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Quantitative Trait Loci on Chromosomes 2p, 4p, and 13q Influence Bone Mineral Density of the Forearm and Hip in Mexican Americans

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ABSTRACT

We performed a genome scan using BMD data of the forearm and hip on 664 individuals in 29 Mexican-American families. We obtained evidence for QTL on chromosome 4p, affecting forearm BMD overall, and on chromosomes 2p and 13q, affecting hip BMD in men.

Introduction: The San Antonio Family Osteoporosis Study (SAFOS) was designed to identify genes and environmental factors that influence bone mineral density (BMD) using data from large Mexican-American families.
Materials and Methods: We performed a genome-wide linkage analysis using 416 highly polymorphic microsatellite markers spaced approximately 9.5 cM apart to locate and identify quantitative trait loci (QTL) that affect BMD of the forearm and hip. Multipoint variance components linkage analyses were done using data on all 664 subjects, as well as two subgroups of 259 men and 261 premenopausal women, from 29 families for which genotypic and phenotypic data were available.
Results: We obtained significant evidence for a QTL affecting forearm (radius midpoint) BMD in men and women combined on chromosome 4p near D4S2639 (maximum LOD = 4.33, genomic $p = 0.006$) and suggestive evidence for a QTL on chromosome 12q near locus D12S2070 (maximum conditional LOD = 2.35). We found suggestive evidence for a QTL influencing trochanter BMD on chromosome 6 (maximum LOD = 2.27), but no evidence for QTL affecting the femoral neck in men and women combined. In men, we obtained evidence for QTL affecting neck and trochanter BMD on chromosomes 2p near D2S1780 (maximum LOD = 3.98, genomic $p = 0.013$) and 13q near D13S788 (maximum LOD = 3.46, genomic $p = 0.039$), respectively. We found no evidence for QTL affecting forearm or hip BMD in premenopausal women.
Conclusion: These results provide strong evidence that a QTL on chromosome 4p affects radius BMD in Mexican-American men and women, as well as evidence that QTL on chromosomes 2p and 13q affect hip BMD in men. Our results are consistent with some reports in humans and mice.
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Key words: genetic, linkage, bone density, forearm, genome scan

INTRODUCTION

OSTEOPOROSIS IS A MAJOR public health concern in the United States; it affects more than 25 million people, leading to more than 1.5 million fractures per year, including 300,000 hip fractures. Furthermore, nearly 25% of individuals experiencing an osteroporotic hip fracture die within 1 year.[1,2] The risk of bone fracture at the forearm or hip is directly related to bone mass, especially peak bone mass,[3–5] which is clearly heritable.[6]

Over the past decade, polymorphisms in several candidate genes, such as the vitamin D receptor, have been

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associated with peak bone mass or bone mineral density (BMD), although the effects of these polymorphisms generally are small.[6,7] A few additional genetic variants having relatively large effects on BMD, such as in the *LRP5* gene,[8,9] have been identified from family studies, although the frequency of these variants in the population seems to be rare. Several genome screens have been performed using BMD or femoral structure data on sibpairs and families.[10–17] From these studies, evidence for quantitative trait loci (QTL) has been reported for spine BMD on chromosomes 1q,[13] 3p,[16] 4q, and 12q[10]; for femoral neck BMD on chromosomes 1p,[11,16,18] 5q, and 11q[12,13]; femoral structure on chromosomes 3q, 4q, 5q, 7q, 8q, 17q, and 19q[14,16]; and forearm BMD on chromosome 2p.[15] With the exception of a QTL for spine BMD that is located near LRP5 on chromosome 11q13.4[8,12] and recent reports of QTL for spine BMD on chromosome 1q[13,19] and chromosome 3p,[20] hip BMD on chromosome 1p,[18,20] and femoral structure on 3q, 7q, and possibly 19q,[16] few of the QTL for BMD or bone structure have been replicated. This result is unsurprising given that bone density and structure are likely to be affected by numerous genes and environmental factors, and most genome scans have sufficient power to detect relatively large QTL effects only.

