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ORIGINAL INVESTIGATION

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A genome-wide scan for loci linked to forearm bone mineral density

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Abstract Osteoporosis is a chronic disorder characterized by low bone mass and fragility fractures. It affects more than 25 million men and women in the United States alone. Although several candidate genes, such as the vitamin-D-receptor gene or the estrogen-receptor gene, have been suggested in the pathogenesis of osteoporosis, the genetic dissection of this disorder remains a daunting task. To search systematically for chromosomal regions containing genes that regulate bone mineral density (BMD), we scanned the entire autosomal genome by using 367 polymorphic markers among 218 individuals (153 sibpairs) from 96 nuclear families collected from three townships of Anqing, China. In these 96 families, DNA samples from both parents were available for 82 (85.4%) families. By using age- and gender-adjusted forearm BMD measurements, a peak on chromosome 2 near *D2S2141*, *D2S1400*, and *D2S405*, a region previously linked to spinal BMD, showed evidence of linkage to both proximal and distal forearm BMD (multipoint LOD=2.15 and 2.14 for proximal and distal forearm BMD, respectively). One region on chromosome 13 (multipoint LOD=1.67) in the proximity of *D13S788* and *D13S800* showed evidence of linkage to distal forearm BMD only. Possible candidate genes included *CALM2* (calmodulin 2) at 2p21.3-p21.1, a putative *STK* (serine/threonine kinase) at 2p23–24, *POMC* (pro-opiomelanocortin) at 2p23.3, and *COL4A1* and *COL4A2* (collagen IV alpha-1 and alpha-2 subunits) at 13q34. Because of the limited sample size, the suggestive evidence of linkage of this study should be considered as tentative and needs to be replicated in other larger populations.

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Introduction

Osteoporosis is a chronic disorder characterized by low bone mass and fragility fractures. It affects more than 25 million men and women in the United States alone (Consensus Development Conference 1993; Melton 1995). Health care expenditures for osteoporotic patients in this country are nearly 13 billion dollars per annum (Melton 1995; Jacobson et al. 1990; Ray et al. 1997). Currently, one third of the world's hip fractures are estimated to occur in Asia (Cooper et al. 1992), and it is projected that 50% of all hip fractures will occur in Asia by the middle of the next century, the majority in China (Lau and Cooper 1996).

Longitudinal and cross-sectional studies have firmly established that low bone mineral density (BMD) is the single most potent risk factor for future osteoporotic fracture (Hui et al. 1989; Ross et al. 1991; Jones and Davie 1998). For instance, in the study of Yang et al. (1996) conducted on a Chinese population, the BMD values of the fracture cases were significantly lower than those of the controls. In particular, the risk of fracture increased exponentially with increased age and reduced BMD. Since osteoporosis is an extending continuum beginning with low BMD and ending with fracture, it is the latent phases of osteoporosis at which risk determination becomes so critical.

Several sociodemographic and environmental factors, e.g., age, years since menopause, body mass index (BMI), cigarette smoking, and alcohol consumption, have been associated with fracture risk in Chinese (Shaw 1993; Lau et al. 1992). By contrast, few genetic studies of osteoporosis or BMD have actually been conducted in China. The goal of the current study has been to search systematically for

chromosomal regions that may harbor genes controlling forearm BMD in a Chinese population. With the support of the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotying Service, we have completed a total genomic screening of 1477 indivudals with parents initially selected for extreme blood pressure values in the offspring. The distal and proximal forearm BMD data were obtained from a subset of these genotyped subjects consisting of 153 sibpairs (218 siblings) from 96 nuclear families and were included in the analyses.

