

A Whole-Genome Linkage Scan Suggests Several Genomic Regions Potentially Containing Quantitative Trait Loci for Osteoporosis

HONG-WEN DENG, FU-HUA XU, QING-YANG HUANG, HUI SHEN, HONGYI DENG, THERESA CONWAY, YONG-JUN LIU, YAO-ZHONG LIU, JIN-LONG LI, HAI-TAO ZHANG, K. M. DAVIES, AND ROBERT R. RECKER

Osteoporosis Research Center (H.-W.D., F.-H.X., Q.-Y.H., H.S., H.D., T.C., Y.-J.L., Y.-Z.L., J.-L.L., H.-T.Z., K.M.D., R.R.R.) and Department of Biomedical Sciences (H.-W.D., F.-H.X., Q.-Y.H., H.S., H.D., Y.-J.L., Y.-Z.L., J.-L.L., H.-T.Z.), Creighton University, Omaha, Nebraska 68131; and Laboratory of Molecular and Statistical Genetics (H.-W.D.), College of Life Sciences, Hunan Normal University, ChangSha, Hunan 410081, People's Republic of China

Osteoporosis is an important health problem, particularly in the elderly women. Bone mineral density (BMD) is a major determinant of osteoporosis. For a sample of 53 pedigrees that contain 1249 sibling pairs, 1098 grandparent-grandchildren pairs, and 2589 first cousin pairs, we performed a whole-genome linkage scan using 380 microsatellite markers to identify genomic regions that may contain quantitative trait loci (QTL) of BMD. Each pedigree was ascertained through a proband with BMD values belonging to the bottom 10% of the population. We conducted two-point and multipoint linkage analyses. Several potentially important genomic regions were suggested. For example, the genomic region near the marker D10S1651 may contain

a QTL for hip BMD variation (with two-point analysis LOD score of 1.97 and multipoint analysis LOD score of 2.29). The genomic regions near the markers D4S413 and D12S1723 may contain QTLs for spine BMD variation (with two-point analysis LOD score of 2.12 and 2.17 and multipoint analysis LOD score of 3.08 and 2.96, respectively). The genomic regions identified in this and some earlier reports are compared for exploration in extension studies with larger samples and/or denser markers for confirmation and fine mapping to eventually identify major functional genes involved in osteoporosis. (*J Clin Endocrinol Metab* 87: 5151–5159, 2002)

LOW BONE MINERAL density (BMD) is an important risk factor for osteoporotic fractures (OF), and osteoporosis is mainly characterized by low BMD (1–3). In the United States alone in 1995, osteoporosis results in more than 1.3 million OF a year, with an estimated direct cost of about 14 billion dollars (4). Extensive data have established that BMD variation is under strong genetic control with heritability estimates ranging from 0.5–0.9 (5–17). Recently, extensive molecular genetic studies (18–29) have been launched to search for genes underlying BMD variation. The extensive results from population association studies of candidate genes so far have been largely inconsistent (30–32). It is known that population admixture may induce false positive (33) or false negative (34) results in association studies of candidate genes.

Compared with the extensive association studies, only a limited few studies of alternative approaches such as linkage studies (20, 23, 24, 35, 36) and transmission disequilibrium test (37, 38) in humans and quantitative trait locus (QTL) mapping (27–29, 39–41) in mice have been conducted to identify genomic regions underlying population BMD variation. The 7 pedigrees studied (35) (identified through probands with low BMD) in an autosomal genome scan contain 74 sibling pairs genotyped for 330 markers. A total of 153 sibling pairs (randomly ascertained with respect to BMD) were studied in the genome-wide scan (36) using 367 markers (with an average heterozygosity of

~0.77). A total of 595 sibling pairs (randomly ascertained) were used (20), with 270 markers (average heterozygosity being ~0.70) genotyped throughout the genome. Sibling pair approach for linkage studies is usually of limited power when sibling pairs are ascertained randomly (42–44). For example, more than 8000 randomly ascertained sibling pairs are necessary in a whole genome scan to detect a major locus with a heritability as large as 30% (44). The limited power for the small samples of the previous sibling pair linkage studies and the few but the largely inconsistent empirical linkage findings warrant that independent studies with alternative linkage approaches and larger samples be conducted. Generally speaking, the linkage detecting power increases rapidly with the marker heterozygosity, reduced genotyping error rate, and marker density that may reflect expected genetic distance between markers and QTLs (45, 46). Sampling through extreme probands may improve the linkage power (47, 48).

In this study, for a relatively large sample of human pedigrees that was recruited through probands with low BMD values, we genotyped 380 highly heterozygous markers across the whole genome for each subject. We performed a whole-genome linkage analysis to identify genomic regions potentially important for BMD variation.

Materials and Methods

Subjects

The study was approved by the Creighton University Institutional Review Board. All the study subjects signed informed consent documents before entering the project. Only healthy people (defined by the

Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; cM, centimorgans; MLS, maximum LOD scores; OF, osteoporotic fractures; pter, p terminal; QTL, quantitative trait locus.

exclusion criteria to be detailed below) were included in the analyses. All the study subjects were Caucasians of European origin as ascertained by questionnaires administered by research nurses. A total of 53 pedigrees with 630 subjects (248 males and 382 females) from 2–4 generations were analyzed. There are more female than male subjects, largely due to the higher propensity to volunteer in a genetic study of this type among females. Among the study pedigrees, 1 is 2-generation, 41 are 3-generation, and 11 are 4-generation pedigrees. The pedigrees vary in size from 3–99 individuals, with a mean of 11.7 (\pm SE = 2.4). The pedigrees were identified through a proband having BMD Z-scores -1.28 or less at the hip or spine. Hence, the probands were selected from the bottom 10% of the population BMD variation with the intended purpose of achieving higher statistical power than random sampling (47, 48). BMD values expressed as Z-scores adjust for age, gender, and ethnic difference in general referent healthy populations. The exclusion criteria for the study subjects were a history of (including past as well as current disease conditions, unless otherwise specified) the medical conditions that were detailed previously (23, 49). The exclusion criteria were assessed by nurse-administered questionnaires and/or medical records and applied most rigorously to potential subjects contacted between ages 25 and 50. About 5.1% of the total people screened were excluded from our recruitment due to their meeting at least one of the exclusion criteria.

Genotyping

For each subject, blood (20 cc) was drawn into lavender cap (EDTA containing) tubes by certified phlebotomists. DNA was extracted by employing a kit (Puregene DNA Isolation Kit, Gentra Systems, Inc., Minneapolis, MN; catalog no. D-5000) following the procedures detailed therein. DNA was genotyped using fluorescently labeled markers commercially available through PE Applied Biosystems (ABI PRISM Linkage Mapping Sets, version 2, Norwalk, CT), as we did before (49, 50). A genetic database management system (GenoDB) (51) was employed to manage the genotype data for linkage analyses. PedCheck (52) (available at http://watson.hgen.pitt.edu/register/soft_doc.html) was employed for checking the conformation to Mendelian inheritance pattern at all the marker loci and for checking the relationships of family members within pedigrees. The genotyping error rate, determined by the procedures described earlier (59, 51, 53), was about 0.3%. A total of 380 markers (including 362 on autosomes) were successfully genotyped. These markers have an average population heterozygosity of approximately 0.79.

