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Genome Screen for Quantitative Trait Loci Contributing to Normal Variation in Bone Mineral Density: The Framingham Study*

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ABSTRACT

A genome-wide scan was performed in a randomly ascertained set of 330 extended families from the population-based Framingham Study to identify chromosomal regions possibly linked to bone mineral density (BMD). A set of 401 microsatellite markers was typed at a 10-centimorgan (cM) average density throughout the genome. BMD was measured at the femoral neck, trochanter, Ward's area, and lumbar spine in 1557 participants of both Framingham cohorts. BMDs were adjusted for age, body mass index (BMI), height, alcohol, caffeine, calcium and vitamin D intakes, smoking, physical activity, and estrogen use in women within each sex and cohort. Strong heritabilities (values between 0.543 and 0.633) were found for the adjusted BMD at all sites. Two-point and multipoint quantitative linkage analyses were performed for each BMD site using the maximum likelihood variance components method. By two-point screening, loci of suggestive linkage were identified on chromosomes 6 and 21, with the maximum $\log_{10}$ of the odds ratio (LOD) scores of 2.34 for the trochanter at D21S1446 and 2.93 for the femoral neck at D6S2427. Lumbar spine BMD had maxima at D6S2427 (LOD = 1.88) and at D12S395 (LOD = 2.08). Multipoint linkage analysis revealed suggestive linkage of trochanteric BMD at a broad (~20 cM) interval on chromosome 21q, with the peak linkage close to D21S1446 (LOD = 3.14). LOD scores were 2.13 at 8q24 with Ward's BMD and 1.92 at 14q21.3 with lumbar spine BMD. This largest genome screen to date for genes underlying normal variation in BMD, adjusted for a large number of covariates, will help to identify new positional candidate genes, otherwise unrecognized. (J Bone Miner Res 2002;17:1718–1727)

Key words: **quantitative trait loci, genome screen, variance component analysis, bone density, Framingham cohorts**

INTRODUCTION

OSTEOPOROTIC FRACTURES of the hip and spine are a major public health problem, occurring at a rate of >1.2 million per year in the United States and accounting for over $13 billion in healthcare expenses in 1995.[1] Low bone mineral density (BMD) in later life is a major contributing factor to osteoporotic fractures. Low BMD in elderly individuals results from low peak BMD, attained during growth, a high rate of bone loss in later life, or a combination of both.[2,3]

BMD is a complex phenotype, which is influenced by both genetics and environment. Genetic factors are important determinants of BMD but have not been elucidated fully. Family studies have shown that some 60–80% of the

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total variation in BMD could be attributable to genetic factors.[2–4] However, the interplay between genetic and environmental factors in bone homeostasis is not well established because of apparently redundant factors and mechanisms.[5] Two complex and opposing processes, namely, formation and resorption, regulate the amount of bone present at any stage of life. Both processes are potentially influenced by different genes, as well as nongenetic factors such as diet, physical activity, smoking, or their interactions. Identification of such genes, which may be specific for compact and trabecular bone, will be important for understanding the underlying mechanisms of bone formation and resorption and for providing molecular targets for future osteoporosis therapies.

Because BMD is measured on a continuous (quantitative) scale, it may be a good candidate for quantitative genetic analysis. Given the likely polygenic influence on BMD, a strong case can be made to identify potential chromosomal regions governing BMD, using a genome search approach with subsequent testing of candidate genes in suggested regions. Genome-wide linkage screens for genes underlying BMD variability have been conducted recently in humans[6–9] and mice.[10–13] However, regions and loci revealed by these studies vary greatly. Furthermore, linkage analyses with microsatellites flanking some of the candidate loci governing bone-related biochemical traits such as the vitamin D receptor[14–16] osteocalcin[17] and type 1 collagen A1 (COL1A1)[14] failed to provide support for linkage of these genes to the BMD phenotypes in several samples.

Loci harboring genes that contribute to the BMD variation may be considered as quantitative trait loci (QTLs). To identify putative QTLs for such complex traits as BMD with the greater power, it is important to investigate a large number of general, rather than nuclear, pedigrees using nonparametric linkage tests.[18,19] Moreover, accounting for the effects of multiple covariates on total variance of the studied trait may increase both the heritability estimate and the likelihood that QTLs providing relatively small effects on the BMD will be revealed.[20] The purpose of this study was to identify potential genetic determinants of BMD at the hip and spine. We report here a genome-wide linkage screen with BMD at four sites, adjusted for multiple known risk factors, in members of 330 healthy white pedigrees, participants of the Framingham Study cohorts, who were ascertained without prior knowledge of their bone density.