In this study, we report the results of a genome scan of data on BMD of the forearm (radius midpoint) and hip (trochanter and femoral neck) in 29 Mexican-American families (664 individuals) that are part of the San Antonio Family Osteoporosis Study (SAFOS). We also performed subgroup analyses in 259 men and 261 premenopausal women. Our study is the first genome scan performed in large (average size = 23 individuals), non-ascertained pedigrees using data on men and women.

MATERIALS AND METHODS

Subjects

Families enrolled into the SAFOS were selected because of their concurrent participation in a follow-up examination of the San Antonio Family Heart Study (SAFHS), a population-based prospective family study of atherosclerosis and its risk factors.[21] Probands for these families were identified from a low-income neighborhood using a house-to-house recruitment procedure. Eligibility criteria for study probands were that they be 40–60 years of age and have large families in the San Antonio area. All first, second, and third degree relatives of each proband and the proband's spouse were invited to participate, irrespective of the proband's (or relative's) medical history.

Recruitment into the SAFOS was held in conjunction with a 4- to 5-year follow-up examination of the SAFHS families. In 1997, all individuals from the 34 largest SAFHS families, a total of 895 individuals, were invited back to participate in a 5-year follow-up examination. Participating subjects received a physical examination in our clinic in the morning after a 12-h fast and were also interviewed about lifestyle and diet practices. Fasting blood samples were collected for biochemical analysis, and a 2-h glucose tolerance test was also performed. Pregnant women were not eligible to participate; women reporting that they were pregnant were rescheduled for examination at least 3 months after their pregnancy. All procedures were approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio, and all subjects gave written informed consent.

The linkage analyses reported in this study were performed using information on 664 individuals from the 29 largest of these families who had both BMD and genotypic data.

Phenotypes

Bone mineral content (BMC) was measured at the femoral neck and trochanter and forearm (radius midpoint) using DXA (1500W; Hologic, Inc., Bedford, MA, USA) as previously described.[22] The areal BMD (g/cm^2) was determined by dividing the BMC (g) by the projected area of the region scanned (cm^2). The short-term in vivo precision of the BMD was determined on 27 subjects who were examined twice on the same day. The precision of the lumbar spine and total hip was 0.009 g/cm^2 (CV% = 1.0%) and 0.007 g/cm^2 (CV% = 0.87%), respectively. The precision of the manufacturer's spine phantom was 0.0017 g/cm^2 (CV% = 0.17%). BMD traits were not Z-scored by sex and age cohorts before analysis. Instead, effects of sex, age, and other covariates were estimated simultaneously as part of the QTL analyses (see Statistical analyses).

Covariates

Height and weight were measured without shoes. Diabetes was diagnosed using the plasma glucose criteria of the World Health Organization[23](i.e., fasting glucose > 140 mg/dl and/or 2-h post load glucose > 200 mg/dl). Subjects were also considered to have diabetes if they self-reported current use of antidiabetic medications. A questionnaire was administered to obtain information about subjects' medical history, medication use, dietary habits, physical activity patterns, and smoking and alcohol consumption behaviors. An extensive reproductive history questionnaire was administered to women that included questions about menstrual cycles and current use of oral contraceptives and estrogens. Women were considered to be menopausal if more than 1 year had elapsed since last menstrual period or if they had undergone surgical menopause, defined as having both ovaries removed.

Dietary calcium intake was assessed by a 104-item food frequency questionnaire designed for this population,[21] and supplemental calcium intake was defined as the number of milligrams of calcium the subject consumed per day as a result of multivitamin or supplemental calcium pills. Physical activity was assessed using a modified version of the Stanford 7-day Physical Activity Recall Instrument to obtain a measure of metabolic equivalents used per week or METS (1 MET equals the energy expenditure of 1 kg of body weight/h).[21]

Genotypes

DNA was isolated from lymphocytes for polymerase chain reaction (PCR) and automated genotyping. The DNA was amplified with fluorescently labeled primer pairs from

MapPairs Human Screening Set Versions 6 and 8 (Research Genetics, Huntsville, AL, USA) that detect highly polymorphic microsatellite markers. PCRs were performed according to the manufacturer's protocol. Aliquots of the PCRs were pooled into multiplexed panels for genotyping with Applied Biosystems (Perkin Elmer, Foster City, CA, USA) Model 377 DNA Sequencers and Genescan and Genotyper DNA Fragment Analysis software.

