

Full-length article

Evidence for major pleiotropic effects on bone size variation from a principal component analysis of 451 Caucasian families¹

Li-jun TAN², Yao-zhong LIU⁵, Peng XIAO⁴, Fang YANG², Zi-hui TANG², Peng-yuan LIU⁴, Robert R RECKER⁴, Hong-wen DENG^{2,3,5}

²Laboratory of Molecular and Statistical Genetics and the Key Laboratory of Protein Chemistry and Developmental Biology of Ministry of Education, Hunan Normal University, Changsha 410081, China; ³Institute of Molecular Genetics and the Key Laboratory of Biomedical Information Engineering of Ministry of Education, Xi'an Jiao Tong University, Xi'an 710049, China; ⁴Osteoporosis Research Center, Creighton University Medical Center Omaha, Nebraska 68131, USA; ⁵Departments of Orthopedic Surgery and Basic Medical Sciences, University of Missouri, Kansas City, Missouri 64108-2792, USA

Key words

bone size; principal component; whole genome linkage scan

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⁵Correspondence to Prof Hong-wen DENG. Phn/Fax 86-731-887-2791. E-mail dengh@umkc.edu

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Abstract

Aim: To identify pleiotropic quantitative trait loci (QTL) influencing bone size (BS) at different skeletal sites in Caucasians. **Methods:** In a sample containing 3899 Caucasians from 451 pedigrees, 410 microsatellite markers spaced ~8.9 cM apart across the human genome were genotyped. Phenotypical and genetic correlations of BS at lumbar spine, hip (femoral neck, trochanter, and intertrochanter regions), and wrist (ultradistal, mid-distal, and one-third distal sites) were determined using bivariate quantitative genetic analysis. A principal component analysis (PCA) was performed to obtain principal component (PC) factors that were then subjected to variance components linkage analysis to identify regions linked to the PC. **Results:** Genetic correlations of BS at different skeletal sites ranged from 0.40 to 0.79 ($P < 0.001$). The PCA yielded a PC named PC_{total}, which explained up to 76% of the total (co)variation of all the BS at the 7 skeletal sites for the whole sample. We identified a QTL influencing the BS of multiple skeletal sites on chromosome 7 at 140 cM [logarithm of odds (LOD)=2.85] in the overall sample. Sex-specific evidence for linkage was observed on chromosome 11 at 53 cM (LOD =2.82) in the male-only data subset. **Conclusion:** Our study identified several genomic regions that may have pleiotropic effects on different skeletal sites. These regions may contain genes that play a critical role in overall bone development and osteoporosis at multiple skeletal sites, hence are biologically and clinically important.

Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength, which predisposes an individual to an increased risk of fractures. Several categories of phenotypes, including bone mineral density (BMD), bone size (BS), geometry, and structure are considered determinants of bone strength^[1-3]. Numerous studies conducted over last 2 decades have documented that BMD is highly heritable^[4,5] and much effort has been done to identify the particular chromosomal loci and genes involved in the determination of BMD variation^[6]. BS is also under strong genetic control, with heritability of over 50%^[7,8]. In contrast, there has been rela-

tively few genetic studies on BS in humans, and the list of quantitative trait loci (QTL) and genes involved in the determination of BS is far from being complete.

Several candidate genes and allelic variants have been shown to be associated with or linked to BS-related phenotypes, for example, collagen type I alpha (*COL1A1*)^[9] and *COL1A2*^[10], low-density lipoprotein receptor-related protein 5^[11], alpha2-HS glycoprotein^[12], estrogen receptor alpha^[13], cytochrome P450c17alpha^[14]. We previously identified several chromosomal regions for BS variation at individual skeletal sites^[15,16]. However, it is unclear if BS variation at multiple skeletal sites is governed by some shared genetic determination since BS

at different sites are highly correlated^[8].

There is also a significant sex bias in the magnitude of BS and incidence of osteoporotic fracture. Women generally have smaller body size and skeletons than men^[17,18]. Furthermore, females show a greater incidence of stress fractures early in life^[19] and fragility fractures later in life, with a female-to-male ratio of approximate 4:1^[20,21]. Thus investigations into the genetic basis underlying the sex effects in BS variation are warranted.

Here, we present the results of genome-wide scan in a large sample containing 3899 Caucasians from 451 pedigrees. To examine the pleiotropic effects on BS at multiple skeletal sites, we used the outcome of a principal component analysis (PCA) conducted on 7 BS measurements. The notable sex bias led us to investigate whether there are sex-specific pleiotropic genes that underlie BS variation.

Materials and methods

Subjects The study was approved by the Creighton University Institutional Review Board (Omaha, NE, USA). All the study subjects signed informed consent documents before entering the project. The sampling scheme and exclusion criteria have been detailed elsewhere^[22]. Briefly, patients with chronic diseases and conditions that might potentially affect bone mass, structure, or metabolism were excluded.