Measurement

BMDs of spine, hip, and wrist were measured by a Hologic 1000, 2000+, or 4500 dual energy x-ray absorptiometry scanner (Hologic Corp., Waltham, MA). All machines are calibrated daily, and long-term precision is monitored with external spine and hip phantoms. Short-term precision in humans as determined by the method of Glüer *et al.* (54) with repeated measurements on at least 27 subjects for a single type of machine is 0.8% for spine BMD, 0.6% for hip BMD, 1.1% for wrist BMD on Hologic 1000; and 0.8% for spine BMD, 1.0% for hip BMD, 2.6% for wrist BMD on Hologic 2000+; and 0.9% for spine BMD, 1.4% for hip BMD, and 2.3% for wrist BMD on Hologic 4500. We chose to study BMD because BMD is the measure most closely correlated with fracture risk (55). In addition, more than 86% and about 98% of BMD variation are attributable to bone mineral content (BMC) variation at the spine and hip, respectively (56), and the majority of the variation of BMD and BMC at the spine, hip, and wrist can be accounted for by one principle component in factor analyses (11). Our analyses (57, 58) show that the genomic regions for BMD and bone size are largely different, whereas those for BMD and BMC are largely the same. For the spine, our quantitative phenotype was combined BMD of L₁₋₄. For the hip, it was total BMD of the femoral neck, trochanter, and intertrochanteric region provided by the instruments. For the wrist, it was ultradistal BMD. All dual energy x-ray absorptiometry machines report BMD in g/cm². Weight was measured at the same visit when the BMD measurements were taken. Data obtained from different machines are transformed to a compatible measurement by an algorithm as in our previous studies (59), and members of the same pedigree are usually measured on the same type of machine.

Statistical analyses

A variance component linkage analysis (60–62) for quantitative traits was performed. The analysis is based on specifying the expected genetic covariances between arbitrary relatives as a function of the identity by descent at a given marker locus. The analysis considers the phenotypic and genetic information from all pedigree members simultaneously. The analysis assumed joint multivariate normality of phenotypic values, additive genetic effects, and no interaction between genes and the residual variation. The common familial environmental effects were assumed to be negligible, which is reasonable and supported by previous studies (7–9, 11, 12). The program employed was SOLAR (Sequential Oligogenic Linkage Analysis Routines) (62), which is available at <http://www.sfbr.org/sfbr/public/software/solar/>. The ascertainment scheme of pedigrees based on the low BMD values of probands was accounted for in the analyses by identifying to the program the probands and their BMD values for each pedigree. The built-in modules of the SOLAR program can account for the ascertainment scheme by using cut-off BMD values of the probands and conditional likelihoods in LOD score computation.

In linkage analysis, age, sex, and weight were adjusted as covariates for raw BMD values (not the Z-scores), as these generally affect BMD variation (10, 63) and tested to be significant in our sample in a statistical screen for important covariates. Z-scores of BMD reported by the Hologic machines adjust for age and sex (but not weight) effects in referent populations provided by the manufacture of the scanners and our BMD phenotype data for linkage analyses are those adjusting the raw BMD data for age, sex, and weight according to their effects in our own sample. Analyses were also performed without adjusting for some or any of these covariates. Adjustment for significant covariates in genetic analyses can generally increase the genetic signal to noise ratio in linkage detection by decreasing the proportion of the residual phenotypic variation attributable to random environmental factors (22). Comparison of the analyses with and without adjustment for a significant covariate may shed light on the genomic regions identified as to their direct importance for the trait *per se* or indirect importance via the influence on the covariates only. The BMD data were tested by graphical methods (64) and found not to deviate from normal distributions. The variance component analyses implemented in SOLAR are quite robust to reasonable violations of normality of the data (65). Using SOLAR, two-point, and multipoint linkage analyses were performed. When a putative QTL is suggested, the proportion of phenotypic variation attributable to this QTL can be estimated by SOLAR. The estimate is usually an upper bound of the genetic effect due to the locus (66).

Results

The basic characteristics of the study subjects stratified by age and sex are summarized and the data are available upon request. Detailed information about the family structure of the study pedigrees has been summarized earlier (49). Mainly, there are 1249 sibling pairs, 1098 grandparent-grandchild pairs, and 2589 first cousin pairs, *etc.* The average Z-scores (\pm SE) of spine, hip, and wrist BMD are -0.12 ± 0.05 , -0.22 ± 0.04 , and 0.09 ± 0.05 , respectively, for all the subjects in the sample, reflecting the sampling scheme of our pedigrees which were ascertained through probands of extremely low BMD values at spine and/or hip.

The results for the chromosomes that had maximum LOD scores (MLS) close to or greater than 2.0 in multipoint linkage analyses at any one of the three skeletal sites were summarized in Fig. 1; the results for the other chromosomes were summarized in Fig. 2. Because the current version of SOLAR does not handle multipoint analyses for X chromosome, we plotted two-point LOD score results for the X chromosome and presented them in Fig. 2 to convey the linkage signal pattern. For the results presented, BMD were adjusted with multiple regression for significant covariates of age, sex, weight, and height. The results (not presented but available