A total of 416 microsatellite markers from 22 autosomes were included in the analysis. The distances between markers were computed from our data using the CRI-MAP software program[24] and verified for consistency with the genetic maps available from the Marshfield Medical Research Foundation (Marshfield, WI, USA) (www.mfldclin.edu/genetics) and University of Southampton (Southampton, UK) (http://cedar.genetics.soton.ac.uk/public_html/gene.html/). The average spacing between markers was 9.5 (Haldane) cM.

Table 1. Characteristics of the SAFOS Population

	Males	*Females*
Total	259	405
Mean age (years)	42.4 ± 16.4 (18–96)	43.0 ± 15.2 (18–89)
Mean BMI (kg/m^2)*	29.5 ± 5.8 (17.6–49.0)	31.6 ± 7.6 (16.3–65.6)
Diabetics (%)	26	25
Smokers (%)	28	15
METS	277.7 ± 58.4	251.7 ± 36.3
Menopause (%)	—	35
Oral contraceptives (%)	—	13
Mean BMD		
Hip (neck)	0.902 ± 0.146	0.845 ± 0.142
Hip (trochanter)	0.759 ± 0.115	0.679 ± 0.115
Forearm (radius midpoint)	0.680 ± 0.066	0.576 ± 0.060

* Range in parentheses.

Statistical analyses

The aim of the current analyses was to determine whether QTL contribute to variation in BMD of the forearm and hip in the San Antonio Mexican-American families in men and women combined, as well as in men and premenopausal women separately. We did not analyze data on postmenopausal women separately because the small sample size ($n = 144$) resulted in pedigree structures that were too sparse to obtain meaningful results. Before the linkage analysis, we used quantitative genetic methods to simultaneously model the total variation in each BMD trait over all and in both subgroups as a function of the mean value, effects attributable to the measured covariates, and the proportions of the remaining variation that could be attributed to the residual additive genetic and unmeasured environmental effects.[21] The purpose of these quantitative genetic analyses was to reduce the amount of unexplained trait variation by accounting for measured effects (e.g., sex, age, sex × age, age^2, sex × age^2, other covariates, and residual heritability) so that the relative proportion of the variability attributable to the QTL would be maximized. Using data on the total 664 individuals (or 259 men or 261 premenopausal women) with data in 29 pedigrees, all parameters were estimated simultaneously by maximum likelihood methods. Significance of the residual heritability and the covariate effects was assessed by comparing the likelihood of a submodel, in which the specific parameter to be tested was fixed at zero, to that of a model in which all parameters were estimated, using the likelihood ratio test, as described in detail elsewhere.[21] This statistic is asymptotically distributed as a χ^2 with one degree of freedom. Because we are primarily interested in detecting genes that affect unmeasured variation, we chose a liberal significance level ($p < 0.10$) for inclusion of covariates. Details of these analyses are presented elsewhere.[22]

Two-point and multipoint genomic scans were performed using a variance components method that has been extended for use on full pedigrees as implemented in SOLAR.[25] Briefly, we estimated the genetic variance attributable to the region around a specific genetic marker (σ^2_m) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. We compared the likelihood of the restricted model, in which $\sigma^2_m = 0$ (no linkage), with that of a model in which the variance caused by the marker is estimated. True multipoint IBD probabilities were computed using the Markov chain Monte Carlo algorithm implemented in Loki.[26] After conducting a "first-pass" linkage analysis to detect QTL influencing each trait, we performed a sequential, or second pass, multipoint linkage analysis in which we accounted for the QTL with the highest LOD score in the linkage model and then included a second QTL effect that was conditional on the presence of the first QTL effect. Sequential multipoint linkage analysis, as this procedure has been called, may help eliminate false positives and/or uncover additional QTL that may be masked by the marginal effects of other QTL.[27] The significance of the second QTL effect was evaluated by comparing the likelihood of the dual QTL model (the full model) to that of the single QTL model (nested model).