All the study subjects were Caucasians of European origin. The sample contained a total of 4256 phenotyped subjects from 451 pedigrees (see Table 1 for their basic characteristics), of whom 3899 subjects were genotyped. The pedigree size varied from 4 to 416 individuals, with a mean (SD) of 11.6 (28.5), providing us an exceedingly large number

of relative pairs (>150 000) informative for the linkage analysis.

Measurements BS (cm²) at lumbar spine (L1-4), hip (femoral neck, trochanter and intertrochanter), and forearm (ultradistal, mid-distal, and one-third distal) were measured by Hologic 1000, 2000+, or 4500 dual-energy X-ray absorptiometry scanners (Hologic, Waltham, MA, USA). All scanners are calibrated daily, and long-term precision is monitored with external phantoms. The short-term BS measurement precision on the Hologic 4500 is reflected by a coefficient of variation (CV) of 1.11%, 3.25%, 4.37%, 6.42%, 2.36%, 1.29%, and 1.88%, respectively, at the spine, femoral neck, trochanter and intertrochanter regions at the hip, as well as ultradistal radius, mid-distal, and one-third distal areas at the forearm. A similar CV was obtained on the Hologic 1000 and 2000+ scanners. BS measurements by different scanners at our center are highly compatible with one another and are well within the precision limits^[23]. In particular, members of the same pedigree were usually measured on the same type of machine, which ensured minimum or no effect on our linkage analyses due to measurements by different scanners. Weight (kg) and height (m) were measured at the same visit of the BS measurement.

Genotyping For each patient, DNA was extracted from peripheral blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). Among all the subjects, 3899 individuals were genotyped with 410 microsatellite markers (including 393 markers for 22 autosomes and 17 markers for the X chromosome) from the Marshfield screening set 14 by the Marshfield Center for Medical Genetics. The markers had an average population heterozygosity of 0.75 and were spaced on average 8.9 cM

Table 1. Basic characteristics of the study subjects.

	Females (n=2520)	Males (n=1736)	Total (n=4256)
Age (years)	47.57±15.98	47.95±16.12	47.72±16.04
Height (m)	1.64±0.07	1.78±0.07	1.70±0.10
Weight (kg)	71.3±16.0	89.4±15.8	78.5±18.2
Body mass index (kg/cm ²)	26.59±5.80	28.04±4.45	27.18±5.38
BS (cm ²)			
Spine	58.58±5.84	70.38±6.47	63.38±8.41
Femoral neck	5.04±0.40	5.91±0.49	5.40±0.61
Trochanter	11.74±2.13	13.67±1.59	11.72±2.11
Intertrochanter	18.28±2.33	25.04±3.30	21.04±4.32
Ultradistal site	3.57±0.37	4.34±0.44	3.89±0.55
1/3 distal site	2.61±0.41	3.19±0.31	2.85±0.47
mid-distal site	6.80±1.02	9.79±1.47	8.04±1.92

Note: BS values are mean±SD of the raw data without adjustment for covariates.

apart. The detailed genotyping protocol is available at http://research.marshfieldclinic.org/genetics/Lab_Methods/methods.html. A database management system, including GenoDB^[24], was employed to manage the phenotype and genotype data for the linkage analysis. GenoDB was also used for allele binning (including setting up allele binning criteria and converting allele sizes to distinct allele numbers), data quality control, and data formatting for PedCheck^[25] and linkage analysis. PedCheck was performed to ensure that the genotype data conform to the Mendelian inheritance pattern at all the marker loci. In addition, we used MERLIN^[26] to detect genotyping errors of unlikely recombination (eg double recombination) in our sample. The genotyping error rate of ~0.03% was determined.

Statistical analyses

Correlation analyses Prior to the genetic analysis, descriptive statistics of the sample were analyzed using SPSS version 11.0 software (SPSS, Chicago, IL, USA). We performed a bivariate quantitative genetic analysis to assess genetic and phenotypic correlations using SOLAR v3.0.4 (Sequential Oligogenic Linkage Analysis Routines) software^[27], following the procedures detailed in our earlier studies^[28].

PCA A PCA was undertaken for the 7 BS phenotypes to avoid the problem of multiple comparisons and redundancy of information, following the method detailed in a previous study^[29]. Briefly, the analysis was performed in a 3-step process: (1) extraction of initial components by use of PCA; (2) rotation of components using the varimax option, resulting in elucidation of factors; and (3) interpretation of factors with loadings. The original data, regardless of sex and age, were used in the PCA. The analysis generated principal component (PC) factors (as linear combinations of the variables) for each individual, and the pedigree members were assumed independent for this analysis. The eigenvalue (which represents the variance explained by each of the PC) of 1 criterion was implemented to determine the number of the components and varimax rotation to achieve a better interpretation of the PC. However, only one PC (PC_{Total}) was extracted in the analysis and there was no need for a further rotation procedure.