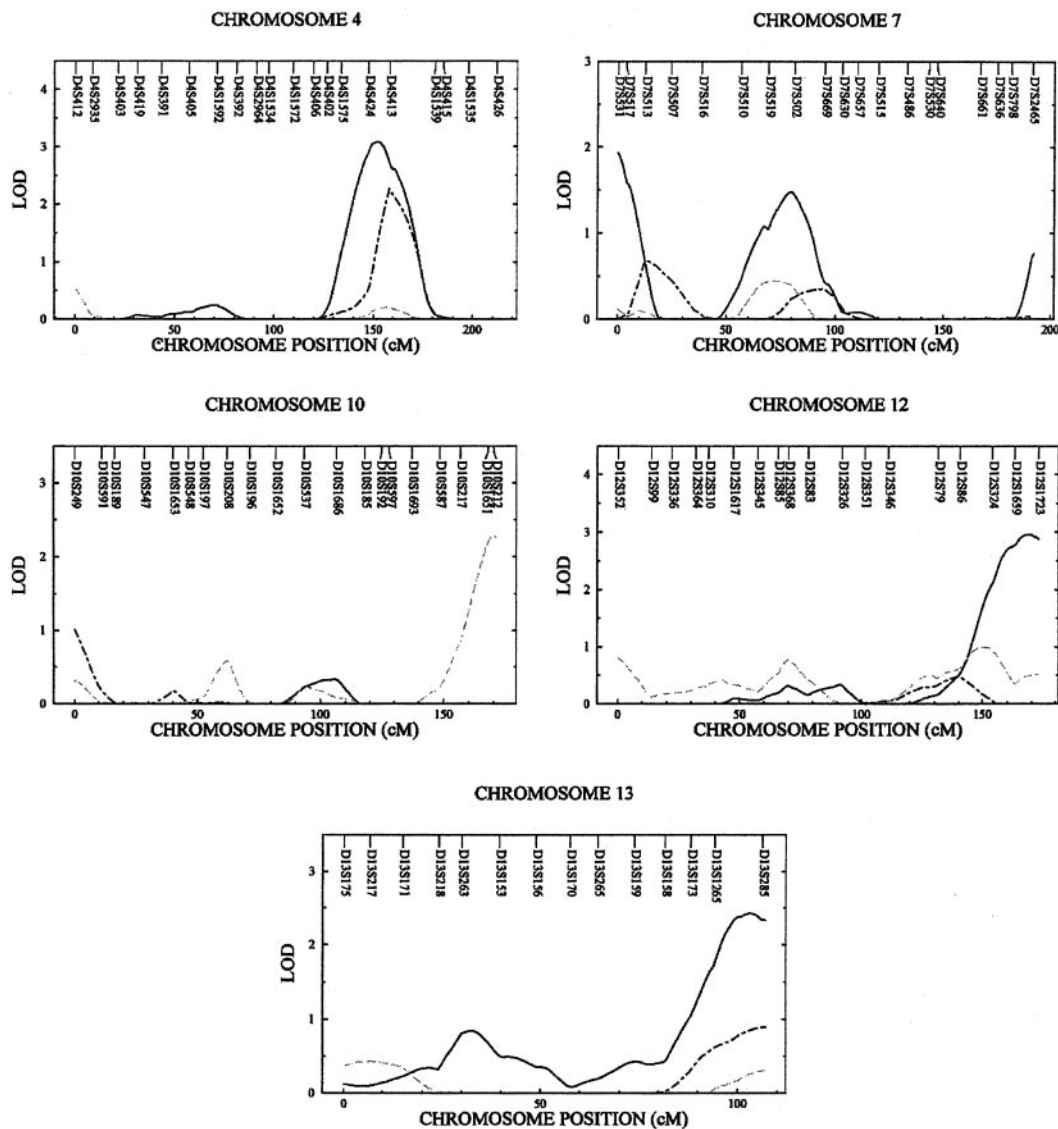


FIG. 1. Multipoint linkage analysis results for the chromosomes that had an MLS close to or greater than 2.0. In Figs. 1 and 2, the *solid line* is for spine BMD, the *dashed line* is for hip BMD, and the *dot dashed line* is for wrist BMD.

upon request) for the linkage analyses for BMD values that were not adjusted for the covariates showed similar pattern in LOD scores and qualitatively the same results, suggesting the importance (if indeed exists) of the genomic regions identified here is direct on BMD rather than indirect through a covariate analyzed. In fact, our whole genome linkage scan results (49) for body mass index (which is weight/height²) revealed genomic regions for body mass index different from those revealed here for BMD.

For the spine BMD, four genomic regions were identified that may contain putative QTLs. Specifically, an MLS of 3.08 at 152 centimorgans (cM) from p terminal (pter) on chromosome 4 in multipoint linkage analyses and a LOD score of 2.12 at the marker D4S413 in two-point linkage analyses were achieved in the genomic region 4q31–32. An MLS of 2.96 at 169 cM from pter on chromosome 12 in multipoint analyses and an MLS of 2.17 at D12S1723 in two-point analyses were achieved in 12q24. An MLS of 2.43 at 103 cM from pter on

chromosome 13 in multipoint analyses and a LOD score of 1.77 at D13S285 in two-point analyses were achieved in 13q33–34. An MLS of 1.93 at 0.0 cM from pter on chromosome 7 in multipoint analyses and a LOD score of 2.28 at D7S531 in two-point analyses were achieved in 7p22. In addition, in two-point analyses, a LOD score of 1.6 was achieved at D15S165 in 15p11; however, the MLS is only 0.76 in the genomic region (14cM from pter on chromosome 15). At the spine, for the putative QTL with the strongest evidence of linkage on 4q31, approximately 30.3% of BMD variation (after adjusting for age, sex, weight, and height) may be attributable to this locus.

For the hip BMD, three genomic regions were identified that may contain putative QTLs. An MLS of 2.29 at 170 cM from pter on chromosome 10 in multipoint analyses and a LOD score of 1.97 at D10S1651 in two-point analyses were achieved in 10q26. In addition, in two-point linkage analyses, a LOD score of 1.69 was achieved at D12S368 on 12q13 and

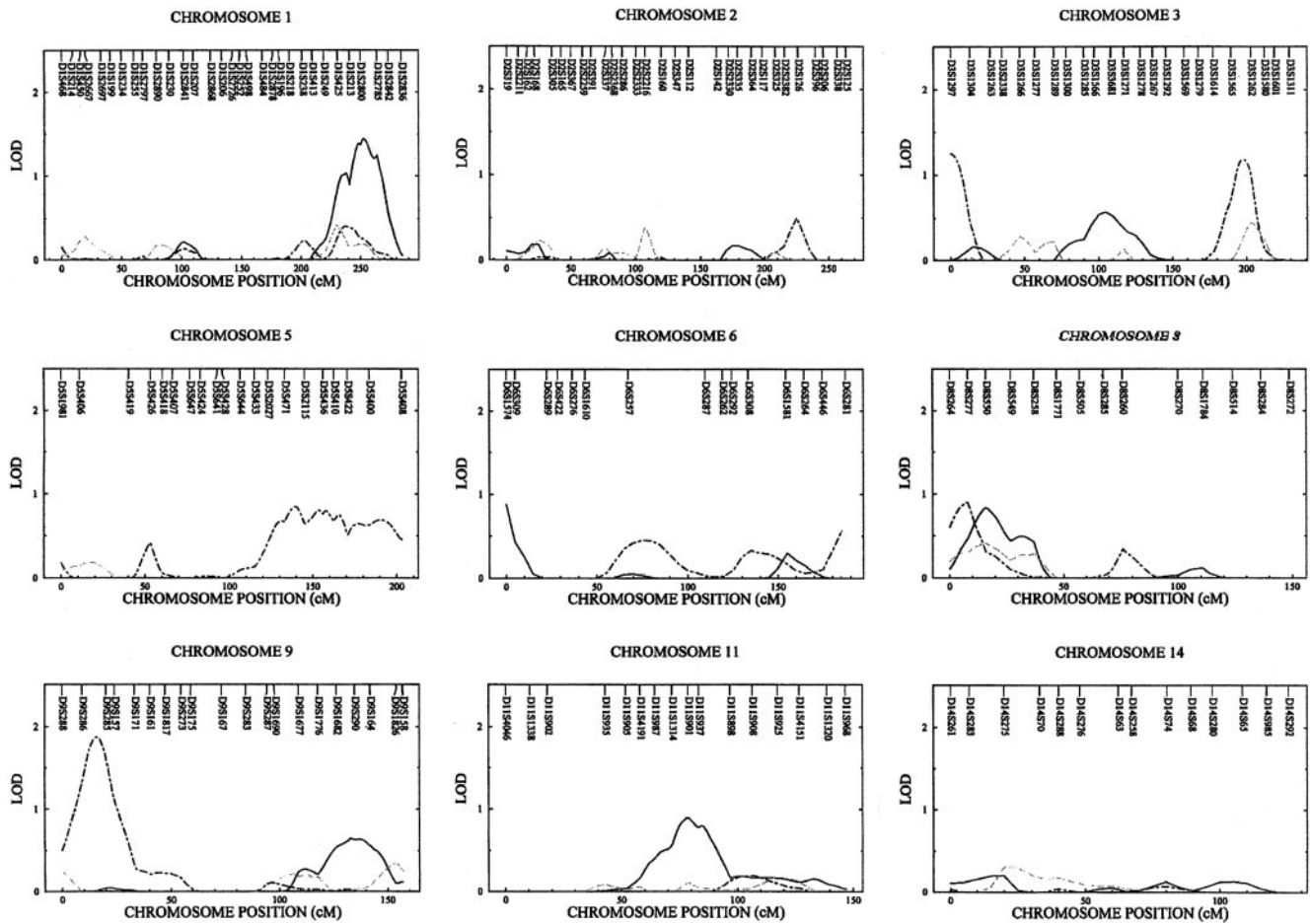


FIG. 2. Multipoint linkage analysis results for the autosomes that had an MLS less than 2.0 and the two-point linkage analysis results for the X chromosome.

a LOD score of 1.58 was achieved at D17S1857 on 17p11, with corresponding multipoint MLSs being 0.99 and 1.2, respectively. At the hip, for the putative QTL with the strongest evidence of linkage on 10q26, approximately 29.2% of BMD variation (after adjusting for age, sex, weight, and height) may be attributable to this locus.