To assess the significance of the multipoint LOD scores for all traits, we generated an empirical distribution of nominal LOD scores for each phenotype. This null distribution was generated by simulating 10,000 unlinked markers and then evaluating evidence for linkage to each marker. All LOD scores given in the text are empirically adjusted LOD scores. In addition, we calculated genomic p values following the suggestion of Lander and Kruglyak.[28]

RESULTS

BMD phenotypic and genotypic data were available for a total of 664 individuals in 29 two- and three-generation pedigrees that ranged in size from 3 to 63 individuals, with a median size of 24. Detailed descriptions of the characteristics of the SAFOS families have been presented elsewhere.[21,22] Briefly, the 405 women and 259 men had a mean age of 43.0 and 42.4 years, respectively (range, 18.5–96.7 years), and a mean body mass index (BMI) of 31.6 and 29.5 kg/m^2, respectively (range, 16.3–65.6 kg/m^2; Table 1).

Approximately 25% of the family members were diabetics, 15% of women and 28% of men were smokers, and 35% of the women were postmenopausal.

Initial analyses of the full (men and women) SAFOS cohort revealed the following covariates to be associated with radius BMD: age, age^2, sex, BMI, METS (per week), diabetes status (present/absent), and supplemental calcium intake (g/day). Together, these variables accounted for 59% of the total variation in radius BMD. The following covariates were significantly associated with femoral neck and trochanter: age, sex, BMI and METS; in aggregate, these variables accounted for 41% and 31% of the total variation of BMD of the femoral neck and trochanter, respectively. After simultaneously incorporating the effects of covariates, the estimated residual heritabilities for BMD of the radius midpoint, femoral neck, and trochanter were 0.44 $\pm$ 0.08, 0.51 $\pm$ 0.07, and 0.51 $\pm$ 0.07, respectively. Thus, as expected, residual heritability estimates for BMD of the forearm and hip were relatively high and highly significant ($p <$ 0.00001).

In men, the covariates age^2, BMI, and diabetes status were significantly correlated with radius BMD and accounted for 14% of the total variation. Femoral neck and trochanter BMD were significantly correlated with age, age^2, BMI, and METS, and these covariates accounted for 31% and 12% of the total variation, respectively. Estimated residual heritabilities of radius, neck, and trochanter BMD were 0.38 $\pm$ 0.19 ($p < 0.01$), 0.67 $\pm$ 0.13 ($p <$ 0.00001), and 0.63 $\pm$ 0.14 ($p <$ 0.00001), respectively.

Analyses of BMD data in premenopausal women revealed that age, age^2, BMI, and diabetes status accounted for 11% of the variation in radius BMD. The covariates age and BMI were significantly correlated with femoral neck BMD, and BMI alone was correlated with trochanter BMD. These covariates accounted for 33% and 21% of the variation in neck and trochanter BMD, respectively. Estimated residual heritabilities in premenopausal women were higher than those in men and were estimated as 0.60 $\pm$ 0.20 ($p <$ 0.00001), 0.68 $\pm$ 0.20 ($p < 0.0001$), and 0.68 $\pm$ 0.20 ($p <$ 0.0001) for radius, neck, and trochanter BMD, respectively.

While simultaneously incorporating the above-mentioned covariates, we next performed multipoint variance components linkage analyses over all men and women to detect QTL influencing BMD of the hip and forearm. The most striking linkage result was for radius midpoint BMD, for which we obtained highly significant evidence (maximum LOD = 4.33, genomic p value = 0.006, Bonferroni-adjusted p value = 0.018) for a QTL on chromosome 4; Table 1). The two-LOD support interval for this QTL ranged between 23 and 45 cM (Fig. 1). The two-point LOD scores for markers in this region were 3.98 for marker D4S2639 (map position 35.4 cM) and 1.20 for marker D4S403 (map position 26.2 cM).