QTL analysis Two-point and multipoint linkage analyses across 22 autosomes and a 2-point linkage analysis on chromosome X were performed using SOLAR. Under polygenic models, covariates age, sex, height, and weight were tested for importance on the PC_{Total} variation. Significant covariates ($P < 0.05$) were used to adjust the PC within the linkage model in SOLAR.

The sex bias observed in BS led us to investigate whether there was sex-specific evidence for linkage in these families.

The original BS data were stratified on the basis of sex, with the male set generated by setting the females to “unknown”, and vice versa for the female data set. The new male ($n_m = 1736$) and new female ($n_f = 2520$) data sets were used in the PCA and the linkage analysis as described earlier. The PCA generated 1 PC from the male-only subset (PC_M) and one PC from female-only data subset (PC_F). Age, height, and weight were tested, and significant factors ($P = 0.05$) were adjusted as covariates in the linkage analysis. Furthermore, the Marshfield sex-specific genetic maps were used in the subgroup analysis.

The normality of distribution of the PC phenotypes was estimated through kurtosis that was 0.63, 0.75, and 0.68 for PC_{Total}, PC_F, and PC_M, respectively. Although the variance component analyses implemented in SOLAR are quite robust to slight deviation from normality (kurtosis < 2.0), we still carried out 10 000 replicates of the overall data set, as well as the male-only and female-only data subsets, using the procedure “lodadj” implemented in SOLAR^[30] to correct for such deviations and to test the robustness of our results. All the results were reported as adjusted logarithm of odds [LOD] scores by multiplying the original LOD scores by a correction constant that were 1.00, 0.95 and 0.95 for PC_{Total}, PC_F and PC_M, respectively. The proportion of expected LOD scores greater than the observed LOD score is the latter’s empirical P -value. Because we conducted 2 independent genome-wide scans for the sex-specific analyses, we conducted a *Bonferroni* adjustment approach following Lander *et al*^[31]. The LOD thresholds increased from 1.90 to 2.45 for suggestive linkage, and from 3.30 to 3.87 for significant linkage.

Results

Table 1 summarizes the basic characteristics of the study subjects. The data showed that males had significant greater values for all the descriptive statistics except for age than females (t -test $P < 0.001$). All of the 7 BS variables were significantly correlated both genetically and phenotypically (Table 2). The genetic and phenotypic correlations of BS at different skeletal sites ranged from 0.40 to 0.79, and from 0.17 to 0.65, respectively. The square of the genetic correlation that approximates shared genetic effects between 2 traits is in the range of 16%–63%, indicating the existence of common genetic factors governing a clustering of BS phenotypes.

Table 3 shows the results of the PCA analysis. For the 7 BS measurements, 1 PC, with eigenvalue = 1.0, was retained in the overall data set, as well as the male-only and female-only data subsets, which accounted for 76%, 49%, and 51% of the total variation of the 7 BS measurements, respectively.

Table 2. Correlations of BS at different skeletal sites in the total sample.

Phenotype	Spine	Femoral neck	Trochanter	Inter-trochanter	Ultradistal site	One-third distal site	Mid-distal site
Spine	1	0.43	0.49	0.48	0.51	0.50	0.57
Femoral neck	0.17	1	0.65	0.60	0.58	0.40	0.62
Trochanter	0.35	0.29	1	0.65	0.57	0.41	0.63
Intertrochanter	0.30	0.23	0.27	1	0.61	0.49	0.61
Ultradistal site	0.31	0.22	0.34	0.29	1	0.53	0.79
One-third distal site	0.34	0.22	0.27	0.28	0.37	1	0.74
Mid-distal site	0.38	0.26	0.38	0.35	0.63	0.65	1

Note: Values in shaded regions are coefficients of genetic correlation for BS variables. Values in unshaded regions are coefficients of phenotypical correlation of the variables. All correlations are significant ($P<0.001$) with BS variables adjusted by significant covariates, such as age, sex, height, weight, and sex-by-age interaction.

Table 3. PCA for BS at various skeletal sites.

	Total sample ($n=4256$) PC _{Total}	Males ($n=1736$) PC _M	Females ($n=2520$) PC _F
Spine	0.836	0.646	0.628
Femoral neck	0.883	0.646	0.667
Trochanter	0.870	0.708	0.689
Intertrochanter	0.850	0.669	0.624
Ultradistal site	0.873	0.853	0.750
One-third distal site	0.848	0.755	0.648
Mid-distal site	0.936	0.700	0.854
Eigenvalue	5.31	3.57	3.41
Total explained Variance (%)	76	51	49

Note: PC loadings, the correlation coefficients between BS measurements and the PC factors, are shown. Eigenvalues represent the variance explained by each of the PC.

