.7 ± 19.7	0.15
Cortical vBMD, mg/cm^3	1031 ± 9.8	1038 ± 13.5	0.26

$n = 7$.