In addition to the possible QTL on 4p, we also obtained suggestive evidence that additional QTL on chromosomes 7q (maximum LOD = 2.24 at position 150 cM near D7S1804) and 12q (multipoint LOD = 2.24 at position 136 cM near D12S2070) may affect BMD of the wrist (Table 2). To further investigate whether QTL in addition to the significant QTL on chromosome 4 influence forearm BMD, we performed a conditional multipoint linkage analysis by conditioning on the putative location of the significant QTL for forearm BMD on chromosome 4 and re-running the multipoint linkage analyses. These analyses revealed no significant evidence for additional QTL affecting forearm BMD, although suggestive evidence for a QTL on chromosome 12q was retained (conditional LOD = 2.35 at position 135 cM; Table 2).

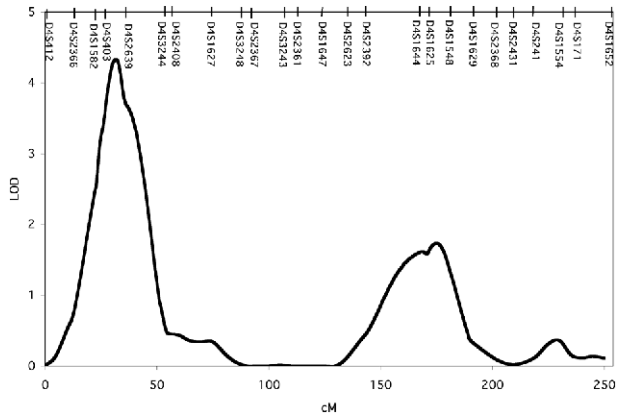

FIG. 1. Multipoint LOD score profile for BMD of the forearm on chromosome 4.

In addition to the conditional linkage analyses, we also performed linkage analyses in the men and premenopausal women separately to determine whether the evidence for the 4p QTL was attributable to variation in either or both groups. We obtained no significant evidence for linkage of forearm BMD in either men or premenopausal women; however, we did obtain LOD >1.80 for each group at a similar location on chromosome 4p (Table 3). This result indicates that the 4p QTL has similar effects on radius BMD in both sexes.

We had no significant evidence for a QTL that influences BMD of the femoral neck or trochanter overall (Table 2) or in premenopausal women (Table 3). However, when we analyzed data on the men (Table 3), we obtained evidence for QTL influencing femoral neck BMD on chromosome 2p near D2S1780 (maximum LOD = 3.98, genomic p = 0.013, Fig. 2) and trochanter BMD on 13q near D13S788 (maximum LOD = 3.46, genomic p = 0.039, Fig. 3). The 13q QTL is interesting because we also obtained suggestive evidence for a QTL (maximum LOD = 2.51) in the same region for neck BMD in men, although after conditioning on the putative chromosome 2p QTL, minimal evidence for this QTL on neck BMD remained (conditional LOD = 1.49, data not shown).

DISCUSSION

Within the past decade, several investigators have reported evidence for QTL influencing BMD and bone structure.[10–19,29] A comparison of results across these studies reveals some consistent evidence for seven or eight possible QTL that affect spine and/or hip BMD or femoral structure, although the evidence for linkage for most studies

