osteoporosis; linkage; quantitative trait loci

OSTEOPOROSIS is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (10). According to the definition of the World Health Organization (WHO) for osteoporosis, established osteoporosis affects 30% of postmenopausal white women in the United States, and the proportion rises to 70% in women over the age of 80 yr (39). The most common clinical outcomes of osteoporosis are fracture of the spine, hip, and wrist. Of these, hip fractures are the most severe, leading to a 12–20% reduction of expected survival (49). The direct cost for hip fracture was around $13.8 billion in the United States in 1995 (46) and £942 million in the United Kingdom in 1998 (51). With rapid economic development and aging of the population, the worldwide health and economic burden of osteoporosis will rise further in the future.

Bone strength is the ultimate determinant of resistance to fracture. In clinical practice it is measured mainly as bone density [estimated as “areal” bone mineral density (BMD) as measured by dual-energy X-ray absorptiometry (DXA)], bone size, and bone quality. As BMD contributes substantially to bone strength and can be practically measured with marked sensitivity and precision, the evaluation of BMD is the most commonly used method for predicting fracture risk in humans. Therefore, BMD is often used as a surrogate phenotype in the search for osteoporosis genes (13, 15, 18, 28, 33, 40, 44, 50). However, since BMD does not take into account the geometrical and material characteristics of bone that also contribute to bone strength, low BMD is not the only important risk factor for osteoporotic fractures (17, 27, 38, 55). Bone size per se is also an important determinant of osteoporotic fractures. First, larger bone size and higher BMD values are both independently associated with stronger bone (38). Patients with spine fractures on average have reduced bone size (24, 37, 53). The deficit in bone size may partly account for the increased bone fragility, more so when accompanied by a deficit in bone mineral content and BMD compared with age-matched controls (17). Second, spinal BMD in females is comparable with that in males; however, females suffer a higher incidence of spine fractures than males (39). This is partially attributable to the fact that female spine bone sizes are 20–25% smaller than male spines after adjusting for body size differences (21). Third, it is known that while a decrease in bone mass may result in a loss of bone strength, the loss may be offset, at least in part, by an increase in bone size, tending to preserve bone strength (11, 48). Identification and characterization of specific loci or genes involved in determining bone-related phenotypes such as bone size not only contribute to a greater understanding of the pathogenesis of osteoporosis, but also lead to the development of better diagnostic, prevention, and treatment strategies for the disease.

Compared with BMD, genetic studies of bone size have been rather rare so far, even though the genetic determination of bone size (heritability) has been characterized as high (14, 20, 42). The heritability of bone size at the spine, hip, and wrist is over 50% (14). In support of this, alleles of the vitamin D receptor gene were shown to be associated with variation in bone size (43), and a whole genome linkage scan in 309 randomly ascertained Caucasian sister pairs have identified a few chromosomal regions (5q, 4q, 17q, 3q, 7q, 9q, and 19p) with significant or suggestive evidence of linkage to femoral bone size (43), and a whole genome linkage scan in 309 randomly ascertained Caucasian sister pairs have identified a few chromosomal regions (5q, 4q, 17q, 3q, 7q, 9q, and 19p) with significant or suggestive evidence of linkage to femoral bone size (43).

To identify chromosomal regions contributing to bone size variation, we conducted a whole genome scan in 53 pedigrees each ascertained through a proband having a BMD Z-score less than -2.0 standard deviation (SD) using 380 microsatellite markers. Lumbar area 1, 2, 3, and 4 at the spine, femoral neck, trochanter, intertrochanter areas at the hip, ultradistal, mid-distal, and one-third distal areas at the wrist were measured by dual-energy X-ray absorptiometry (DXA), and adjusted for age, height, weight, and sex. Two-point and multipoint linkage analyses were performed for skeletal bone size at each site and their composite measurements using the SOLAR package. Two chromosomal regions (1q22 and 10q21) were identified with significant evidence of linkage (LOD > 4.32) to one-third distal area, and three were identified with suggestive evidence of linkage (LOD > 2.93) to bone size in one skeletal site. Our results indicated that the low power of QTLs mapping for composite phenotypic measurements may result from genetic heterogeneity of complex traits.