Materials and methods

Subjects

All the study subjects were recruited from Anqing in Anhui Province, China. Anqing stretches for about 80 km along the north bank of the Yangtze river. It has three urban areas and eigth rural counties, with a total area of 15,000 km^2. Available records indicate that the Anqing district was settled 2000 years ago. The total population in 1990 was 5.8 million (10% urban and 90% rural). Because of limited public transportation, the population has a very stable base and is extremely homogeneous with respect to ethnicity, dietary habits, lifestyles, and environmental factors. Initial recruitment yielded 1477 individuals (804 offspring from 337 nuclear families, and 673 out of 674 parents) from three townships in Anqing: Huaining, Wangjiang and Susong. The offspring of these selected families comprised extreme sibling pairs for blood pressure values. A resting systolic blood pressure (SBP) or diastolic blood pressure (DBP), from at least 3 readings, in the top or bottom of the age-adjusted decile at three separate screenings was used to define high or low blood pressure status; all offspring were aged 15–55 years. Between April 1997 and April 1998, a clinical laboratory for peripheral dual energy X-ray absorptiometry (pDXA) measurements was set up in each of the townships Huaining, Wangjiang, and Susong. An invitation letter from each center was sent to the township residents who had been enrolled in the genetic study of blood pressure. Forearm BMD data were obtained from 218 offspring and their available parents in 96 nuclear families who participated during this period of time and were included in the final analyses. The distribution of these 218 offspring is presented in Table 1. Sociodemographic questionnaires concerning life style, reproductive history, physical activity, diet, and use of medication or herbal preparations were administered by well-trained interviewers. We compared the major demographic and clinical variables in the non-participants and participants of the 804 initially recruited offspring. The mean± standard deviations (SDs) of age, height, weight, BMI, SBP, and DBP of the non-participated siblings were 29.8±6.6 years, 1.61±0.08 m, 57.71±10.29 kg, 22.16±3.13 kg/m^2, 124.22±21.12 mm Hg, and 70.99±16.54 mm Hg, respectively. These values were similar to those of the 218 siblings who participated in the current study (age: 30.3±6.34 years, height: 1.63±0.08 m, weight: 58.57±8.50 kg, BMI: 22.13±2.67 kg/m^2, SBP: 126.72±19.46 mm Hg, and DBP: 72.60±15.50 mm Hg; see Table 2). Informed consent was provided prior to any testing, and approval for this study was received from the Institutional Review Board of the Harvard School of Public Health and Anqing. Blood pressure, height, and weight were obtained from all individuals prior to forearm BMD measurements by means of standardized protocols.

Table 1 Distribution of families

Father	Mother	No. of offspring			Total no. of nuclear families
		2	3	4	
Yes	Yes	63	15	4	82
Yes	No	7	0	1	8
No	Yes	3	1	0	4
No	No	2	0	0	2
Total no. of nuclear families		75	16	5	96
Total no. of individuals		150	48	20	218

Bone mineral density by using pDXA

The forearm proximal and distal scans were performed on five pDXA machines (Norland, Ft. Atkinson, Wis.) as described previously (Xu et al. 1998). Scans were repeated on the same subject within 10 min to assess reproducibility of both proximal and distal sites. Data were reported as BMD in grams/cm^2. Area, length, width, and bone mineral content were also noted. Overall, the pDXA method used in this study had a precision error of about 2% (Xu et al. 1998) and was comparable to the previously reported 2.6% (Leboff et al. 1992).

Phlebotomy

Forearm venous blood samples were obtained from all study participants via venipuncture. Samples were collected in 10-ml vacutainer tubes containing EDTA (2 tubes) and citrate (2 tubes). Tubes were kept on ice and subsequently centrifuged for 10 min in a tabletop refrigerated centrifuge at 4000 rpm. Plasma was subsequently removed from the cell pellet by pipetting. All samples were frozen and stored at –85°C.

DNA extraction

DNA extraction was performed at the Anhui Meizhong Institute for Biomedical Science and Environmental Health. Isolation of genomic DNA was performed with Puragene DNA isolation kits (Gentra Systems, Minneapolis, Minn.) by a modification of a previously described method (Buffone and Darlington 1985). This DNA extraction protocol typically yielded 300 µg DNA from 10 ml whole blood with an $OD_{260}/OD_{280}\approx1.8$. After extraction, half of the DNA sample was sent to the Program for Population Genetics for genetic analysis, and the other half was stored at –85°C as a backup sample.