MATERIALS AND METHODS

Sample

Subjects eligible for this investigation are drawn from the Framingham Heart Study, which began in 1948 with the primary goal of evaluating risk factors for cardiovascular disease. Participants from the Framingham original cohort, initially aged 28–62 years, have been examined every 2 years since that time. The Framingham Osteoporosis Study, started at biennial examination cycle 20 (1988–1989), involved 1164 surviving original cohort members (448 men and 716 women), aged 68–98 years, nearly all (98%) of whom were white.[21] The Framingham cohort participants follow the same age- and sex-specific population proportions found in the general population of Framingham, MA.[21] Details and descriptions about the Framingham Osteoporosis Study have been reported by Hannan et al.[21,22]

In 1971, the Framingham Offspring Study was initiated with the intent to evaluate the role of genetic factors in the etiology of coronary artery disease and it included a total sample size of 5124 subjects.[23] The offspring cohort is comprised of 71% of all eligible adult offspring of couples from the original Framingham cohort. There were no differences in age, blood pressure, cholesterol, weight, and smoking history between parents whose children volunteered for the offspring cohort versus parents whose offspring did not volunteer. These offspring cohort members also participated in the Framingham Osteoporosis Study at either their examination cycle 6 or 7. Informed consent was obtained from participants of each cohort before entry into the study, which was approved by the Boston University Institutional Review Board for Human Subjects Research.

Bone density measurements

Details of the BMD measurements taken in 1988–1989 and in 1992–1993 have been published previously.[21,22] The original cohort participants underwent bone densitometry of lumbar (L2–L4) spine, femoral neck, trochanter, and Ward's area by dual photon absorptiometry using a GE Lunar DP-3 (GE Lunar, Madison, WI, USA) at examination 20 (1988–1989) or by DXA by GE Lunar DPX-L at examination 22 (1992–1993). For the offspring cohort, the GE Lunar DPX-L was used to measure BMD (g/cm^2) in 1996–2000. As noted in the original study reporting the Framingham Osteoporosis Study, the CV over 2 years in normals for the DP-3 was 2.6% for the femoral neck, 2.8% for the trochanter, 4.1% for the Ward's area, and 2.2% for the lumbar spine [21]; these values are consistent with the CV reported for DP-3 by other researchers. For the DPX-L, CVs were 1.7% (femoral neck), 2.5% (trochanter), 4.1% (Ward's area), and 0.9% (lumbar spine).[22]

To maximize the sample size of the original cohort, we used BMD measurements from examination 22 for 34 original cohort members missing BMDs at examination 20. We previously showed high correlations between dual photon absorptiometry and DXA.[24] However, because of a small but consistent shift in BMD values between the two technologies, femoral BMDs were adjusted for the change in equipment from DP3 to DPX-L technology, using published corrections, based on cross-calibrations of the two instruments using our Framingham Study subjects.[22]

Out of a total of 1702 genotyped individuals from 330 pedigrees with family sizes ranging from 2 to 29 genotyped individuals, 1557 had BMD measurements. The sample with genotyping and BMD measurements (77% offspring members and 23% cohort members) included the following relative pairs: 834 parent-offspring, 279 grandparent-grandchild, 691 sibling and 7 half-sibling, 452 cousin, and 390 avuncular.

Detection of microsatellite markers

A 5- to 15-ml blood sample was collected from each participating individual for either genomic DNA extraction or lymphocyte culture or both. For all Framingham subjects, DNA was obtained from whole blood specimens collected between 1988 and 1989. In the original cohort, DNA was extracted by phenol-chloroform extraction and stored at −80°C. In the offspring cohort, DNA was extracted from peripheral lymphocytes using the Qiagen Blood and Cell Culture DNA Maxi kit (Qiagen GmbH, Hilden, Germany).

The National Heart Lung and Blood Institute (NHLBI) Mammalian Genotyping Service in Marshfield, WI, genotyped a set of 401 microsatellite markers covering the genome at an average density of 10 centimorgans (cM) and having an average heterozygosity of 0.77 (Screening Set, version 8a; Marshfield Medical Research Foundation, Marshfield, WI, USA).[25,26] Marker order and map positions were obtained from the Marshfield electronic database (http://research.marshfieldclinic.org/genetics/). The genotyping error rate in the Marshfield lab is <1%.

Other measurements

Potentially confounding variables measured at the time of bone density measurement were obtained for each participant, along with overall medical history. Details of these measurements have been published previously.[21,22] These variables included age, sex, height, weight, body mass index (BMI), alcohol intake, caffeine consumption, calcium and vitamin D intake, smoking status, physical activity, and for women, estrogen use.

In brief, adjustment for age is necessary because BMD reaches a peak in the third or fourth decade of life and declines with age in both sexes. Age squared was considered in the models to account for nonlinear age effects.[15,27] Weight was measured using a standardized balance beam scale. Height (without shoes) was measured to the nearest ¼ in using a stadiometer. BMI was calculated in kilograms per squared meter. Michels et al.[28] have suggested that use of BMI and height is sufficient for body size and body composition adjustments in epidemiological studies of bone mass.