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Qing-Yang Huang, Fu-Hua Xu, Hui Shen, Hong-Yi Deng, Theresa Conway, Yong-Jun Liu, Yao-Zhong Liu, Jin-Long Li, Miao-Xin Li, K. Michael Davies, Robert R. Recker, and Hong-Wen Deng. Genome scan for QTLs underlying bone size variation at 10 refined skeletal sites: genetic heterogeneity and the significance of phenotype refinement. Physiol Genomics 17: 326–331, 2004. First published March 23, 2004; 10.1152/physiolgenomics.00161.2002.—To identify quantitative trait loci (QTLs) underlying variation in bone size, we conducted a whole-genome linkage scan in 53 pedigrees with 630 subjects using 380 microsatellite markers. Lumbar area 1, 2, 3, and 4 at the spine, femoral neck, trochanter, intertrochanter areas at the hip, ultradistal, mid-distal, and one-third distal areas at the wrist were measured by dual-energy X-ray absorptiometry (DXA), and adjusted for age, height, weight, and sex. Two-point and multipoint linkage analyses were performed for skeletal bone size at each site and their composite measurements using the SOLAR package. Two chromosomal regions (1q22 and 10q21) were identified with significant evidence of linkage (LOD > 4.32) to one-third distal area, and three were identified with suggestive evidence of linkage (LOD > 2.93) to bone size in one skeletal site. Our results indicated that the low power of QTLs mapping for composite phenotypic measurements may result from genetic heterogeneity of complex traits.

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than or equal to −1.28 at the hip or spine. Herein we report results of a genome-wide linkage scan with the spine bone size, hip bone size, wrist bone size, and 10 refined components (lumbar 1 area, lumbar 2 area, lumbar 3 area, lumbar 4 area, femoral neck area, trochanter area, intertrochanter area, ultradistal radius area, mid-distal area, and one-third distal area). These linkage results are an important first step in localizing and identifying the genes influencing bone size.

SUBJECTS AND METHODS

Subjects. The study subjects came from an expanding database being created to search for genes underlying osteoporosis risk that is underway in the Osteoporosis Research Center of Creighton University. All study subjects were whites of European origin. Fifty-three pedigrees with 630 subjects (248 males and 382 females) from two to four generations were analyzed. The pedigrees range from 3 to 99 individuals, with a mean (SE) of 11.7 (2.4). In total, there were 1,249 sibling pairs, 1,098 grandparent-grandchild pairs, and 2,589 first cousin pairs. Each pedigree was identified through a proband having a BMD Z-score less than or equal to −1.28 at the hip or spine. Only healthy people were included in the analysis. The exclusion criteria (any disease, treatment, or condition that would be a nongenetic cause of low bone mass) are detailed in Deng et al. (14). About 5.1% of the total people screened were excluded from our study sample and were not counted in the 53 pedigrees and total of relative pairs. The study was approved by the Creighton University Institutional Review Board. All of the study subjects signed consent documents before entering the project.

Genotyping. Genomic DNA was extracted from whole blood using Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN). The 401 dinucleotide markers we used are commercially available through Perkin-Elmer Applied Biosystems (ABI Prism Linkage Mapping Sets Version 2). We successfully genotyped 380 markers (including 362 on autosomes). These markers have an average population heterozygosity of 0.79 and are spaced, on average, ∼8.6 cM. PCR cycling conditions followed those suggested in the ABI Prism Linkage Mapping Sets Version 2. PCRs for each marker were performed separately, and products were combined before gel electrophoresis. Genotyping was performed using Applied Biosystems automated DNA sequencing systems (models 377 and 310; Perkin Elmer-ABI, Foster City, CA) running the GeneScan and Genotyper software programs for allele identification and sizing. GenoDB, a genetic database management system developed by us (35), was employed to manage the phenotype and genotype data for linkage analyses. GenoDB is also employed for allele binning (including setting up allele binning criteria and converting allele sizes to distinct allele numbers), data quality control, and data formatting for PedCheck (45) and linkage analyses by SOLAR (“sequential oligogenic linkage analysis routines”) (3). Marker data for each pedigree were checked for Mendelian inheritance. Mendelian inconsistencies were detected and eliminated by using the PedCheck program (http://watson.hgen.pitt.edu/register/soft_doc.html). The genotyping error and missing rate, determined by the procedures described earlier (25), was about 0.3%. All reported family relationships have been confirmed with the genotyping results. The chromosomal orders and the intervals of markers were obtained from the ABI Prism Linkage Mapping Sets Version 2, which was based on the 1996 Genethon map (16).