Table 2 Clinical characteristics of siblings of the study sample

Variable	*n*	Mean	SD	Median	Interquartile range
Age (years)	218	30.3	6.3	29.7	25.7–33.8
Height (cm)	218	162.6	8.1	163.0	156.0–169.0
Weight (kg)	218	58.6	8.5	58.0	53.0–65.0
BMI (kg/m^2)	218	22.1	2.7	21.8	20.3–23.7
Proximal forearm BMD (g/cm^2)	218	1.01	0.52	0.85	0.70–0.95
Distal forearm BMD (g/cm^2)	218	0.60	0.56	0.43	0.31–0.51

Table 3 Chromosomal regions linked (LOD≥1.2) to adjusted forearm BMD traits[a]

Phenotype	Chromosome	Markers in proximity to the interval	Peak Z_w	Peak LOD	Peak cM[b]
Proximal	2	*D2S2976, D2S1400, D2S405*	3.15	2.15	50.0
Distal	2	*D2S2976, D2S1400, D2S405*	3.14	2.14	50.0
	13	*D13S788, D13S800*	2.77	1.67	55.0

[a] Forearm BMD values were adjusted for age, age^2 and sex
[b] Distance from pter

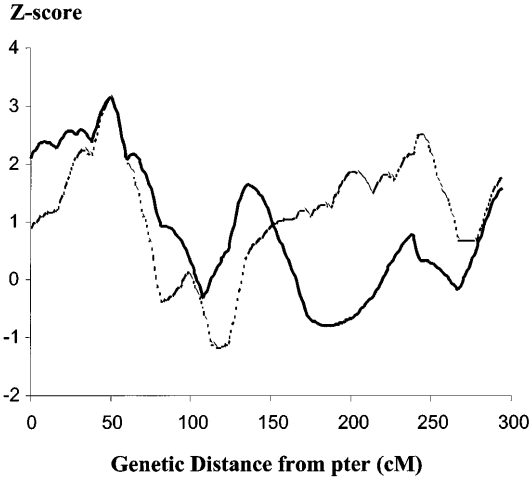

Fig. 1 Nonparametric multipoint linkage analysis Z scores for proximal forearm BMD (*solid line*) and distal forearm BMD (*dotted line*) plotted against genetic distance (chromosome 2)

Genotyping

Genotyping of Weber screening set 9 markers (Yuan et al. 1997) for the 1477 individuals was performed by the NHLBI Mammalian Genotyping Service in Marshfield, Wis., by using fluorescent-based detection. Protocol details can be found at http://www.marshmed.org/genetics/. Average heterozygosity was 0.74, and only 3 markers had completeness values of less than 90%. For a subset of DNA samples, we re-genotyped 7 markers, yielding a total of 2838 genotypes in our laboratory, and found 99.8% concordance with the genotypes provided to us.

Statistical analyses

Descriptive statistical analyses were performed by using the procedures of the SAS Institute (Cary, N.C.). The distal and proximal forearm BMD did not approximate a normal distribution, even after $\log_{10}$ transformation. To control for the effects of age and sex, multiple linear regression analyses were used in the linkage analyses of the adjusted BMD values. Allele frequencies were estimated from 673 parents genotyped for a larger genetic investigation of blood pressure (see above) from which the present subset of subjects was drawn. Because of the non-normality and heteroscedasticity of the adjusted or unadjusted proximal and distal forearm BMD measurements, the traditional parametric quantitative trait locus (QTL) mapping methods would not have been appropriate. Multipoint analyses were performed by using the nonparametric function of MAPMAKER/SIBS program version 1.0 (Kruglyak and Lander 1995) to detect linkage. Our analyses used all possible sibpairs.

The proportion of alleles identical by descent was estimated, and single-marker nonparametric sibpair linkage analyses were performed by using the SIBPAL procedure of the Statistical Analysis for Genetic Epidemiology package (S.A.G.E.; version 2.6, release 2.2; S.A.G.E. 1994) for markers with a LOD>2.0 (corresponding to a Z score greater than 3.04).