Total alcohol consumption (oz/week) was calculated based on a published equation using a self-report of the intake of beer, wine, and mixed drinks per week, providing alcohol equivalent in grams per week.[29] Smoking was assessed as current cigarette smoker (smoked regularly in the past year) at the time of BMD measurement, former smoker, or never smoked tobacco. Caffeine intake, incorporating coffee and tea intake, was computed in units equal to the number of cups of coffee per day plus ½ times the number of cups of tea per day as suggested by Kiel et al.[30] Dietary intakes of calcium and vitamin D (including supplements) were assessed using the 126-item Willett food frequency questionnaire.[31,32] These data then were converted to food and nutrient intake. Reports with overall food energy intakes of <600 kcal or >4000 kcal or with data missing for more than 12 food items were excluded.

Physical activity was examined using two questionnaires: one for the Framingham original cohort and the other for the Framingham offspring group. The original cohort was asked to complete the Framingham Physical Activity Index (PAI), a weighted 24-h score of typical daily activity based on hours spent doing heavy, moderate, light, or sedentary activity as well as sleeping.[33,34] The Framingham offspring group was asked to complete the Physical Activity Scale for Elderly (PASE) questionnaire.[35] Both questionnaires produce indices of physical activity using validated items that are appropriate for older respondents.

For women, estrogen-replacement therapy (ERT) use was evaluated as current use, former use, and never used oral conjugated estrogen, patch, or cream.

Statistical analysis

Multiple linear regression analysis was used in each sex and cohort separately using PROC GLM in SAS version 6.12 (SAS Institute Inc., Cary, NC, USA) to generate standardized residuals for each subject that account for effect of confounding variables. Variables used as covariates for BMD included age, age squared, BMI, height, intakes of alcohol, caffeine, calcium and vitamin D, smoking, and physical activity, as well as ERT (for women only).

The regression analyses were performed in the full sample of subjects with BMD measures so that the standardized residual would be more stable and would reflect the deviation of an individual's value from the mean of those with comparable covariates. Subsequent genetic analyses using these standardized residuals were performed in the subsample of subjects from the 330 pedigrees who were included in the genome scan.

Modeling of BMD for genetic analyses was performed also in each sex and cohort with a reduced number of covariates, namely, age, age squared, BMI, height, and ERT in women, which are commonly used in BMD studies.

Quantitative trait linkage analysis

The marker genotype data were used to verify the familial relationships among the subjects using the SIB_KIN program of the ASPEX package (ftp://lahmed.stanford.edu/pub/aspex/index.htm) and random genotyping errors were identified and eliminated with the GENTEST program (Southwest Foundation for Biomedical Research, San Antonio, USA), a precursor of INFER, in PEDSYS (http://www.sfbr.org/sfbr/public/software/pedsys/pedsys.html). When random errors were detected, the marker information was eliminated from the nuclear family. Once the data were clean, identical-by-descent (IBD) status was inferred for all individuals based on data from available relatives.

All genetic analyses were conducted in SOLAR[36] using the standardized residuals resulting from the multiple linear regression analyses. Because the standardized residual BMD phenotypes showed some skewness, we applied a correction for a nonnormal distribution, as implemented in SOLAR, by addition of a *t*-distribution parameter. First, we estimated heritability (h^2) of BMD at four bone regions—femoral neck, Ward's area, trochanter, and lumbar spine—for each phenotype using residuals from both models of adjustment for covariates. SOLAR uses maximum likeli-