For the wrist BMD, four genomic regions were identified that may contain putative QTLs. An MLS of 2.26 in multipoint analyses at 158 cM from pter on chromosome 4 and a LOD score of 2.53 at D4S413 in two-point analyses were achieved in 4q32. An MLS of 1.87 in multipoint analyses at 16 cM from pter on chromosome 9 and an LOD score of 1.74 in two-point analyses at D9S285 were achieved in 9p22–24. An LOD score of 1.99 was achieved at D17S1852 in 17p13, and a LOD score of 1.82 in two-point analyses was achieved at D3S1297 in 3p26, with corresponding MLSs of 1.48 and 1.25 achieved in multipoint analyses in the respective genomic regions. At the wrist, for the putative QTL with the strongest evidence of linkage on 4q32, approximately 25.5% of BMD variation (after adjusting for age, sex, weight, and height) may be attributable to this locus.

Discussion

Some of the regions we identified are supported by earlier findings or contain important candidate genes (Tables 1 and

2). For the spine BMD, four genomic regions (4q31–32, 7p22, and 13q33–34, and 12q24) were identified. Drake *et al.* (2001) (35) detected a QTL region for femur BMD with an LOD score greater than 2.3 in mice in regions homologous to human chromosome 12q24. IGF1, TBX3, and TBX5 genes that are of importance in BMD metabolism are located in the 12q24 region (Table 1). Several studies have suggested the importance of IGF1 in osteoporosis (67, 68). The genomic region 13q33–34 has previously (36) been linked to forearm BMD with a LOD score of 1.67 (Table 2). Potential candidate genes in this region include COL4A1 and COL4A2 (Table 1). Additionally, a candidate gene IL6 was mapped to 7p22. Duncan *et al.* (24) obtained a two-point LOD score of 1.7 at D12S83 for lumbar spine BMD. Our results for D12S83 do not provide supporting evidence of linkage. However, for the marker D12S368 (in 12q13) that is 8.2 cM from D12S83, a two-point LOD score of 1.69 is obtained for the hip BMD in our analyses. The genomic region near D12S83 and D12S368 contain a number of candidate genes, including the prominent vitamin D receptor gene (Table 1). For the three genomic regions (10q26, 12q13, and 17p11) identified for hip BMD, in mice in a genomic region homologous to human chromosome 10q26, Klein *et al.* (26) identified a QTL region for whole body BMD ($P = 0.0093$) and Beamer *et al.* (39) identified a QTL region for vertebral BMD with a LOD score of 5.01.

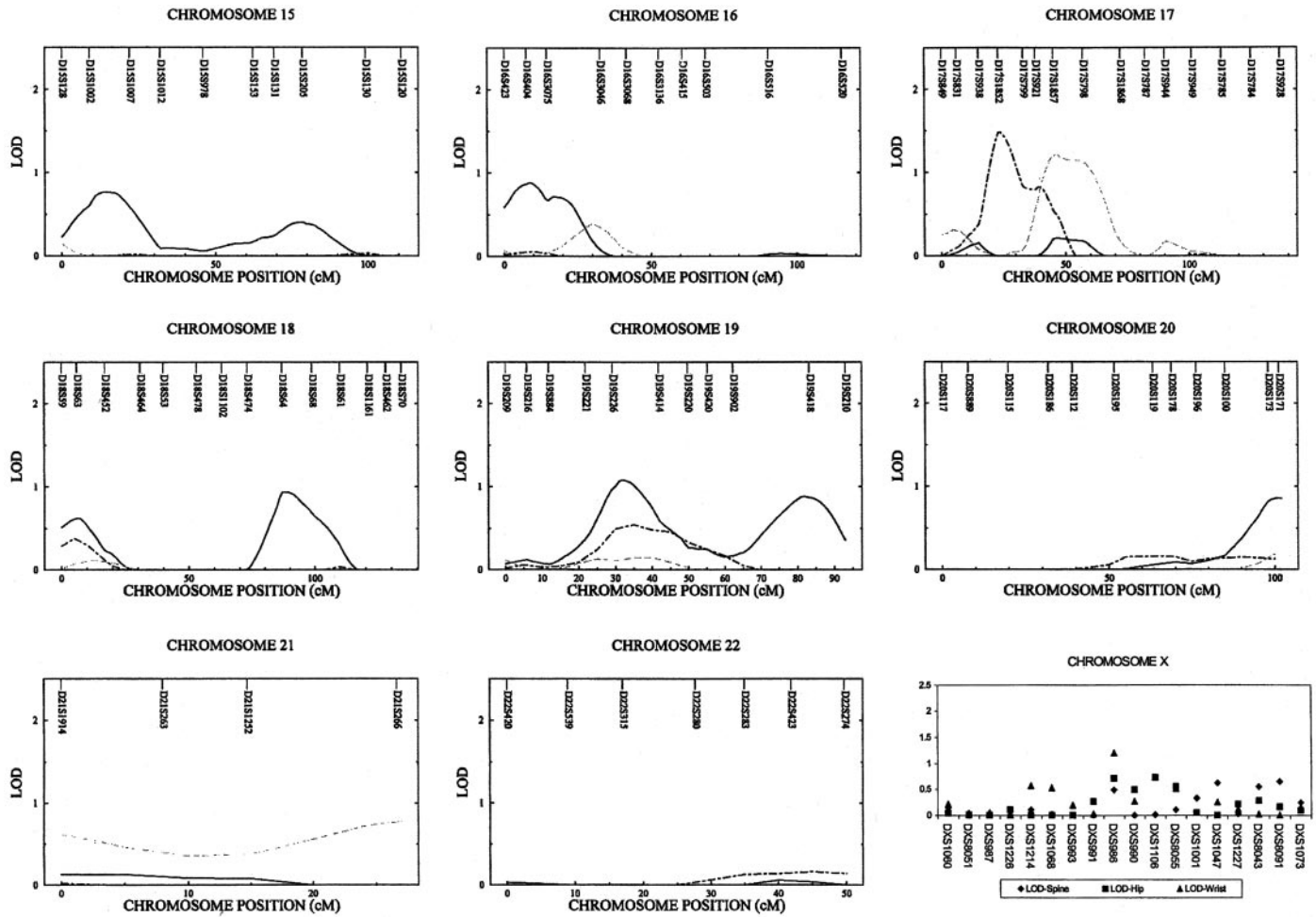


FIG. 2. Continued.

For the four genomic regions (3p26, 4q32, 9p22–24, and 17p13) identified for wrist BMD, 4q32 coincides with the region identified for spine BMD, and 17p13 coincides with the region identified for hip BMD in our sample. These results suggest that some genetic factors underlying BMD variation at different skeletal sites are located closely in the human genome or are shared. In the region homologous to human chromosome 3p26, Beamer *et al.* (39) identified in mice a QTL region for femoral BMD with a LOD score of 4.56. Devoto *et al.* (35) reported a LOD score of 2.34 near D17S261 for hip BMD, which is in the region 17p11–13 identified for hip and wrist BMD in our study. In the region homologous to human chromosome 17p11–12 in mice, Beamer *et al.* (39) identified a QTL region for femoral and vertebral BMD variation with LOD scores of 6.76 and 2.98, respectively. A QTL for femur peak bone mass in the mouse genomic region homologous to human chromosome 17p11–13 was identified with a LOD score of 10.8 (40).