Power simulations of genome scan

The significance of the results from nonparametric QTL analysis (Kruglyak and Lander 1995) of the genome-scan data was evaluated by using simulation. Parental alleles at markers across the genome were simulated according to population allele frequencies and were allowed to drop down at random to the offspring, taking into account linkage between markers on the same chromosome. The value of the nonparametric Z statistic was calculated at 10 equally spaced positions within each map interval, with the same methods of Kruglyak and Lander (1995). This process was repeated for 2000 simulated replicates, allowing the estimation of significance levels (i.e., *P* values) down to 0.01 with reasonable accuracy (standard errors were approximately 0.002 for the region that reached a significance level of 0.01, and approximately 0.005 for the region with a significance level of 0.05).

Pointwise significance levels were evaluated by examining the distribution of the simulated Z statistics at a single map position (results were found to be consistent across all map positions). Genome-wide significance levels were calculated by examining the distribution of the maximum Z statistic obtained in each replicate from all 22 chromosomes.

Results

Table 2 presents the clinical characteristics of the 218 offspring (136 men, 82 women) comprising the 153 sibpairs. The phenotypes of the parents are not presented, because only the sibpair phenotype data were needed in the linkage analyses.

By using age- and gender-adjusted forearm BMD measurements, an interval on chromosome 2 covering *D2S2976–D2S405* (Table 3, Fig. 1) showed evidence of linkage to both proximal and distal forearm BMD values (multipoint LOD=2.15 for proximal forearm BMD, and multipoint LOD=2.14 for distal forearm BMD), whereas one region of interest on chromosome 13 (Table 3, Fig. 2) in the proximity of *D13S788* and *D13S800* showed evidence of linkage to distal forearm BMD only (multipoint LOD=1.67). The interval on chromosome 2 therefore provided the strongest evidence of linkage to both proximal and distal forearm BMD obtained in this study. Single-point analyses of *D2S405* showed a significant negative relationship between the squared difference of adjusted distal forearm BMD and mean allele sharing $\hat{\pi}$ (β=–0.253, P<0.05). A marginally significant relationship was demonstrated between the squared difference of adjusted proximal forearm BMD and $\hat{\pi}$ (β=–0.285, P=0.06).

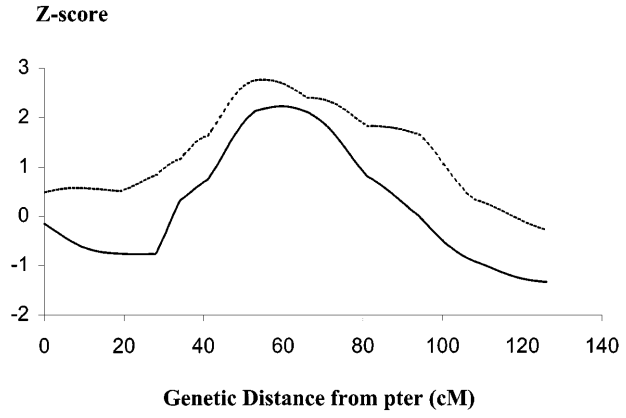

Fig. 2 Nonparametric multipoint linkage analysis Z scores for proximal forearm BMD (*solid line*) and distal forearm BMD (*dotted line*) plotted against genetic distance (chromosome 13)

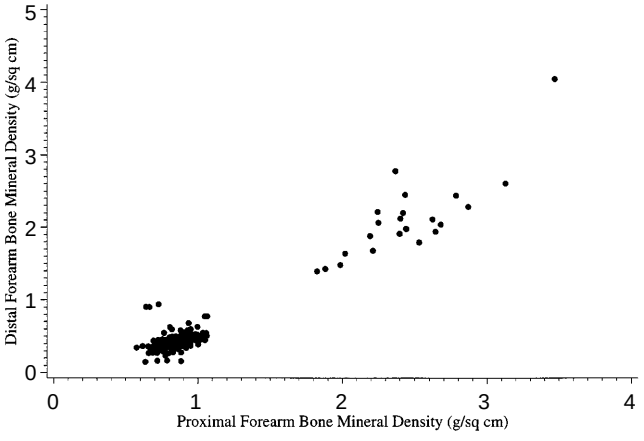

Fig. 3 Plot of individual values of distal forearm BMD against proximal forearm BMD