Phenotyping. Lumbar areas 1, 2, 3, and 4 at the spine; femoral neck, trochanter, and intertrochanter areas at the hip; and ultradistal, mid-distal, and one-third distal areas at the wrist were measured chiefly by a Hologic 1000, 2000+, or 4500 scanner (Hologic, Waltham, MA). Bone density data obtained from different machines (105 from Lunar, and 32 from Norland) were transformed to a compatible measurement using the transformation formula described in Ref. 22, which was based on linear regression rules and was demonstrated to be reliable and efficient to calibrate bone measurements by different DXAs in our previous study (47). Members of the same pedigree were usually measured on the same type of machine. The spine bone size was expressed as the combined projected bone area of lumbar 1–4. The hip bone size was expressed as the combined projected bone area of the femoral neck, trochanter, and intertrochanteric area. The wrist bone size was expressed as the sum of the projected bone area of the ultradistal radius, mid-distal, and one-third distal radius. All DXA machines report bone size as area measurements in units of square centimeters. Usually, for the hip and wrist, the nondominant body side was measured. The coefficients of variation (CV) of the bone size measurement on the Hologic 4500 were 2.49, 2.26, 2.68, 2.32, 3.25, 4.37, 6.42, 2.36, 1.29, and 1.88%, respectively, at the lumbar areas 1, 2, 3, and 4, the femoral neck, trochanter, intertrochanter, ultradistal radius, and mid-distal and one-third distal areas, respectively. Height and weight were measured on the visit at which the bone size measurements were taken.

Statistical analyses. The variance component linkage analysis for quantitative traits was utilized (3–5). The program employed was SOLAR (http://www.sfrb.org/solar/). Although the analysis assumed joint multivariate normality of phenotypic values, the variance-component analyses implemented in SOLAR are generally robust to reasonable distributional violations (1, 26). Bone size, or their composite phenotype measurements (spine, hip, and wrist bone size) at 10 sites, had skewness coefficients of −0.23 to +0.73 and kurtosis coefficients of −0.52 to +0.76. Age, sex, weight, and height were used as covariates to adjust for bone size values in linkage analysis. Two-point and multipoint linkage analyses were performed for bone size at each skeletal site. According to the criteria of Lander and Kruglyak (34), the categories of significant and suggestive linkage correspond to LOD scores of at least 3.3 and 1.9, respectively. Empirical P values for single trait were estimated by simulation, using the procedure lodadj implemented in SOLAR (8). This approach samples the null distribution (the distribution of LOD scores obtained under the no linkage hypothesis) so that a sorted array of LOD scores is obtained and the proportion of LOD scores greater than the observed LOD score is the empirical P value. A total of 10,000 replicate samples were generated. Because 10 bone sizes that are highly correlated were analyzed, correcting for multiple analysis was performed as described by Camp and Farnham (9). First, we estimated the number of effectively independent analyses. Second, we established the genome-wide thresholds of “significant” and “suggestive” evidence for linkage for 10 traits, which were LOD scores of 4.32 and 2.93, respectively. The thresholds were conservative compared with those that were determined by performing simulations (9).

RESULTS

The pairwise phenotypic correlations among bone size at 10 skeletal sites, their composite measurements, and their heritabilities are summarized in Table 1. The correlation coefficient between femoral neck area at the hip and one-third distal area at the wrist is 0.349, and others are over 0.5. The square of the phenotypic correlation that approximates the proportion of variation of one trait that can be attributable to the other is in the range of 12–78%. Heritabilities of bone size at 10 skeletal sites and their composite measurements range from 29% (lumbar 4 area) to 76% (one-third distal area), indicating that a substantial portion of the variation in bone size is attributable to additive genetic factors. Results for the chromosomes giving maximum LOD > 2.93 in multipoint linkage analyses at any one of the 10 skeletal sites or their composite phenotypic measurements are shown in Table 2. Results for chromosomes giving significant evidence of linkage (LOD > 4.32) in multipoint linkage analyses at any one of the 10 skeletal sites are presented in Fig. 1.
One-third distal area 10q21 (83) 4.9 1.00