TABLE 1. CHARACTERISTICS OF THE FRAMINGHAM OSTEOPOROSIS STUDY POPULATION

	Original (examination 20)		*Offspring*	
Characteristic	*Men (n = 444)*	*Women (n = 707)*	*Men (n = 1295)*	*Women (n = 1657)*
Age (years ± S.D.)	75.78 ± 5.06	76.26 ± 5.10	59.36 ± 9.61	58.71 ± 9.53
Height (in ± SD)	67.08 ± 2.73	61.52 ± 2.51	68.86 ± 2.63	63.44 ± 2.49
BMI (kg/m^2 ± SD)	27.11 ± 3.99	26.48 ± 4.89	28.67 ± 4.37	27.24 ± 5.56
Alcohol intake (oz/week ± SD)	3.2 ± 4.6	1.7 ± 2.8	3.3 ± 4.3	1.5 ± 2.5
Caffeine consumption (U/day ± SD)	1.58 ± 1.56	1.53 ± 1.37	2.35 ± 2.26	1.89 ± 1.83
Calcium intake (mg/day)	766.6 ± 497.8	824.3 ± 477.9	811.3 ± 422.0	1012.7 ± 544.1
Vitamin D intake (IU mg/day)	557.9 ± 407.5	546.6 ± 358.7	235.6 ± 142.3	233.2 ± 134.9
Smoking history (%)				
Never smoked	42.2	60.9	66.9	53.5
Former smoker	49.2	28.6	20.1	32.2
Current smoker	8.6	10.5	13.0	14.2
Estrogen supplement—women only (%)				
Never used	—	63.5	—	72.0
Formerly used	—	35.5	—	0.5
Currently using	—	1.0	—	27.5
Physical activity[a]	33.7 ± 6.3	33.13 ± 5.25	155.40 ± 86.49	134.08 ± 71.51
BMD				
Femoral neck	0.873 ± 0.143	0.717 ± 0.114	0.979 ± 0.138	0.875 ± 0.143
Trochanter	0.845 ± 0.149	0.623 ± 0.128	0.891 ± 0.141	0.719 ± 0.136
Ward's area	0.681 ± 0.165	0.556 ± 0.124	0.786 ± 0.159	0.729 ± 0.171
Lumbar spine	1.335 ± 0.224	1.071 ± 0.191	1.329 ± 0.207	1.159 ± 0.201

[a] PAI in original cohort, examination 20; PASE in all other.

hood methods to estimate variance components for the polygenic genetic effect and random individual environmental effects.[37] Accounting for the effects of as many covariates as possible before genetic analysis may decrease variation because of known effects and may increase the likelihood that QTLs providing relatively small effects on the total variance will be found in the analysis.[20] Heritability of BMD levels is defined here, in the "narrow sense," as the proportion of the total trait variance attributable to the additive effects of genes.

Second, we carried out a quantitative trait linkage analysis using the standardized residuals of BMD phenotypes with a set of 401 microsatellite markers. Linkage analysis implemented in SOLAR assesses whether relatives who are genetically more similar at a particular locus also have more similar bone density.

In SOLAR, the variance-components model, initially proposed by Amos[38] for nuclear pedigrees, is extended to general pedigrees of arbitrary size and complexity.[36] The hypothesis of linkage was evaluated by testing whether the variance attributable to the QTL is significantly greater than zero. Model parameters were obtained using maximum likelihood estimates, and the likelihood estimates of nested models were compared using a likelihood ratio test. The $\log_{10}$ of the ratio of the likelihood of the polygenic and marker-specific models produces the LOD score, the traditional measure of genetic linkage.

The two-point variance component approach has been expanded to multipoint linkage analysis using IBD sharing by relative pairs at genotyped loci, with a constrained linear function to impute sharing at arbitrary points along the chromosome.[36] Thus, LOD scores were computed at 1-cM intervals along each autosome; observed allele frequencies in genotyped individuals were used in the genome screen. In multipoint analysis, a locus with the highest LOD score was included in the model, and then the analysis for a second locus was repeated, conditional on the locus found in the previous scan included in the model. Multipoint linkage analysis implemented in SOLAR has been shown to be more powerful than the two-point analyses, because the former contains information from adjacent markers. Multipoint linkage analysis also provided an unbiased estimate of QTL location.[36]

No ascertainment correction of likelihood was made because our pedigrees represent a community-based sample that was selected without regard to an individual's bone density.

RESULTS

Table 1 displays descriptive statistics of pedigree members from both cohorts by examination and sex. As expected, the younger offspring group had greater BMD at all skeletal sites compared with original cohort members (Table 1). In each cohort, male participants had greater average BMD at all skeletal sites compared with females. Table 2 shows results of regression models performed in all available members of each cohort and sex, including 10 (11 for women) covariates. As is evident, regression models vary

Table 2. Results of Stepwise Multiple Regression Analyses of BMD Phenotypes With Covariates (Age, Age Squared, BMI, Height, Alcohol, Caffeine, Smoking, Physical Activity, Calcium, Vitamin D, and ERT in Women)

	Original (examination 20)				*Offspring*			
Cohort	*FN*	*TROCH*	*WRD*	*LS*	*FN*	*TROCH*	*WRD*	*LS*
Men								
R^2	0.179	0.095	0.160	0.164	0.196	0.163	0.180	0.084
n	430	420	429	325	1285	1285	1284	1286
Women								
R^2	0.241	0.239	0.190	0.243	0.372	0.407	0.343	0.273
n	688	684	688	527	1642	1642	1642	1654

FN, femoral neck; TROCH, trochanter; WRD, Ward's area; LS, lumbar spine.