A significant correlation was found (Fig. 3) between the proximal and the distal forearm BMD values among the 218 siblings studied ($r_{proximal,distal}$=0.96, P<0.001). The distribution of siblings' blood pressure against their forearm BMD data can be seen in Figs. 4–7. No significant relationships can be demonstrated between either proximal or distal forearm BMD values to either SBP or DBP values ($r_{proximal,SBP}$=0.06, P>0.10; $r_{proximal,DBP}$=0.04, P>0.10; $r_{distal,SBP}$=0.04, P>0.10; $r_{distal,DBP}$=0.04, P>0.10).

The simulated genome scan yielded the following results. For adjusted proximal forearm BMD, the nonparametric QTL Z scores of 3.654, 3.710, 3.759, 3.862, and 4.009 have genome-wide significance levels of 0.05, 0.04, 0.03, 0.02, and 0.01, respectively. For adjusted distal forearm BMD, the nonparametric QTL Z scores of 3.695, 3.773, 3.840, 3.960 and 4.165 have genome-wide significance levels of 0.05, 0.04, 0.03, 0.02 and 0.01, respectively. These criteria are slightly less conservative (i.e., lower) than the suggested criteria of Lander and Kruglyak (1995), who have shown that a Z score of 4.100 corresponds to a genome-wide P value of 0.05. The highest Z scores of the

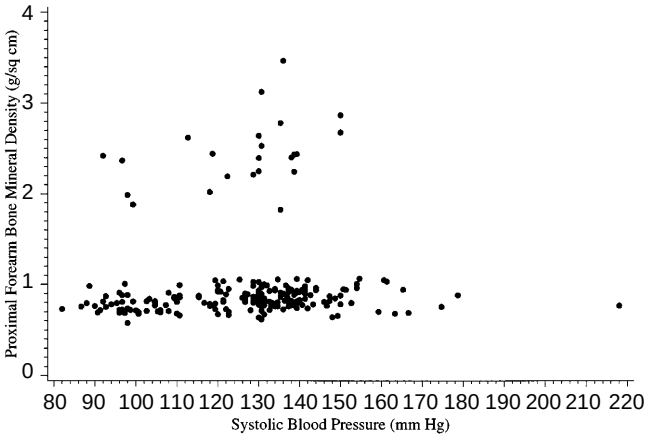

Fig. 4 Plot of individual values of proximal forearm BMD against systolic blood pressure

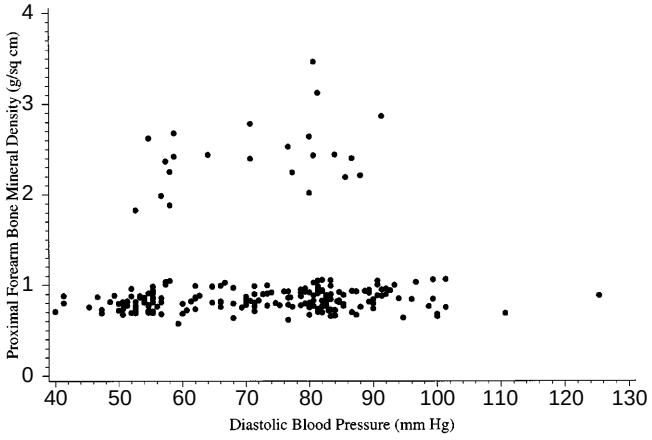

Fig. 5 Plot of individual values of proximal forearm BMD against diastolic blood pressure