TABLE 2. MAXIMUM MULTIPOINT LOD SCORES FOR BMD OF THE FOREARM AND HIP IN 24 MEXICAN-AMERICAN FAMILIES

	BMD phenotype							
	Femoral neck		*Trochanter*		*Forearm*		*Forearm (conditional analysis)*	
Chromosome	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*
1	0.91	15	0.70	185	0.45	15	0.26	270
2	0.91	180	1.51	29	0.99	278	1.21	280
3	0.94	5	0.97	10	0.99	38	0.48	35
4	0.51	0	0.57	70	**4.33**	**32**	0.93	175
5	0.06	5	0.13	95	0.52	85	0.61	85
6	0.83	190	**2.27**	**190**	0.26	210	0.31	155
7	0.61	55	0.31	130	**2.24**	**150**	1.40	154
8	1.06	71	0.26	50	0.75	10	0.84	10
9	1.13	123	1.75	141	1.14	78	0.66	80
10	1.29	152	1.55	200	0.69	100	0.46	100
11	0.53	165	0.81	10	0.60	5	0.62	0
12	0.02	80	0.14	45	**2.24**	**136**	**2.35**	**135**
13	1.08	107	0.86	20	0.32	25	0.55	0
14	1.17	39	0.64	35	0.12	80	0.22	75
15	0.25	0	0.33	20	0.10	15	0.18	85
16	0.00	—	0.44	145	0.00	—	0.03	60
17	0.67	165	0.79	90	1.12	163	1.30	157
18	0.21	120	0.03	50	0.52	0	0.46	0
19	0.35	105	0.47	100	1.46	0	0.79	0
20	0.47	75	0.12	60	0.50	20	0.35	80
21	0.14	70	1.39	61	0.00	—	0.00	—
22	0.29	20	0.06	25	0.02	20	0.00	—

LODs > 2.00 are in bold.

is not statistically significant, that is, the genomic p value >0.05. These possible loci include (1) a QTL for spine BMD that is located near LRP5 on chromosome 11q13.4,[8,12] (2) a QTL for spine BMD on chromosome 1q,[13,19] (3) a QTL for spine BMD on 3p,[20] (4) a QTL for hip BMD on chromosome 1p,[18,20] (5) a QTL on chromosome 4q32 that affects BMD of the hip,[11] spine, and forearm,[10] and (6) QTL on 3q, 7q, and possibly 19q[16] that affect femoral structure. We did not obtain any evidence that these eight possible QTL affected forearm or hip BMD in our Mexican-American families, possibly because of the relatively low power of our study to detect QTL with modest effects. Simulation studies indicate that we have only 55% power (at LOD $\geq$ 2.0) to detect a QTL that accounts for $\leq$20% of the residual phenotypic variation of trait, although we have 78% power to detect at least suggestive evidence (LOD $\geq$ 2.0) for a QTL that accounts for 25% of the residual phenotypic variation. QTL whose effects on BMD variation are expressed predominantly in women (or men) only, or predominantly in younger individuals, are not likely to account for a large proportion of the population variance. Failure to replicate linkages across populations could also be attributable to differences in ethnic backgrounds between study populations and/or differences in ascertainment schemes with different age and sex compositions. Although we did not detect linkage in our Mexican-American families to any of the above QTL, we did detect evidence of linkage to at least four regions that are consistent with reported QTL for BMD in mice,[29] as well as results of genome scans[10,11,15] and association studies in humans.[30,31]

In men, we detected evidence that a QTL between D13S788 and D13S800 (50.8 and 72.8 Mb) on chromosome 13q14–13q22 (http://genome.ucsc.edu) affects BMD of the trochanter (LOD = 3.46) and hip neck (LOD = 2.51). Intriguingly, Beamer et al.[29] performed QTL analyses on female mice from an F2 cross from two inbred mouse strains that differed in femur BMD and reported very strong evidence (LOD = 16.3) for a QTL that affects femur BMD in a region homologous to human chromosome 13q14–21. Although no obvious candidate genes are present in this region, the concurrence of strong linkage signals in mice, as well as in men, is encouraging. Deng et al.[10] also reported suggestive evidence (LOD = 2.43) for a QTL for spine BMD on 13q33–13q34, but this may represent a different QTL.