However, comparing the few earlier whole-genome linkage studies of BMD in humans (20, 35, 36) and our study here, most of the results cannot be confirmed by each other. Table 2 outlines the comparison of the earlier results and ours. Our results seem to replicate the linkage findings on 4q and 13q (Table 2) because confirmation of a previously reported link-

age in an independent study involves *P* value criteria different from the whole-genome linkage scan (69). Replication of whole genome linkage studies is difficult due to the potential polygenic nature of inheritance and the limited power associated with the current whole-genome linkage studies (23). A preliminary comparison of mice and human mapping studies for osteoporosis has been made earlier (70). Mitchell *et al.* (71) studied serum osteocalcin concentrations, which is associated with bone loss and turn over rates that are related to BMD. A whole genome search in 429 subjects from 10 randomly ascertained large multigeneration pedigrees identified linkage of genomic regions on 16q and 20q to serum osteocalcin concentrations (71). These two regions differ from those regions identified for BMD variation in earlier whole genome studies (20, 35, 36) and our results here.

The power and the robustness of the linkage results critically depend on the study sample sizes employed, among several other things. Generally, the larger the sample, the higher the power and thus the more robust the results. For the same number of subjects involved, pedigree studies may have dramatically increased power over sibling pair studies (72). The LOD scores reported by earlier whole-genome linkage scan studies with smaller samples are sometimes higher than our current study (Table 2). However, this does not

TABLE 1. Markers and genomic regions with LOD scores > 1.5 in two- or multi-point analyses and the identified candidate genes

a. Two-point analyses						
Skeletal site	Marker	Chromosomal location	LOD score	Candidate genes	Cellular function	
Spine	D4S413	4q32 (157.9 cM from pter)	2.12	IL6	Regulating osteoclast and osteoblast function (79)	
	D7S531	7p22 (7.8 cM from pter)	2.28			
	D12S1723	12q24 (167.2 cM from pter)	2.17	IGF1	Prenatal and postnatal growth regulation	
				TBX3		Involved in posterior elements of limb development
D13S285	13q34 (112.8 cM from pter)	1.77	TBX5	Involved in heart and anterior elements of limb development		
			COL4A1, COL4A2	Collagen of basement membrane associated with laminin, entactin, and heparan sulfate proteoglycans to form basement membranes that separate epithelium from connective tissue		
Hip	D15S165	15p11 (20.2 cM from pter)	1.6	FGFR2	Involved in tyrosine kinase activation and signal transduction	
	D10S1651	10q26 (178.3 cM from pter)	1.97			
	D12S368	12q13 (69.9 cM from pter)	1.69	VDR	Involved in the synthesis of osteocalcin, the most abundant noncollagenous protein in bone, a major regulator for bone and calcium metabolism	
				COL2A1	Collagen of cartilage and vitreous	
				ITGA7	Specific cellular receptor for the basement membrane proteins laminin-1, -2, and -4; provide indispensable linkage between muscle fibers and extracellular matrix; clustering of integrin and Hox genes implies parallel evolution of these gene families	
	HOXC4, HOXC6	HOXC4, HOXC6	HOXC4, HOXC6	HOXC4, HOXC6	Determine embryonic cell fate; involved in mouse anterior and posterior limb patterning	
					HOXC5	Determine embryonic cell fate; involved in patterning of mouse anterior limb; a target for regulation by retinoic acid and HOX homeoproteins
					HOXC8	Determine embryonic cell fate; involved in mouse anterior and posterior patterning and innervation of the limb; may be involved in chondrocytic differentiation
					HOXC9, HOXC10, HOXC113	Determine embryonic cell fate; involved in mouse posterior limb patterning
	Wrist	D17S1857	17p11 (44 cM from pter)	1.58	HOXC12	Determine embryonic cell fate
D3S1297		3p26 (2.5 cM from pter)	1.82			
D4S413		4q32 (157.9 cM from pter)	2.53			
D9S285		9p22 (27.9 cM from pter)	1.74			
D17S1852		17p13 (23.2 cM from pter)	1.99			
b. Multipoint						
Skeletal site	Chromosome location		MLS	Candidate genes	Cellular function	
Spine	4q31 (152 cM from pter)		3.08	See above	See above	
	7p22 (0 cM from pter)		1.93			
	12q24 (169 cM from pter)		2.96			
Hip	13q33 (103 cM from pter)		2.43	See above	See above	
	10q26 (170 cM from pter)		2.29			
Wrist	4q32 (158 cM from pter)		2.26	See above	See above	
	9p24 (16 cM from pter)		1.87			

Unless otherwise specified, the candidate genes (if indicated) in the genomic regions, together with their cellular functions, are identified via the skeletal gene database (80).

TABLE 2. Summary and comparison of linkage results reported

Studies	BMD	Marker or genomic regions	LOD score or MLS (authors)	Our LOD score			Closest marker (cM)	
				Hip	Spine	Wrist		
Devoto <i>et al.</i> (35)	Hip	1p36	2.29	0.00	0.30	0.15		
		D1S450	3.51	0.21	0.01	0.01		
		D1S214	2.62	0.04	0.00	0.00		
		4q32–34	2.28	3.08	0.20	2.26		
		D4S1535	2.74	0.00	0.01	0.00		
		D7S558	2.99	0.00	0.14	0.05	D7S657 (1.23)	
		D17S261	2.34	1.58	0.76	0.08	D17S1857 (1.9)	
		D18S42	2.58					
		D18S70	2.14	0.07	0.00	0.00		
		CD3D	>1.9					
		Spine	2p23–24	2.25	0.20	0.21	0.01	
			D2S149	2.07	0.11	0.00	0.19	D2S305 (4.83)
			D2S144	1.49	0.00	0.00	0.00	D2S165 (2.13)
			D2S71	1.72	0.00	0.00	0.00	D2S112 (2.9)
D4S1539	2.95		0.00	0.00	0.38			
Niu <i>et al.</i> (36)	Proximal and distal forearm	2p21.1–24	2.15	0.20	0.21	0.01		
		D2S1400		0.14	0.33	0.1	D2S168 (1.33)	
	Distal forearm	D2S405		0.00	0.00	0.00	D2S165(0.54)	
		13q21–34	1.67	2.43	0.32	0.89		
		13S788		0.00	0.24	0.00	D13S153 (0)	
		D13S800		0.00	0.47	0.0	D13S156 (0.54)	
Koller <i>et al.</i> (20)	Spine	1q21–23 (D1S484)	3.86	0.00	0.00	0.01		
		6p11–12 (D6S462)	2.13	0.13	0.09	0.56	D6S257 (19.1)	
		11q12–13 (D11S987)	1.65	0.00	0.47	0.00		
		22q12–13 (D22S423)	0.99	0.00	0.29	0.02		
		5q33–35 (D5S422)	2.23	0.00	0.00	0.05		
Duncan <i>et al.</i> (24)	Femoral neck	11q12–13 (D11S935)	2.16	0.44	0.11	0.00		
		D3S3559	1.5	0.00	0.00	0.00	D3S1277 (6.42)	
		D3S1289	2.7	0.42	0.00	0.04		
	Lumbar spine	D4S429	1.8	0.00	0.53	0.02	D4S1575 (1.05)	
		D17S807	1.7	0.42	0.00	0.00	D17S944 (3.38)	
		D12S83	1.7	0.34	0.01	0.00		