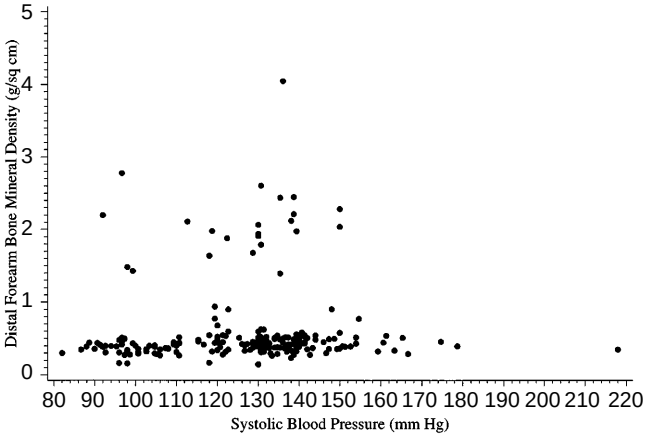

Fig. 6 Plot of individual values of distal forearm BMD against systolic blood pressure

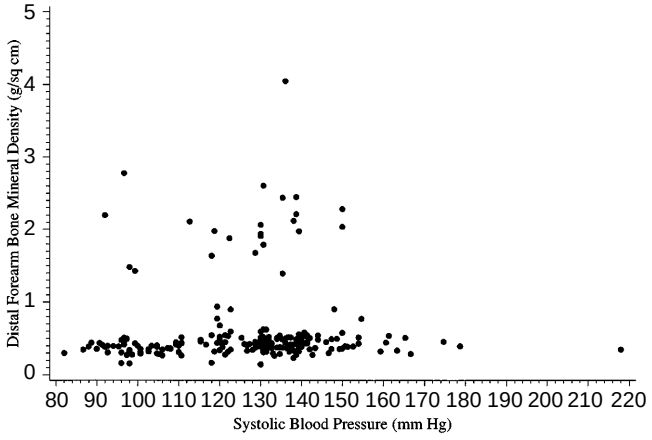

Fig. 7 Plot of individual values of distal forearm BMD against diastolic blood pressure

current study are 3.15 (proximal site) and 3.14 (distal site). Thus, the genome-wide significance levels are 0.235 for adjusted proximal forearm BMD and 0.281 for adjusted distal forearm BMD. In other words, according to our simulations, for adjusted proximal forearm BMD, 23.5% of the time, we obtain a Z score greater than 3.15, and for adjusted distal forearm BMD, 28.1% of the time, we obtain a Z score greater than 3.14.

In terms of pointwise *P* values, our simulations suggested that the distribution of the Z score for both adjusted proximal and distal forearm BMD values is close to normal, at least as far as the tail probabilities that are greater than 0.01 are concerned. If we assume normality, the values are 0.00084 and 0.00082 for adjusted proximal and distal forearm BMD values, respectively.

A rough estimate of power was calculated as follows. Basically, one needs to consider the asymptotic distribution of the Z score statistic. Under the null hypothesis of no linkage, it is distributed as normal (0,1), i.e., with a mean of 0 and a variance of 1. Under the hypothesis of linkage, it should be approximately distributed as normal, where the best estimate of the mean is given by our data, i.e., mean = 3.15 (proximal site) or 3.14 (distal site). According to our calculations, the probability of obtaining suggestive evidence of linkage (i.e., a Z score of 3.179, $P = 0.00074$), if this is the true mean, is 0.484, whereas the probability of obtaining significant evidence of linkage (i.e., a Z score of 4.1, $P = 0.000022$), if this is the true mean, is 0.169. Thus, the estimated powers are 48.4% for suggestive, and 16.9% for significant, evidence of linkage.

Discussion

In order to identify potential genetic determinants of forearm BMD, we have performed an autosomal genomic scan to search for evidence of linkage to forearm BMD phenotypes in 218 offspring from 96 nuclear families from China. Nonparametric analyses of the sibpair data have revealed several regions with evidence of linkage to aspects of calcium metabolism that predict forearm BMD in Chinese populations of Anqing. Genes controlling for BMI may also be involved in BMD regulation and in the determination of the risk of osteoporotic fractures (Willing et al. 1997), since BMI is significantly correlated with the achievement of peak BMD values (Sowers et al. 1991). As a result, controlling for the effect of BMI on BMD can constitute an overadjustment in elucidating the genetic factors underlying BMD. Therefore, in our multivariate model, we have only included age (both the linear term and the quadratic term) and sex as the covariates.