In addition to the possible QTL on 13q, we also detected evidence in men (maximum LOD = 3.98, genomic p = 0.013) that a QTL for hip neck BMD is near D2S1780, which is at 33Mb on chromosome 2p25 (http://genome.ucsc.edu). Although the results are not strong, Niu et al.[15] and Devoto et al.[11] detected suggestive evidence for a QTL located on chromosome 2p21–24 that affects BMD of the forearm (LOD = 2.15) and spine (LOD = 2.25), respectively. Furthermore, in mice, Beamer et al.[29] detected weak evidence (LOD = 2.89, p = 0.05) for a QTL influencing femur BMD in this region. If these observations are correct, they suggest that a possible QTL on 2p may

TABLE 3. MAXIMUM MULTIPOINT LOD SCORES FOR BMD OF THE FOREARM AND HIP IN MALES AND PREMENOPAUSAL FEMALES

	BMD phenotype											
	Males						*Premenopausal females*					
	Forearm		*Neck*		*Trochanter*		*Forearm*		*Neck*		*Trochanter*	
Chromosome	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*	*LOD*	*Position*
1	0.63	75	1.29	72	1.84	73	0.43	215	0.17	195	0.37	200
2	1.07	96	**3.98**	**0**	1.81	186	1.07	196	0.67	150	1.47	282
3	1.23	234	1.81	55	0.62	245	0.13	43	0.43	130	0.66	7
4	1.87	23	1.09	3	0.71	10	1.81	29	0.29	75	0.56	230
5	0.31	0	0.08	85	0.37	75	0.27	165	0.43	125	0.26	70
6	0.75	210	1.09	64	0.61	205	0.36	210	0.87	60	0.31	60
7	0.81	145	0.34	130	0.15	130	0.80	155	1.83	58	0.34	105
8	0.37	115	**2.15**	**48**	1.31	43	0.98	15	0.48	180	0.07	170
9	2.12	80	0.52	75	0.37	70	0.01	125	0.37	85	0.85	130
10	0.58	100	0.99	155	0.83	165	0.40	150	0.86	130	1.55	142
11	0.76	0	0.60	15	0.96	10	1.61	25	0.14	70	0.76	170
12	0.64	10	0.83	65	0.72	40	0.86	160	0.00	—	0.00	—
13	0.57	70	**2.51**	**60**	**3.46**	**55**	0.61	50	0.23	110	0.05	130
14	0.36	80	0.39	110	0.56	65	0.01	30	1.06	43	0.49	60
15	1.42	1	0.14	105	0.42	45	0.24	50	0.57	40	0.64	105
16	0.00	—	0.49	145	0.37	145	0.02	35	0.67	45	0.46	65
17	0.29	15	1.42	27	1.06	29	0.83	160	1.28	140	0.07	165
18	0.47	0	0.68	75	0.11	140	0.36	110	0.37	120	0.03	50
19	0.94	0	0.82	110	1.14	103	0.42	100	0.01	35	0.40	75
20	0.17	20	0.36	100	1.24	100	**2.18**	**8**	0.11	0	0.17	55
21	0.00	—	0.05	0	0.20	55	0.21	25	0.30	70	0.71	65
22	0.27	45	1.12	35	0.46	35	0.12	0	0.23	50	0.56	20

LODs > 2.00 are in bold.

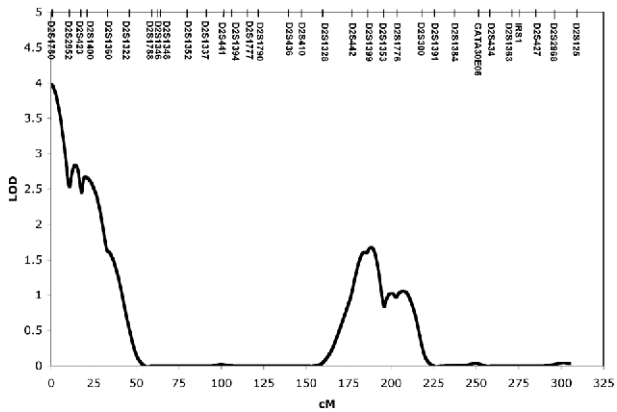

FIG. 2. Multipoint LOD score profile for BMD of the hip and neck in men on chromosome 2.