For those markers that were reported by the other studies but not genotyped in ours, we reported the LOD scores for our markers (the identity given) that are closest to them and give the genetic distance (found through the database “Comprehensive human genetic maps of Marshfield” at http://research.marshfieldclinic.org/genetics/Map_Markers/maps/indexmap.html) between the markers in other studies and the closest markers of ours in *parentheses*. Markers D18S42 and CD3D were not mapped onto the Genethon and Marshfield human genetic linkage maps (<http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/humansearch.html>), and hence we are not able to identify our closest markers to them. MLS denotes for maximum LOD scores in the genomic regions.

indicate that those studies are necessarily more powerful and their results are more robust than our current study. Multiple testing and polygenic effects, among other things, may easily yield statistical fluctuation of LOD scores, particularly in small samples (23). A concrete empirical example that demonstrates statistical fluctuation of LOD scores in small study samples is as follows. A sibling pair study with 374 sibships reported a maximum LOD score of 3.50 near D11S987 for linkage to femoral neck BMD variation (73). However, a subsequent linkage study (20) with 595 sibling pairs reported a LOD score less than 2.2 at D11S987. Replication studies with even larger samples for the genomic region near D11S987 yielded no evidence of linkage to this region (23, 74, 75). Hence, the higher LOD score of the study (73) does not imply that the study (73) is more powerful and the results are more robust than other studies (20, 23, 74, 75) with larger samples but smaller LOD scores. None of our LOD scores meet the criteria (69) for definite linkage. However, the larger sample sizes of our study may render our results statistically more robust than some of the earlier studies with smaller samples. Simulation studies specific for our study pedigrees show that if using a LOD score of 3.0 as a significant criterion,

we may achieve statistical powers of 42.5%, 76.5%, and 94.5% for a QTL of a heritability of 15%, 20%, and 25%, respectively.

Age, sex, and weight are well-known major factors that are associated with BMD changes (63). Age (as age and age² terms), sex, and weight are adjusted as covariates, which, by the basic principle of statistics and statistical modeling (64), effectively controls for their effects on BMD in our study sample. Age effects in sibling pair studies may be more rigorously controlled experimentally by selecting siblings similar in age and of ages between 20 and 45 (20). However, even for the age range of 20–45, BMD still changes with age (60), and the age of peak bone mass reached may vary from individual to individual. Hence, statistical testing and adjustment of age effects is still necessary in sibling pair studies (36). Using multigeneration large pedigrees for the linkage study of BMD, the age range covers those of peak bone mass reached and those in the elderly resulting from the subsequent bone loss after the peak bone mass is reached. The natural process of menopause in women and other potential age-related physiology effects such as osteophytes in the lumbar spine may compromise our ability to fully adjust age effects on BMD. Future measures such as obtaining information about other potentially significant factors

such as menopause, measuring lumbar spine BMD with lateral scans, detailed recording of therapeutic effects (such as with estrogen in postmenopausal women) may ameliorate potentially confounding effects associated with large age ranges of study subjects and other factors. BMD in the elderly is most relevant to osteoporotic fractures that occur mainly after age 50 (3). Hence, a study that covers a large range of ages (particularly those for the elderly) has a scope that may be able to reveal potentials QTLs not only relevant to peak bone mass but also relevant to bone loss. Our study and those that confine to a more narrow age range aimed at QTL mapping for peak bone mass (20) may be able to complement each other in terms of disentangling genes for peak bone mass and those (if different) for subsequent bone loss. QTL mapping that involves measurement of BMD at different development stages (29) for the same individual organism may provide a way to distinguish peak bone mass QTLs from bone loss QTLs.

The genomic regions identified in this and all previous studies need to be subject to extension studies with more samples and denser markers for confirmation. The consistency and inconsistency of the genomic regions identified may serve as a basis for further endeavors and exploration of some focused genomic regions with larger samples and/or alternative methods (38). Implementation and comparison of studies with various designs and populations and various species may help facilitate confirmation of the results obtained. While QTL mapping in model organisms such as mice is undoubtedly useful, the results found in one species may not always transcend across to other species as shown by several known examples in studies of other phenotypes such as obesity (70). This may not be all unexpected at least because the natural selection challenges and thus evolutionary trajectories of genetic architecture of various species are not all the same during the long time independent evolutionary history. Once linkage to a genomic region is confirmed, subsequent fine mapping, a daunting task, will be pursued. Theoretically, examining the pattern of linkage disequilibrium using a dense panel of markers within positively identified genomic regions can narrow the relatively large genomic regions (~30 cM) identified in linkage analyses to regions less than 1 cM (76, 77), a size of the genomic region that is amenable for physical cloning of QTLs. If our ultimate goal is to find genes important for osteoporotic fractures and BMD is just used as a surrogate measure, the genes to be identified important for BMD may eventually need to be tested for their significance for and relevance to osteoporotic fractures (3, 78).

Acknowledgments

We thank graduate students and postdoctoral fellows in Dr. Deng's lab for their involvement in the genotyping effort. Technicians involved in DNA extraction in the Osteoporosis Research Center of Creighton University are appreciated. Helpful comments from anonymous reviewers are greatly appreciated.

Received March 26, 2002. Accepted June 25, 2002.

Address all correspondence and requests for reprints to: Hong-Wen Deng, Ph.D., Osteoporosis Research Center, Creighton University, 601 North 30th Street, Suite 6787, Omaha, Nebraska 68131. E-mail: deng@creighton.edu.

Investigators of this work are partially supported by grants from Health Future Foundation, NIH Grants (K01-AR-02170-01, R01-AR-45349-01, R01-GM-60402-01A1, P01-DC-01813-07), grants from State of Nebraska Cancer and Smoking Related Disease Research Program, U.S.

Department of Energy grant DE-FG03-00ER63000/A00, Creighton University, grants (30025025 and 30170504) from National Science Foundation of China, a grant (25000612) from HuNan Normal University, and a grant (25000106) from the Education Ministry of China.