Table 3. Heritability Estimates of BMD Phenotypes After Adjustment for Covariates

FN	*TROCH*	*WRD*	*LS*
$h^2 \pm$ SE	$h^2 \pm$ SE	$h^2 \pm$ SE	$h^2 \pm$ SE
0.543 ± 0.059	0.581 ± 0.058	0.582 ± 0.054	0.633 ± 0.064
$n = 1447$	$n = 1447$	$n = 1447$	$n = 1434$

FN, femoral neck; TROCH, trochanter; WRD, Ward's area; LS, lumbar spine.

between the offspring and original cohort of both sexes in respect to the pattern and magnitude of potential covariate contribution to each BMD phenotype. In the women of both cohorts, percent of variation explained by covariates (R^2 = 0.190–0.407, Table 2) was much higher than in men for all phenotypes (R^2 = 0.084–0.196). The amount of variation explained by covariates was highest for trochanter BMD (except in original cohort men at exam 20), followed by femoral neck BMD. Most of the variation in BMD phenotypes was explained by age, anthropometrics, and ERT in women ($p < 0.001$). Height, in particular, explained 0.27–0.29 of the BMD variance in men and 0.44–0.47 in women. In original cohort men, smoking was also an important covariate with BMD ($p < 0.05$), and in their female counterparts, physical activity, alcohol, and vitamin D significantly contributed to the total explained variance ($p < 0.05$). In the offspring, besides age and anthropometry, other important covariates were physical activity in men and ERT, alcohol, and caffeine intake in women ($p < 0.05$).

Table 3 presents the SOLAR estimates of the heritability of BMD and size of pedigree sample for four skeletal sites. A strong familial resemblance with h^2 ranged 0.543–0.633 was demonstrated for all studied phenotypes. In comparison with adjustment for a restricted number of covariates (age, age squared, BMI, height, and ERT in women), the inclusion of seven additional covariates in the model yielded some increase in h^2 values, ranging 3.4% for lumbar spine to as much as 10.4% for Ward's area. However, a drop in the sample size caused by missing data on potential covariates was observed with adjustment for a larger number of covariates (110 individuals lost at femoral sites and 68 at lumbar spine).

Two-point linkage analysis of BMD at the four skeletal sites using SOLAR identified several regions with LOD scores above 2.2 (lower threshold for suggestive linkage by Lander and Kruglyak[39]). The maximum LOD scores attained in the analysis of BMD adjusted for covariates were 2.93 on D6S2427 for femoral neck BMD and 2.34 on D21S1146 for trochanteric BMD (Table 4). Markers D12S395 and D6S2427 failed to show suggestive linkage with lumbar spine (LOD scores 2.08 and 1.88, respectively). Linkage for Ward's area BMD with D8S373 had LOD = 1.77, which falls below the suggestive threshold by Lander-Kruglyak.[39] Table 5 shows the number of two-point LOD scores with some indication of linkage (LOD > 1.0) and suggestive linkage by Lander-Kruglyak's criteria, observed per autosome for all four phenotypes.

In the multipoint analysis, the maximum LOD score of 3.14 occurred for trochanteric BMD close to D21S1446 in cytogenetic band 21q22.13–21qter. This LOD score surpasses the common criteria for genome-wide linkage significance (LOD = 3.0). Overall, from marker D21S2055 to 21qter, at an interval spanning ~20 cM, LOD scores were all higher than 2.20 (Table 6 and Fig. 1). Another marginal suggestive linkage was at chromosomal region 8q24, near D8S373, which provided an LOD = 2.13 with Ward's area. No substantial multipoint linkage was shown for femoral neck BMD (Table 4). For lumbar spine, marginal suggestive linkage was obtained at 14q21.3, at an interval from 50 to 54 cM, with LOD scores up to 1.92. There also was some indication of linkage at 12q23 in multipoint analysis of spinal BMD (LOD = 1.72), and linkage of a similar magnitude was found for Ward's area BMD in region 16p13.2 (LOD = 1.75). It should be noted here that the region at 6pter, which was marked by high LOD scores for femoral neck and spinal BMD in two-point analysis, did not provide any evidence of linkage in multipoint analysis.

Multipoint linkage analysis has been suggested to be more powerful than the two-point one and is used here to corroborate the two-point findings. In Table 6 we provide results of both two-point and multipoint linkage for trochanter BMD with chromosome 21. As shown in Table 6, when adjacent markers show evidence of linkage, the multipoint LOD score is increased.

The other approach to BMD modeling, namely, adjustment for a reduced number of covariates, showed the same

TABLE 4. RESULTS OF TWO-POINT AND MULTIPOINT GENETIC LINKAGE ANALYSIS OF BMD PHENOTYPES ADJUSTED FOR COVARIATES (AGE, AGE SQUARED, BMI, HEIGHT, ALCOHOL, CAFFEINE, SMOKING, PHYSICAL ACTIVITY, CALCIUM, VITAMIN D, AND ERT IN WOMEN)

