Quantitative Trait Loci for Bone Density in Mice: The Genes Determining Total Skeletal Density and Femur Density Show Little Overlap in F2 Mice

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Received: 28 September 2001 / Accepted: 8 February 2002 / Online publication: 4 September 2002

Abstract. Bone mineral density variation is a highly heritable trait and is the best predictor of skeletal fragility. Total skeletal density was determined by PIXIMUS™, and femur density was determined by pQCT. The data were analyzed for quantitative trait loci (QTL) to determine if bone density at a specific skeletal site (femur) would identify new gene loci or the same gene loci as total body (PIXIMUS™). In order to show concordance and differences in QTL for total body bone density versus femur bone density, we performed a genome-wide scan from 633 (MRL × SJL) F2 mice. The bone mineral density (BMD) data from pQCT were used to identify nine QTL on chromosomes 1, 3, 4, 9, 12, 17, and 18, while nine QTL on chromosomes 1, 2, 4, 9, 11, 14, and 15 were identified by PIXIMUS™ data, accounting for 32.5% and 30.4% variation in F2 mice, respectively. QTL on chromosomes 1, 2, 3, 9, 11, 12, 14, 15, 17, and 18 are unique to our study, as they have never been described before. Chromosome 1 (D1Mit33 and D1Mit362) had similar QTL between pQCT and PIXIMUS™. Several QTL were identified for both femur and total body BMD but only two QTL were common for both of these phenotypes. This suggests that genes regulating bone density differ depending on the skeletal site analyzed.

Key words: Peripheral quantitative computed tomography — PIXIMUS™ — Quantitative trait loci — Bone mineral density

In contrast to the existing BMD QTL studies [1, 2, 5, 14], the concordance and similarities between QTL that are site specific and total body have never been reported. This lack of information is partly due to the fact that QTL studies are expensive, making them difficult to be repeated in the same laboratory. It is important to understand how QTL information obtained by pQCT (femur) relates to that by PIXIMUS (total body). Because total body BMD (PIXIMUS) includes the femur BMD, we hypothesized that QTL identified by pQCT will also be present in the total skeleton (PIXIMUS). Here we present data to show (1) many more BMD QTL (nine from the femur and nine from the total skeleton) were identified using both pQCT and PIXIMUS on the same F2 mice rather than either instrument alone; and (2) only two QTL from the total skeleton (PIXIMUS) were the same as those from the femur (pQCT).

Materials and Methods

Mice

The progenitor strains of MRL/MpJ females and SJL/J males were obtained from The Jackson Laboratory (Bar Harbor, ME). F1 mice from MRL/MpJ and from SJL/J were crossed to get the F2 population. These inbred strains of mice were selected from our study of 20 different strains that were used for ear phenotype studies [11]. Twenty, 4-week-old MRL/MpJ female mice and 10 SJL/J male mice were housed at the Animal Research Facility, J.L. Pettis VA Medical Center, Loma Linda, CA, under the standard condition of 14 h light, 10 h darkness, ambient temperature of 20°C, and relative humidity of 30–60%. All F1 and F2 mice (MRL/MpJ × SJL/J) crosses were bred at the Animal Research Facility. The experimental protocols were in compliance with animal welfare regulations and approved by the JL Pettis VA Medical Center, Loma Linda, CA.

Bone Mineral Density (BMD) Measurement

By pQCT. The mid-shaft femur density was determined using a peripheral quantitative computed tomography (pQCT) system from Stratec XCT Research M™ (Norland Medical System, Fort Atkinson, WI). The precision of this instrument...
for densitometry of mouse bone has been determined to be 1.2% by repeated placement and measurement of a single femur [2, 9].

By PIXIMUS. A PIXIMUS densitometer (LUNAR Corporation, Madison, WI) was used for measurement of whole body BMD. It is a rapid (5-minute image acquisition) and very precise small animal densitometer, with CV (the percentage of measurement error) of 4.9% for total skeletal BMD, and 4.1% CV for femur region of interest (ROI) BMD. Routine calibration was performed daily with a defined standard (phantom). Before measurement, mice were anesthetized with an IP dose of 50/10 mg/kg Ketamine/Xylazine solution and then placed on a specimen tray and put in a PIXIMUS imaging area for analysis. After measurement a ROI rectangle was moved and resized to cover the whole body, excluding the animal’s head. The PIXIMUS software automatically calculated the whole body BMD and recorded the data in Microsoft Excel files [10].

Genetic Analysis

The (MRL/MpJ × SJL/J) F2 population was sacrificed at 7 weeks of age. A Wizard Genomic DNA kit (Promega, Madison, WI) was used to prepare DNA according to the manufacturer’s instructions. Polymerase Chain Reaction (PCR) primers were purchased from Research Genetics (Huntsville, AL) to perform a genome-wide scan of the F2 mice population. Amplification was performed using Promega reagents (Promega, Madison, WI). PCR cycling conditions included 4 min at