The proximal site of the forearm comprises almost exclusively cortical bone, whereas the distal site of the forearm comprises a mix of trabecular and cortical bone. Thus, although the two traits are highly correlated (Fig. 3), we have analyzed them separately, because the genetic components of BMD at each site are probably different. As shown in Figs. 4–7, there is essentially no correlation between either proximal or distal forearm BMD with blood pressure values among the study subjects. Thus, our data are not based on individuals at only the upper tail (or only the lower tail) of the blood pressure distributions, and therefore, the conclusions of this study do not only apply to those with extremely high or low blood pressure.

With regard to the power simulations of the genome scan, one of the reasons that we have a less conservative criterion is that we are only looking at 22 autosomes rather than 23. The results of Lander and Kruglyak (1995), quoted in Table 1, assume 23 chromosomes and a genome length of 33 Morgans. The Lander and Kruglyak criteria for suggested linkage are a lod score of 2.2 or a pointwise *P* value of 0.00074. This is calculated to be equivalent to one expected false positive per genome scan or a genome-wide *P* value threshold of 0.632. Our highest Z scores (3.15 for proximal and 3.14 for distal) correspond to genome-wide *P* values of 0.281 and 0.235, respectively, which clearly are more significant than 0.632 and thus certainly fall above the suggestive linkage threshold. We can recalculate the pointwise threshold by using the formulae presented by Lander and Kruglyak (1995), for the case of 22 chromosomes and a total genome length of 31 Morgans (i.e., 23 chromosomes minus the X chromosome). In this case, one expected false positive per genome scan or a genome-wide *P* value threshold of 0.632 is equivalent to a pointwise threshold of approximately 0.0008 for suggestive evidence of linkage (rather than 0.00074 for the case of 23 chromosomes and 33 Morgans). Our pointwise results for the two highest Z scores are close to 0.0008 and so could again be argued to give suggestive evidence of linkage, although the power of our study is rather limited.

With adjusted forearm BMD values, the highest LOD score observed in this genome scan was 2.15 on chromosome 2p21.1–24 region (for both proximal and distal sites). Recently, Devoto and colleagues (1998) have performed a genome-wide screen with 330 autosomal microsatellite markers in 74 independent sibpairs from 7 large pedigrees in which many individuals have low BMD. They have used the same equipment (DXA) for BMD determination as that

Table 4 A summary of previous linkage results in the promising chromosomal regions

Phenotype	Chromosome	Critical interval (cM, distance from pter)	Cytogenetic location	Previous linked phenotypes
Proximal	2	18–57	2p21.1–p24	Spinal BMD Serum leptin level Congenital glaucoma
Distal	2	40–57	2p21.1–p24	Spinal BMD Serum leptin level Congenital glaucoma
	13	47–71	13q21–13q34	Macular dystrophy

Table 5 Reported mutations of the candidate genes for controlling adjusted forearm BMD traits[a]

Candidate gene	Cytogenetic location	Missense/nonsense mutations	Splicing mutations	Regulatory mutations	Other mutations	Function of mutations
Proximal site						
CALM2	2p21.3-p21.1	0	0	0	0	–
STK	2p23-p24	0	0	0	0	–
POMC	2p23.3	1	0	1	1	Obesity/adrenal insufficiency
Distal site						
CALM2	2p21.3-p21.1	0	0	0	0	–
STK	2p23-p24	0	0	0	0	–
POMC	2p23.3	1	0	1	1	Obesity/adrenal insufficiency
COL4A1	13q34	0	0	0	0	–
COL4A2	13q34	0	0	0	0	–