References

- Cummings SR, Kelsey JL, Nevitt MC, O'Dowd KJ 1985 Epidemiology of osteoporosis and osteoporotic fractures. *Epidemiol Rev* 7:178–208
- Melton LJ, Kan SH, Frye MA, Wahner HW, O'Fallon WM, Riggs BL 1989 Epidemiology of vertebral fractures in women. *Am J Epidemiol* 129:1000–1011
- Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM, Zhou Y, Deng HY, Heaney RR, Recker RR 2000 Genetic determination of Colles' fractures and differential bone mass in women with and without Colles' fractures. *J Bone Miner Res* 15:1243–1252
- Ray NF, Chan JK, Thamer M, Melton III LJ 1997 Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: Report from the National Osteoporosis Foundation. *J Bone Miner Res* 12:24–35
- Dequeker J, Nijs J, Verstraeten A, Geusens P, Gevers G 1987 Genetic determinants of bone mineral content at the spine and radius: a twin study. *Bone* 8:207–209
- Slemeda SW, Christian JCC, Williams CJ, Norton JA, Johnston CC 1991 Genetic determinants of bone mass in adult woman: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. *J Bone Miner Res* 6:561–567
- Sowers MR, Boehnke M, Jannausch ML, Crutchfield M, Corton G, Burns TL 1992 Familiality and partitioning the variability of femoral bone mineral density in woman of child-bearing age. *Calcif Tissue Int* 50:110–114
- Gueguen R, Jouanny P, Guillemin F, Kuntz C, Poureil J, Siest G 1995 Segregation analysis and variance components analysis of bone mineral density in health families. *J Bone Miner Res* 10:2017–2022
- Deng HW, Stegman MR, Davies M, Conway T, Recker RR 1999 Genetic determination of peak bone mass (PBM) at hip and spine and common familial environmental effects on bone qualities. *J Clin Densitom* 2:251–263
- Deng HW, Chen WM, Conway T, Zhou Y, Davies KM, Stegman MR, Deng HY, Recker RR 2000 Determination of bone mineral density of the hip and spine in human pedigrees by genetic and life-style factors. *Genet Epidemiol* 19:160–177
- Deng HW, Livshits G, Xu FH, Conway T, Davies KM, Deng HY, Recker RR 2002 Evidence of a major gene for bone mineral density/content in human pedigrees identified via probands with extreme bone mineral density. *Ann Hum Genet* 66:61–74
- Krall EA, Dawson-Hughes B 1993 Heritability and life-style determinants of bone mineral density. *J Bone Miner Res* 8:1–9
- Livshits G, Karasik D, Kobylansky E 2001 Major gene control of bone mineral density in two human populations. *J Bone Miner Res* 17:152–161
- Lutz J, Tesar R 1990 Mother-daughter pairs: spinal and femoral bone densities and dietary intakes. *Am J Clin Nutr* 52:872–877
- Smith DM, Nance WE, Kang KW, Christian JC, Johnston CC 1973 Genetic factors in determining bone mass. *J Clin Invest* 52:2800–2808
- Pocock NA, Eisman JA, Hopper JA, Yeates MG, Sambrook PN, Eberl S 1987 Genetic determinants of bone mass in adults. A twin study. *J Clin Invest* 80:706–710
- Dequeker J, Nijs J, Verstraeten A, Geusens P, Gevers G 1987 Genetic determinants of bone mineral content at the spine and radius: a twin study. *Bone* 8:207–209
- Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA 1994 Prediction of bone density from vitamin D receptor alleles. *Nature* 367:284–287
- Johnson ML, Gong GD, Kimberling W, Recker SM, Kimmel DB, Recker RR 1997 Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). *Am J Hum Genet* 60:1326–1332
- Koller DL, Econs MJ, Rodriguez LA, Christian JC, Hui SL, Morin P, Conneally PM, Joslyn G, Peacock M, Johnston CC 2000 Genome scan for QTLs contributing to normal variation in bone mineral density and osteoporosis. *J Clin Endocrinol Metab* 85:3116–3120
- Deng HW, Li J, Li JL, Johnson M, Davies M, Recker RR 1998 Change of bone mass in postmenopausal Caucasian women with and without hormone replacement therapy is associated with vitamin D receptor and estrogen receptor genotypes. *Hum Genet* 103:576–585
- Deng HW, Li J, Li JL, Johnson M, Recker RR 1999 Association of VDR and ER genotypes with bone mass in postmenopausal women: different conclusions with different analyses. *Osteoporos Int* 9:499–507
- Deng HW, Xu FH, Conway T, Deng XT, Davies KM, Li JL, HY Deng, Recker RR 2001 Is population bone mineral density linked to the marker d11s987 on chromosome 11q12–13? *J Clin Endocrinol Metab* 86:3735–3741
- Duncan EL, Brown MA, Sinsheimer J, Bell J, Carr AJ, Wordsworth BP, Wass JAH 1999 Suggestive linkage of the parathyroid receptor type 1 to osteoporosis. *J Bone Miner Res* 14:1993–1999
- Audi L, Carcía-Ramírez M, Carrasosa A 1999 Genetic determinants of bone mass. *Horm Res* 51:105–123

26. Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES 1998 Quantitative trait loci affecting peak bone mineral density in mice. *J Bone Miner Res* 13:1648–1656
27. Beamer WG, Shultz K-L, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ, Donahue LR 1999 Quantitative trait loci for bone density in C57BL/6j and CAST/Eij inbred mice. *Mamm Genome* 10:1043–1049
28. Benes H, Weinstein RS, Zheng W, Thaden JJ, Jilka RL, Manolagas SC, Shmookler Reis RJ 2000 Chromosomal mapping of osteopenia-associated quantitative trait loci using closely related mouse strains. *J Bone Miner Res* 15:626–633
29. Shmookler Reis RJ, Benes H, McClure T, Kang P, Weinstein RS, Shelton RS, Jilka RL, Manolagas SC 2001 The effect of sex on genetic determinants of pre- and post-maturity bone accrual in mice. *J Bone Miner Res* 16(Suppl):S351
30. Eisman JA 1995 Vitamin D receptor gene alleles and osteoporosis: an affirmative view. *J Bone Miner Res* 10:1289–1293
31. Peacock M 1995 Vitamin D receptor gene alleles and osteoporosis: a contrasting view. *J Bone Miner Res* 10:1294–1297
32. Gong GD, Sterns HS, Cheng SC, Fong N, Mordeson J, Deng HW, Recker RR 1999 On the association of bone mass density and Vitamin-D receptor genotype polymorphisms. *Osteoporos Int* 9:55–64
33. Deng HW, Chen WM, Recker RR 2001 Population admixture: detection by Hardy-Weinberg test and its quantitative effects on linkage disequilibrium methods for localizing genes underlying complex traits. *Genetics* 157:885–897
34. Deng HW 2001 Population admixture may change, mask or reverse genetic effects of genes underlying complex traits. *Genetics* 159:1319–1323
35. Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP, Sereda L, Hall S, Considine E, Williams CJ, Tromp G, Kuivaniemi H, Ala Kokko L, Prockop DJ, Spotila LD 1998 First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4q. *Eur J Hum Genet* 6:151–157
36. Niu T, Chen C, Cordell H, Yang J, Wang B, Wang Z, Fang Z, Schork NJ, Rosen CJ, Xu X 1999 A genome-wide scan for loci linked to forearm bone mineral density. *Hum Genet* 104:226–233
37. Keen RW, Snieder H, Molloy H, Daniels J, Chiano M, Gibson F, Fairbairn L, Smith P, MacGregor AJ, Gewert D, Spector TD 2001 Evidence of association and linkage disequilibrium between a novel polymorphism in the transforming growth factor β 1 gene and hip bone mineral density: a study of female twins. *Rheumatology* 40:48–54
38. Deng HW, Shen H, Xu FH, Zhang HT, Deng HY, Li Y, Conway T, Davies KM, Recker RR 2001 Test of linkage and association of vitamin D receptor gene, osteocalcin gene, and parathyroid hormone gene to bone mineral density in Caucasian pedigrees. *J Bone Miner Res* 17:678–786
39. Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ, Rosen CJ 2001 Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6j and C3H/Hej inbred strains of mice. *J Bone Miner Res* 16:1195–1206
40. Shimizu M, Higuchi K, Bennett B, Xia C, Tsuboyama T, Kasai S, Chiba T, Fujisawa H, Kogishi K, Kitado H, Kimoto M, Takeda N, Matsushita M, Okumura H, Serikawa T, Nakamura T, Johnson TE, Hosokawa M 1999 Identification of peak bone mass QTL in a spontaneously osteoporotic mouse strain. *Mamm Genome* 10:81–87
41. Drake TA, Schadt E, Hannani K, Kabo MJ, Krass K, Colinao V, Greaser LE, Goldin J, Lusis AJ 2001 Genetic loci determining bone density in mice with diet-induced atherosclerosis. *Physiol Genomics* 5:205–215
42. Risch N, Merikangas K 1996 The future of genetic studies of complex human diseases. *Science* 273:1516–1517
43. Allison DB 1997 Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet* 60:676–690
44. Xiong MM, Krushkal J, Boerwinkle E 1998 TDT statistics for mapping quantitative trait loci. *Ann Hum Genet* 62:431–452
45. Risch N 1990 Linkage strategies for genetically complex traits II the power of affected relative pairs. *Am J Hum Genet* 46:229–241
46. Sobel E, Papp J, Lange K 2002 Detection and integration of genotyping errors in statistical genetics. *Am J Hum Genet* 70:496–508
47. Risch N, Zhang H 1995 Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science* 268:1584–1589
48. Deng HW, Li J 2002 The effect of selective sampling on transmission disequilibrium test of a quantitative trait locus. *Genet Res* 79:161–174
49. Deng HW, Deng H, Liu YJ, Liu YZ, Xu FH, Shen H, Conway T, Li JL, Huang QY, Davies KM, Recker RR 2002 A genome wide linkage scan for QTLs for obesity phenotypes. *Am J Hum Genet* 70:1138–1151
50. Deng HW, Zhou Y, Johnson M, Li J, Recker RR 2000 Product difference of multiplex and singleplex PCR and its practical implications for human genetic studies. *Biotechnology* 29:298–308
51. Li JL, Deng HY, Lai DB, Recker RR, Deng HW 2001 Towards high-throughput genotyping: a dynamic and automatic software for manipulating large-scale genotype data using fluorescently labeled dinucleotide markers. *Genome Res* 11:1304–1314
52. O'Connell JR, Weeks DE 1998 PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266
53. Huang QY, Xu FH, Shen H, Deng HY, Liu YJ, Liu YZ, Li JL, Recker RR, Deng HW 2002 Mutation patterns at dinucleotide microsatellite loci in humans. *Am J Hum Genet* 70:625–634
54. Glüer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK 1995 Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. *Osteoporosis Int* 5:262–270
55. Black DM, Cummings SR, Genant HK, Nevitt MC, Palermo L, Browner W 1992 Axial and appendicular bone density predict fractures in older women. *J Bone Miner Res* 7:633–638
56. Deng HW, Xu FH, Davies KM, Heaney RR, Deng HY, Recker RR, Bone mineral density, bone mineral content and bone size in fractured and non-fractured females and their relationship. *J Bone Miner Metab*, in press
57. Shen H, Xu FH, Zhang HT, Deng HY, Conway T, Xia XH, Chen J, Liu YZ, Liu YJ, Huang QY, Davies KM, Recker RR, Deng HW 2001 A large-scale whole-genome linkage scan identifies several genomic regions with QTLs underlying bone size variation. *J Bone Miner Res* 16(Suppl):S152
58. Deng HW, Xu FH, Shen H, Zhang HT, Deng HY, Conway T, Xia XH, Chen J, Liu YZ, Liu YJ, Huang QY, Davies KM, Recker RR 2001 A large-scale whole-genome linkage scan suggests several genomic regions containing QTLs for BMD variation. *J Bone Miner Res* 16(Suppl):S167
59. Recker R, Lappe J, Davies K, Heaney 2000 Characterization of perimenopausal bone loss: a perspective study. *J Bone Miner Res* 16:1965–1973
60. Amos CI 1994 Robust variance-components approach for assessing genetic linkage in pedigrees. *Am J Hum Genet* 54:535–543
61. Amos CI, Zhu DK, Boerwinkle E 1996 Assessing genetic linkage and association with robust components of variance approaches. *Ann Hum Genet* 60:143–160
62. Almasy L, Blangero J 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211
63. Deng HW, Li JL, Li J, Davies MK, Recker RR 1998 Heterogeneity of bone mass density across skeletal sites and its clinical implications. *J Clin Densitom* 1:339–353
64. Sokal RR, Rohlf FJ 1995 Biometry. 3rd ed. New York: W. H. Freeman and Co.
65. Williams JT, Van Eerdewegh P, Almasy L, Blangero J 1999 Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. *Am J Hum Genet* 65:1134–1147
66. Göring Harald HH, Terwilliger JD, Blangero J 2001 Large upward bias in estimation of locus-specific effects from genomewide scans. *Am J Hum Genet* 69:1357–1369
67. Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Vlikenburg HA, Pols HA, Slagboom PE, van Duijn CM 1998 A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam study). *Ann Rheum Dis* 57:371–374
68. Rosen CJ, Kurkland ES, Vereault D, Adler RA, Rackoff PJ, Craig WY, Witte S, Rogers J, Bilezikian JP 1998 Association between serum insulin-like growth factor-1 (IGF-1) and a simple sequence repeat in IGF-1 gene: implications for genetic studies of bone mineral density. *J Clin Endocrinol Metab* 83:2286–2290
69. Lander E, Kruglyak L 1995 Genetic dissection of complex traits: guidelines for interpreting the reporting results. *Nat Genet* 11:241–247
70. Recker RR, Deng HW 2002 The role of genetics in osteoporosis. *Endocrine* 17:55–66
71. Mitchell BD, Cole SA, Bauer RL, Iturria SJ, Rodriguez EA, Blangero J, MacCluer JW, Hixson JE 2000 Genes influencing variation in serum osteocalcin concentrations are linked to markers on chromosomes 16q and 20q. *J Clin Endocrinol Metab* 85:1362–1366
72. Williams JT, Blangero J 1999 Power of variance component analysis to detect quantitative trait loci. *Ann Hum Genet* 63:545–563
73. Koller DL, Rodriguez LA, Christian JC, Slemenda CW, Econs MJ, Hui SL, Morin P, Conneally PM, Joslyn G, Curran ME, Peacock M, Johnston CC, Foroud T 1998 Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12–13. *J Bone Miner Res* 13:1903–1908
74. Karasik D, Myers RH, Hannan MT, Gadnon DR, Cupples LA, Herbert A, Kiel DP 2001 Genome screen for quantitative trait loci contributing to bone mineral density: Framingham Osteoporosis Study. *J Bone Miner Res* 16(Suppl 1):S166
75. Mitchell BD, Kammerer CM, Schneider JL, Cole SA, Hixson JE, Perez R, Bauer RL 2001 A quantitative trait locus on chromosome 4p influences variation in bone mineral density. *J Bone Miner Res* 16(Suppl 1):S167
76. Deng HW, Chen WM, Recker RR 2000 QTL fine mapping by measuring and testing for Hardy-Weinberg and linkage disequilibrium at a series of linked marker loci in extreme samples of populations. *Am J Hum Genet* 66:1027–1045
77. Deng HW, Chen WM 2000 QTL fine mapping in extreme samples of finite populations for complex traits with familial correlation due to polygenes. *Am J Hum Genet* 67:259–261
78. Deng HW, Mahaney MC, Williams J, Li J, Conway T, Davies KM, Li JL, Deng HY, Lai DB, Recker RR 2002 Relevance of the genes for bone mass variation to susceptibility to osteoporotic fractures and its implications to gene search for complex human diseases. *Genet Epidemiol* 22:12–25
79. Ho NC, Jia L, Driscoll CC, Gutter EM, Francomano CA 2000 A skeletal gene database. *J Bone Miner Res* 15:2095–2122
80. Zmuda JM, Cauley JA, Ferrell RE 1999 Recent progress in understanding the genetic susceptibility to osteoporosis. *Genet Epidemiol* 16:356–357