	Marker		*Location*	*FN*		*TF*		*Ward*		*LS*	
Chromosome	*Marshfield name*	*D number*	*(cM)*	*Two-point*	*Multipoint*	*Two-point*	*Multipoint*	*Two-point*	*Multipoint*	*Two-point*	*Multipoint*
5p14	GATA134B03	D5S2845	36	<1.0	—	1.75	<1.0	—	—	—	—
6p21.2	GGAA15B08	D6S2427	55	**2.93**	<1.0	<1.0	—	<1.0	—	1.88	<1.0
8q24.3	UT721	D8S373	164	<1.0	—	1.05	1.26	1.77	**2.13**	<1.0	—
12q24.2	GATA4H01	D12S395	137	<1.0	<1.0	1.41	1.07	<1.0	—	**2.08**	1.72
14q31	GGAA10C09	D14S587	56	—	—	—	—	—	—	1.07	**1.92**
16p13.2	ATA41E04	D16S2616	11	—	<1.0	—	1.05	1.33	1.75	1.07	<1.0
21q22.2	GATA188F04	D21S2055	40	<1.0	—	1.53	**2.39**	<1.0	—	<1.0	—
21qter	GATA70B08	D21S1446	58	—	—	**2.34**	**3.14**	—	—	<1.0	—

Bold fonts indicate LOD scores >1.90; blank indicates LOD scores ≤ 0.5.

maxima as those mentioned previously; thus, only the results of the model with the maximal number of covariates are shown in the Tables 4–6 and reported here. However, notable LOD scores for linkage of all BMD phenotypes, especially of lumbar spine BMD, were lower when BMD was adjusted for a restricted number of covariates in the same pedigree sample.

DISCUSSION

To identify potential genetic determinants of BMD at four skeletal sites, we have performed an autosomal genomic scan in members of 330 pedigrees from the Framingham Study. This is one of the largest autosomal genome screens performed for BMD to date, which included 401 polymorphic marker loci with an average spacing of 10 cM, with <5% of the intervals being >15 cM.

This investigation indicated that BMD as measured by absorptiometry was highly heritable; h^2 values between 0.54 and 0.63 were found for four sites. Variance component analyses of the pedigree data have revealed several regions with evidence of linkage to BMD. We report suggestive two-point linkage of BMD at the femoral neck to 6pter, trochanteric BMD to 21qter, and possible linkage of lumbar (L2–L4) spine BMD to chromosomal regions 12q23 and 6pter. For Ward's area, no suggestive linkage was shown here. In the multipoint analysis, strong evidence of linkage was provided in region 21qter with trochanteric BMD (LOD = 3.17), and a suggestive linkage (with LODs > 2.2) with the latter phenotype at an interval spanning ~20 cM from 21q22.2 to 21qter. Other regions of possible linkage were at chromosome 8q24 with Ward's area (LOD = 2.13) and at chromosome 14q with lumbar spine BMD (LOD > 1.90 at interval 50–52 cM). No indication of multipoint linkage was found for femoral neck BMD.

Previous simulation analyses of power in the Framingham sample indicated that we had ~80% power to detect a locus with LOD ≥ 2.0 when it accounts for 25% heritability or more.[40] Our heritability estimates for BMD at all sites were almost twice as high. Moreover, empiric *p* values were determined for this study by genome-wide simulations. Four hundred simulated markers were generated by SOLAR using our pedigrees under the hypothesis of no linkage and analyzed by two-point analysis. In the analyses, only subjects who have BMD data measured were included; all others were given missing values. Of the 400 markers, the mean number of observed two-point LOD scores > 1.0 was 5 and LOD scores > 2.0 was 2; none had an LOD score > 2.9 (which corresponds to $p < 0.0025$).

In our sample we did not detect loci (apart from the D6S2427 locus) with substantial pleiotropic effect on BMD at multiple sites across the skeleton. However, because of the absence of multipoint evidence for linkage in this chromosomal region to either femoral neck or spinal BMD, it remains unclear whether there is, in fact, a susceptibility locus in this region. Each of the maxima detected on chromosomes 8, 12, 16, and 21 appeared to affect BMD at primarily one skeletal site, not all of the skeletal sites studied. Both Devoto et al.[6] and Koller et al.[9] also found

Table 5. Number of Loci With Possible Linkage (Two-Point LOD Scores > 1.0) Per Chromosome, for All BMD Phenotypes

	Femoral neck BMD		*Trochanter BMD*		*Ward's area BMD*		*Lumbar spine BMD*	
Chromosomes[a]	*Lod > 1.0*	*Lod > 2.2*	*Lod > 1.0*	*Lod > 2.2*	*Lod > 1.0*	*Lod > 2.2*	*Lod > 1.0*	*Lod > 2.2*
1	1							
2			1					
5			2					
6		1			1		1	
7							1	
8			2		1		1	
9	1		1				2	
12	1		1				2	
14							5	
15					1		1	
16			2		1			
17	1				1			
18	1							
20			1					
21			2	1				
Total	5	1	12	1	5	0	12	0

[a] Only chromosomes with possible linkage shown.