[a]Reported in the Online Mendelian Inheritance in Man (OMIM, TM) Center for Medical Genetics, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.) 1998. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/, and The Human Gene Mutation Database, World Wide Web URL: http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html (Krawczak and Cooper 1997)

used in our study. It is noteworthy that one of their suggestive linkages for spinal BMD maps to 2p23–24 (LOD=2.25), which actually overlaps the promising region 2p21.1–24 that we have pinpointed for both proximal and distal forearm BMD among our Chinese sibpairs (Table 4). In addition, Comuzzie et al. (1997) have found that the 2p21 region is strongly linked with serum leptin levels (LOD=4.95) in Mexican Americans. Sarfarazi and colleagues (1995) have also mapped the primary congenital glaucoma (GLC3) to the 2p21 region in a group of 17 GLC3 families (combined LOD=11.50; Table 4). A number of candidate genes residing in this chromosomal segment are listed in Table 5. *CALM2*, the gene encoding calmodulin 2, is located in the vicinity of *D2S405* on chromosome 2. Previous studies have suggested a role of calmodulin activity in osteopenia in animal models (Lehman et al. 1984). Calmodulin is also involved in the differential control of osteoblast proliferation and differentiation and may influence forearm BMD (Siddhanti and Quarles 1994). In addition, a serine/threonine kinase (*STK*; Genebank accession no. D87119) is considered as a candidate gene in this region (Devoto et al. 1998; Table 5). Pro-opiomelanocortin (*POMC*), a precursor for adrenocorticotropic hormone, is located on 2p21 and is another candidate; it acts on the cortex of the adrenal glands, leading to the production of glucocorticoid. Glucocorticoids diminish calcium absorption and increase renal calcium excretion. Thus, *POMC* mutations, which have previously been associated with obesity and adrenal insufficiency (Krude et al. 1998), may also play a role in controlling forearm BMD traits. The use of additional markers within the 2p21.1–24 region may narrow down this critical interval for fine mapping purposes.

The chromosome 13q34 region has previously been linked to Stargardt's macular dystrophy (LOD=4.55), which is characterized by diminished visual acuity and funduscopic abnormalities (Zhang et al. 1994; Table 4). Potential candidate genes in the region of interest include *COL4A1* and *COL4A2*, which encode collage IV alpha-1 and alpha-2 subunits, respectively. So far, no functional mutations in these genes have been reported (Table 5). It should be noted that all candidate genes presented in Table 5 are highly speculative because of the moderate linkage findings.

The previously studied candidate genes for BMD include the vitamin-D (1,25-dihydroxyvitamin D3)-receptor gene (*VDR*) on 12q12-q14 (Tsai et al. 1996), the estrogen receptor gene (*ER*) on 6q25.1 (Sano et al. 1995), the *COL1A1* gene on 17q21.31-q22.05 (Uitterlinden et al. 1998), and the *COL1A2* gene on 7q21.3-q22.1 (Spotila et al. 1991). However, negative results have been reported for

COL1A1, *COL1A2*, *VDR* (Willing et al. 1997), and *ER* (Han et al. 1997) polymorphisms. We have obtained no evidence that any of these regions are linked to forearm BMD.

Our study has several strengths. First of all, BMD is an objective trait, and BMD determinations by pDXA are extremely accurate and precise (Bonnick 1996). Low BMD at a peripheral site, such as the forearm, represents a strong predictor for osteoporotic fracture risk (Gardsell et al. 1989). Second, our study population is homogeneous with regard to ethnicity and a variety of environmental factors involving the lack of transportation (with the exception of bicycles) in Anqing. Third, our study is novel, since it represents the first sibpair study of forearm BMD by means of the genome-wide screening method.

There are several limitations to this study. First, the forearm BMD measurement is an intermediate phenotype for osteoporosis, and our study endpoints are not based on clinical manifestations of osteoporosis. Second, our study has a small sample size, which limits its power. Third, the sibpairs in this study are selected on the basis of blood pressure levels, not on the basis of extreme low or high forearm BMD values. Thus, this study is inefficient, because of potential confounding or selection bias. Nonetheless, the suggestive evidence obtained in the current study lays the basis for future genome-scan studies.

In summary, the results of this autosomal genome scan reveal a couple of chromosomal regions that may be linked to adjusted forearm BMD in the Chinese. The suggestive evidence of linkage on chromosome 2 needs to be replicated in extended populations. In the future, we aim to carry out a whole genome scan on a sufficiently large number of sibpairs concordant for osteoporotic fractures (with parents) to pinpoint chromosomal regions linked to osteoporosis.

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