L1 area 2p25 (22) 2.98 1.06 × 10⁻⁴ 0.90 STK, POMC
L3 area 11q15 (2) 3.68 1.90 × 10⁻⁴ 0.20 PTH, CALCA, IGFl, P57
Ultradistal area 17q23 (77) 3.01 9.80 × 10⁻⁴ 0.84 COL1A1, HOXB1-B13, TBX2, SOX9, NOG, GH1
One-third distal area 10q21 (83) 4.9 1.00 × 10⁻⁶ 0.01 COL1A1, BMP1A, NFkB2, FGF2
1q22 (176) 4.78 1.00 × 10⁻⁶ 0.02 CTSK, DDR2, THBS3

Values are means ± SE, where noted; h², heritability.

DISCUSSION

Genome-wide searches for loci influencing complex human traits and diseases such as bone size and osteoporosis are often plagued by low power and interpretive difficulties (2). One approach to remedy these difficulties is to subdivide phenotypes into intermediate phenotypes. In the present study, we conducted a genome scan for QTLs underlying bone size variation in 53 pedigrees ascertained through a proband having BMD Z-scores less than or equal to −1.28 at the hip or spine. We analyzed the refined components (lumbar areas 1, 2, 3, and 4, femoral neck, trochanter, and intertrochanter areas; ultradistal, mid-distal, and one-third distal areas) and composite phenotypic measurements (spine bone size, hip bone size, and wrist bone size) separately. For the refined components, we identified two chromosomal regions (1q22 and 10q21) with significant evidence of linkage and three chromosomal regions with suggestive evidence of linkage. It seems that QTL mapping for composite phenotypic measurements has low power compared with the refined components (Table 2). Substantial increases in power to map loci (dissection of a novel locus and increase in LOD score) can be obtained by phenotype refinement.
An important finding of our study was genetic heterogeneity of bone size among spine, hip, and wrist sites, as well as within spine, hip, and wrist sites, which may reflect differences in their developmental and genetic control. One classic example is "short fingeredness," a heritable trait caused by a dominant mutation in a single gene. A defect in this one gene reduces the second bone of the second finger, which means that the normal gene controls the size and shape of just this one bone (see figure 1.1 in Ref. 21). Recently, genes affecting osteoblast and osteoclast differentiation have been identified (54). Different QTLs identified here may influence bone size at the refined skeletal sites by upregulating or downregulating the expression of these genes. Previous studies have indicated that high phenotypic correlations do not mean high genetic correlations. Deng et al. (12) have shown that although there exists significant phenotypic correlation between BMD and osteoporotic fractures, the genetic correlation between them is low and not significant. Significant phenotypic correlations are largely due to environmental correlations. Although there exists significant correlation between the skeletal sizes, whether there exist significant genetic correlations between them is unknown.

Even at the phenotypic level, the square of the phenotypic correlation that approximates the proportion of variation of one trait that can be attributable to the other is in the range of 12–78% from the data in Table 1. Genetic heterogeneity of bone size at refined skeletal sites may at least partially explain the low power of QTL mapping of composite phenotypic measurements and difficulties in replication of genome scan linkage results observed in mapping complex disease.

In the search for genes underlying complex traits, the researcher is faced with the problem of how to set up the significant level. In our study, many markers and correlated phenotypes have been tested. To control the genome-wide false-positive rate, we employed the method of Camp and Farnham (9) to get the genome-wide \( P \) value for all the traits (experiment wide). Our results indicated that the rate of false positives for 10 phenotypes in a whole genome scan was \( \sim 0.01–0.9 \) per genome scan at regions with LOD scores greater than 2.93 in multipoint analyses for bone size variation.