Table 6. Two-Point and Multipoint LOD Scores of Trochanter BMD Linkage With Microsatellite Markers on Chromosome 21

Marker		*Location*	*LOD scores*	
Marshfield name	*D number*	*(sex averaged cM)*	*Two-point*	*Multipoint*
GATA11C12	D21S1432	7.7	0.000	0.000
GGAA3C07	D21S1437	16.3	0.146	0.015
GATA129D11	D21S2052	27.0	0.000	0.026
ATA27F01	D21S1440	41.5	0.943	2.389
GATA188F04	D21S2055	44.4	1.534	2.554
GATA70B08	D21S1446	62.0	2.336	3.142

only site-specific peaks, which suggests that minimal pleiotropy, if any, exists between femoral and spinal BMD.

This is one of the largest genome screens and it will help to identify new positional candidate genes, which may be unrecognized from bone biology knowledge. For example, recent identification mutation in low-density lipoprotein receptor-related protein 5 (LRP5) gene, which is responsible for the high bone mass,[41] was triggered by linkage of this phenotype with chromosome 11q12–13.[42] However, no work done to date in population samples, including this study, justifies much discussion of candidate genes. Genome scans reveal quite large candidate chromosomal regions instead of pinpointing identification of candidate genes.

Chromosomal regions that were linked with BMD in our study contain several genes with possible involvement in the genetic regulation of bone mass. The region underlying our broad (~20 cM) interval for linkage on chromosome 21q22.2–21qter includes the structural gene for collagen type VI (COL6A1 and COL6A2). Collagen type VI is present in areas of bone development such as fetal bone and the growth plate; its content diminishes in osteoporotic bone.[43] According to the Online Mendelian Inheritance in Man (OMIM) map, this gene was placed exactly in the same location as marker D21S1446 on our map (at 58 cM), which coincides with our highest peak signal for linkage with trochanteric BMD.

Two bone morphogenetic proteins (BMPs), namely, BMP-4 and BMP-6, are located near the region of multipoint linkage of spinal BMD on chromosome 14q22[44] and femoral neck and spinal BMD are located at 6pter-6p21,[45] respectively. At 8q24, a locus for osteoprotegerin, a member of the tumor necrosis factor receptor superfamily, was mapped at 164 cM,[46] which coincides with maximum of multipoint linkage for Ward's area BMD (and weak linkage with trochanter BMD). There also is one potential candidate gene for bone formation, which is located very close to our region of peak linkage for lumbar spine BMD on 12q23 (and weakly linked with trochanter BMD). This is the gene for insulin-like growth factor 1, located at 134.5 cM. In region 16p13.2, which indicated some linkage with Ward's area, there are no known candidate genes.

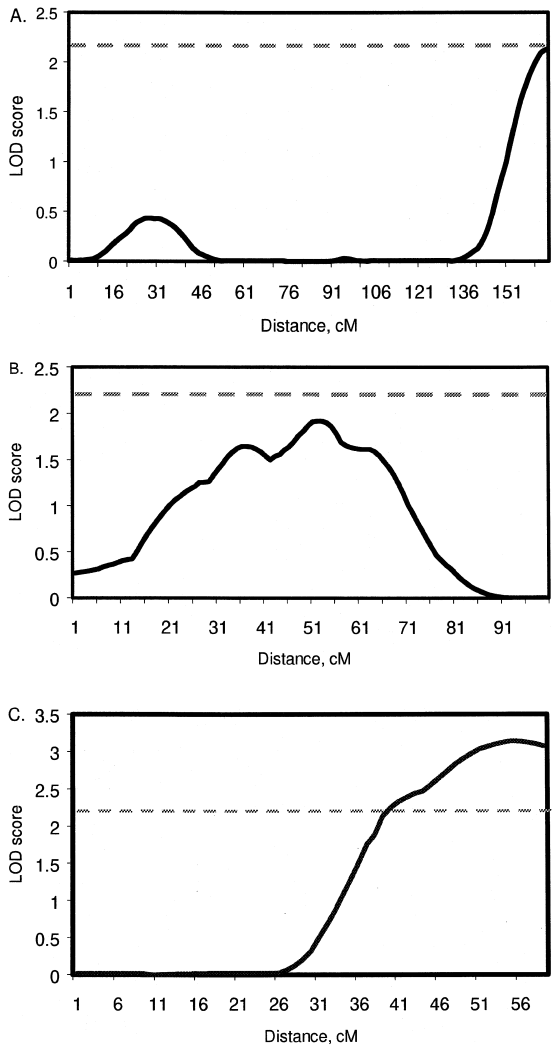

FIG. 1. Multipoint linkage results with BMD for the chromosomal regions genotyped in the Framingham pedigrees. Results are shown for (A) chromosome 8 with Ward's area BMD, (B) chromosome 14 with lumbar spine BMD, and (C) chromosome 21 with femoral trochanter BMD.