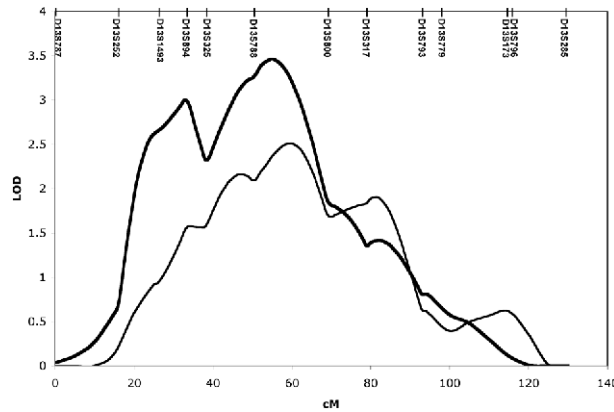

FIG. 3. Mulitpoint LOD score profile of BMD of the trochanter (thick line) and neck (thin line) in men on chromosome 13.

have pleiotropic effects on BMD of the hip, forearm, and spine.

We obtained suggestive evidence (maximum LOD = 2.35) that a QTL for forearm BMD in men and women resides near D12S2070, which is located at 115 Mb on chromosome 12q24 (http://genome.ucsc.edu). This result also is interesting because two other research groups[10,17] reported suggestive evidence for potential QTL for spine BMD on chromosome 12q24 near locus D12S395 (LOD = 2.08)[17] and locus D12S1723 (maximum LOD = 2.96).[10] These two markers are located at 120 and 132 Mb, respectively, on chromosome 12q24 (http://genome.ucsc.edu). If these results are true, they could indicate that a locus on 12q24 has pleiotropic effects on BMD of the forearm and spine. One potential candidate gene that could have pleiotropic effects on the spine and forearm is insulin-like growth factor 1 (*IGF-1*), which is located at 102 Mb on 12q23 (http://genome.ucsc.edu). IGF-1 is involved in regulation of

growth and has been associated with BMD and osteoporosis in humans and with BMD in mice.[30,32]

Although replication of linkage has been reported for a few possible QTL influencing spine and hip BMD and femoral structure, no replications have been reported for QTL influencing forearm BMD, although possible QTL on 4q and 2p may have pleiotropic effects on spine and forearm BMD.[10,11,15] The lack of any consistent reports of QTL for forearm may have several causes. First, there are fewer reports of genome scan analyses of BMD for the forearm than for the hip or spine, and in several studies, individuals were ascertained on low BMD of the spine or hip. Second, the residual heritability of wrist BMD is lower, at least in our study, than BMD of the hip and spine,[22] thus requiring larger sample sizes to achieve significant results. Furthermore, the lower residual additive heritability indicates that environmental factors have a larger effect on forearm BMD, a factor that may complicate detection of QTL in populations in which few environmental factors are measured.

As described above, we report strong evidence for QTL associated with radius BMD that lies on chromosome 4 between markers D4S403 and D4S2639 (maximum multipoint LOD = 4.33, genomic p value = 0.006). Several genes within this region have known or potential roles in skeletal metabolism, including peroxisome proliferative activated receptor gamma, coactivator 1 (*PPARGC1*), superoxide dismutase 3, extracellular (*SOD3*), and heparin-binding growth factor binding protein (*HBP17*).

Of the candidate genes identified to date, *PPARGC1* seems of particular interest because the peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors that regulate transcription of various genes. PPAR-γ is activated by fatty acids and eicosanoids and plays a major role in adipocyte differentiation, and its actions in energy homeostasis can be modulated by *PPARGC1*. Furthermore, osteoblasts and adipocytes share a mesenchymal precursor cell[33,34] whose differentiation can be modulated by PPARG2. Ogawa et al.[31] confirmed endogenous expression of PPARG2 protein as well as its transcript in primary osteoblasts derived from rat calvariae and tested the association of a silent *PPARG2* exon 6 polymorphism with BMD in a postmenopausal Japanese population. Subjects carrying a *T* allele (CT and TT) at this locus had a lower BMD than did the group carrying the CC genotype. These observations, coupled with the close proximity of *PPARGC1* locus to the peak of our linkage signal, implicate PPARGC1 as a strong positional candidate gene that influence variation in forearm BMD.

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