The strongest evidence for linkage in our sample was to chromosomes 1q22 (multipoint LOD = 4.78) and 10q21 (multipoint LOD = 4.9) with one-third distal area. The region of
linkage on chromosome 1q extends over an ~40-cM interval between D1S196 and D1S2726, where consistent evidence for linkage across multiple markers was obtained in the two-point analyses (data not shown, Fig. 1). The region homologous to human 1q21–23 was implicated in BMD variation in the mouse (6, 7). Chromosome 10q includes a variety of plausible candidate genes, bone morphogenetic protein receptor, type 1A (BMPR1A), fibroblast growth factor receptor 2 (FGFR2), nuclear factor κB, subunit 2 (NFKB2), and collagen type Vα3 (COLUMBAR13A1) (Table 2). Klein et al. (30, 31) reported two QTLs for whole body BMD in the mouse in regions homologous to human 10q21–24 (P < 0.0007) and 10q23–26 (P < 0.0093). Beamer et al. (7) also reported a QTL for L5 with a LOD score of 5.01 in mice in the regions homologous to human 10q25–26.

Chromosome 11p15, which showed suggestive evidence of linkage with lumbar 3 area, is of particular interest because it includes several well-known candidate genes, insulin-like growth factor 2 (IGF2), parathyroid hormone (PTH), P57, and calcitonin (CALCA), which have previously been associated with BMD or osteoporotic fracture (18, 41, 52). The region 17q23, where we observed suggestive evidence for linkage to the ultradistal radius area (multipoint LOD = 3.01), contains several interesting candidate genes. These include type 1 collagen (COL1A1), chondroadherin, and HOXB (Table 2). The gene COL1A1 is the only gene for which there is convincing evidence to suggest it plays a role in the determination of bone density and fracture risk (19, 36). Chondroadherin is involved in cartilage formation and maintenance (29). HOX genes have evidence to suggest it plays a role in the determination of bone architecture in several interesting candidate genes. These include type 1 collagen (COL1A1), chondroadherin, and HOXB (Table 2). The gene COL1A1 is the only gene for which there is convincing evidence to suggest it plays a role in the determination of bone density and fracture risk (19, 36). Chondroadherin is involved in cartilage formation and maintenance (29). HOX genes have evidence to suggest it plays a role in the determination of bone architecture in numerous animal systems (21). Koller et al. (33) have also reported significant linkage of this region with femur head width (LOD = 3.6). Region 2p25 showed suggestive evidence of linkage with lumbar 1 area (multipoint LOD = 2.98) and some signals of linkage with femoral neck area (multipoint LOD = 1.89). Beamer et al. (7) detected a QTL for femur BMD (LOD = 2.89) in mice in the regions homologous to human 2p22–25. More importantly, Devoto et al. (15) reported a multipoint LOD score of 2.25 for 2p23–24 for spinal BMD, and Niu and colleagues (44) found linkage evidence of 2p23–24 with forearm BMD with a LOD score of 2.15 in a Chinese population. The candidate genes in this region include serine threonine kinase (STK) and pro-opiomelanocortin (POMC).

Several other regions deserve mention. 4q22–23 showed some signals of linkage to lumbar 2 area (multipoint LOD = 1.92). Koller et al. (33) also reported evidence of linkage of chromosome 4q with femur neck axis length (LOD = 3.9) and with the midshaft femur width (LOD = 3.5). Additionally, 19p11–13 and 9q21 that showed some signals of linkage to the intertrochanter and ultradistal radius areas, respectively, correspond to regions identified by Koller et al. (33).

In summary, we have identified two chromosomal regions with significant evidence of linkage and three chromosomal regions with suggestive evidence of linkage to bone size or their composite phenotypic measurements (spine, hip, and wrist bone size) at 10 sites, and we have demonstrated the genetic heterogeneity of complex traits. Furthermore, we found some interesting overlaps between our QTL linkage results and results from Koller et al. (33). We have targeted these regions for further replication, fine mapping, and gene-identification studies. Bone size is an important risk factor for osteoporosis. Identification of QTLs underlying bone size variation represents our effort in searching for osteoporosis genes using various relevant phenotypes (including osteoporotic fractures per se). We believe that this sort of multipronged approach is useful in identifying genes underlying osteoporosis or any complex genetic disease.

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