Previously, chromosome 11q12–13 was suggested to be of interest because of the mapping of several Mendelian bone density phenotypes to this candidate region.[41,47] However, we could not support linkage within the 11q12–13 region with any of the BMD phenotypes in this study (analogous findings by Deng et al.[47]). Similarly, no linkage was found with candidate genes such as vitamin D and estrogen receptors, osteocalcin, and COL1A1. This may be ascribed to the difference of relative effects of various genes on phenotypic variation in BMD among populations, which limits the generalizability of results from one population to others.

Our findings also do not overlap with other genome scans of the same phenotypes such as Devoto et al.'s[6] and Koller et al.'s.[8,9] Possible explanations for the disagreement in results between different studies include (1) dissimilarity in subject recruitment (patients from a population-based study in Framingham vs. families identified via probands with low BMD and (2) adjustment for a greater number of potential confounders in our study.

It should be noted in this connection that this study is unique because of the number of characteristics taken into account for covariation with BMD. It is known that accounting for the effects of maximal number of covariates frequently increases the heritability estimate of a studied trait, which in turn can increase the likelihood that QTLs providing relatively small effects on the total BMD variance will be revealed.[20] In this study, we adjusted BMD not only for age, age squared, BMI, height, and estrogen use in women, as was done in many other quantitative genetic studies of BMD, but also to such important individual factors as alcohol, caffeine, calcium and vitamin D consumption, smoking, and physical activity. All these variables are known to co-vary with BMD in many studies.[22,48] Including a large number of covariates in models for BMD adjustment (as suggested by Zee et al.[15]), creates a decrease in sample size because of missing measurements (68–110 cases lost in our case). This loss of sample size may be justified in part by an accompanying increase in both heritability estimates and LOD scores, as is evident in this study. More importantly, genes related to some of the covariates also may be involved in BMD regulation. For example, body size (height and weight) and serum vitamin D likely have a substantial genetic component and are significantly correlated with BMD values.[49–51] As a result, controlling for the effect of, for example, BMI on BMD may eliminate the contribution, if any, of genes governing BMI/BMD covariation. By eliminating the contribution of as many covariates as possible, our analysis focuses on the pure contribution of loci governing BMD only.

In our linkage analysis, BMD was considered as a quantitative trait and thus no designation of affected status or penetrance of the trait was required. The variance decomposition approach incorporated in SOLAR accommodates information from the entire pedigree and therefore is more powerful than sib-pair or nuclear pedigree methods that include only similarities between restricted types of relative pairs. Model-free linkage analysis, performed in this study, examined the genetic factors influencing a phenotype without any phenotype-genotype model specification. The latter model is particularly appropriate to osteoporosis, because there are no known major genes determining the BMD phenotypes studied here.[4] However, because Mendelian transmission was suggested previously for BMD measured on the hand phalanges[52] and for spinal BMD,[53] it may be suggested that further linkage analysis should consider also model-based methods.

There are several potential limitations of this study. First, the variance-component approach assumes multivariate normality. Although it may be somewhat insensitive to skewness in the data, it is susceptible to substantially different outliers, which may derive from extreme discordant pairs of relatives. We addressed this issue by applying a correction for a nonnormal distribution, as implemented in SOLAR, by addition of a *t*-distribution parameter. Results of these analyses were almost identical to those obtained without any correction.

A second limitation is that the Framingham Osteoporosis Study was performed in a general population; thus, despite the fact that almost all the subjects are white, unavoidable effects of ethnic admixture and subsequent genetic nonhomogeneity may diminish our power to reveal exact chromosomal location of genetic sources of BMD.

Third, there are certain recognized artifacts in older persons that we were unable to account for, such as aortic calcification, ossification of ligaments, and lumbar osteophytes, which may impact the foregoing results for the lumbar spine BMD phenotype.

Finally, the last potential limitation is that individual genes may influence only male or female BMD, while such effects may be masked in a mixed-gender study such as we report here. Also, in our analysis, we did not differentiate between effects caused by genes controlling peak bone mass or rate of bone loss, and no terms of genotype/age interaction[54] were examined in this study.

The chromosomal regions of our suggestive linkages for some phenotypes are broad, indicating that more than one candidate locus underlying studied BMD phenotypes may map to these regions. Fine mapping of putative QTLs should be performed to refine their genetic positions in these regions. Elucidation of the genes contributing to normal variation in BMD together with gene-gene and gene-environmental interactions will likely strengthen risk assessment algorithms for osteoporosis that would include combined information on the individuals' genetic and environmental profiles and provide molecular targets for future therapeutic interventions for osteoporosis and associated fractures.

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