The PC loadings (which are the correlation between each variable and the component) ranged from 0.84 to 0.94 for the

total sample, 0.65 to 0.85 for females, and 0.62 to 0.85 for males, respectively.

In the variance component analysis of the PC, the heritability of PC_{Total}, PC_F, and PC_M were (mean±SEM) 0.64±0.03, 0.59±0.03, and 0.56±0.03 (data not shown), respectively, revealing that PC were under significant genetic influences. The results of the genome-wide linkage analysis in the overall sample are provided in Figure 1 and Table 4. The strongest linkage signal was detected on chromosomes 7q34 (140 cM, LOD=2.85, empirical $P<0.0001$). Sex-specific analyses resulted in additional evidence for linkage on chromosome 11 at 53 cM (Figure 2), with a LOD score of 2.82 ($P<0.0001$).

Discussion

Previously, we reported a whole genome linkage scan for BS variation in 451 Caucasian pedigrees^[17]. Several genomic regions were suggested to be linked to BS variation. To detect pleiotropic genomic regions for BS, we conducted a second genome-wide linkage scan in the same sample. In this study, significant and high-genetic correlations of BS at multiple skeletal sites were observed, implying that BS at

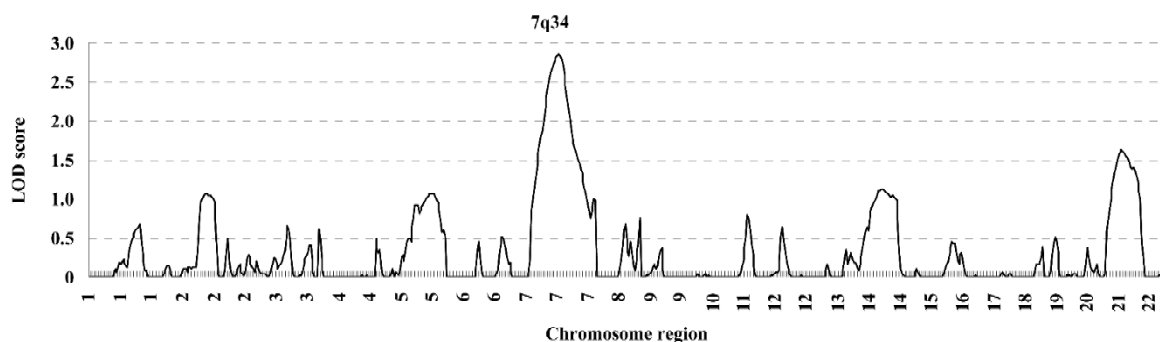
**Figure 1.** Genome-wide linkage signals on autosomes in the overall sample.

Table 4. Genomic regions of suggestive linkage to PC phenotypes.

Location	Marker	LOD scores (2 point/multipoint)		
		PC _{Total}	PC _F	PC _M
5q23(99 cM)	GATA62A04	–	–	2.63/2.39
7q34(140 cM)	GATA63F08P	2.71/2.85	2.21/1.79	–
8q24(218 cM)	UT721M	–	1.91/2.30	–
11p11(53 cM)	ATA9B04N	–	–	1.57/2.82
21q21(31 cM)	GATA129D11N	–	2.08/1.85	–

Note: Numbers in parentheses are genetic distance of the linkage peak from the p-terminal. Markers listed are the nearest marker genotyped from the linkage peak.

different skeletal sites may share some genetic determinations. We then performed PCA and whole genome linkage scan, and identified a suggestive QTL influencing the BS of multiple skeletal sites on chromosome 7q34, with a LOD score of 2.85 at 140 cM.

PCA is a variable reduction procedure that extracts uncorrelated factors from correlated variables to obtain an

independent PC that explains the vast majority of the correlated phenotypic variations. In this study, the joint analyses of the 7 correlated BS measurements may allow us to identify the loci which have common effects to a set of BS measured at different sites. However, the genetic effects at pleiotropic loci/genomic regions identified are not allowed to possibly have different contributions to each BS measured. In our PCA we extracted a PC factor (PC_{Total}) which explains over 76% of the total BS variation of multiple sites. Strong evidence of linkage was detected on chromosome 7q34 for the combined BS phenotypes, but no linkage evidence was found for BS at each individual skeletal site at this region (data not shown), suggesting that PC may provide more information for linkage analysis than each individual trait.

Chromosome 7q34 is an interesting region since the importance of this genomic region has been highlighted in previous studies. In a white sibling sister pairs study, Koller *et al* found a significant linkage peak at this region (LOD=5.0) for femur head width^[32] Kato *et al* also mapped Silver–Russell syndrome, which involves short stature and dysmorphology, to chromosome 7q34^[33]. Three candidate genes present in the 1 LOD unit drop support interval (11 cM) of the 7q34

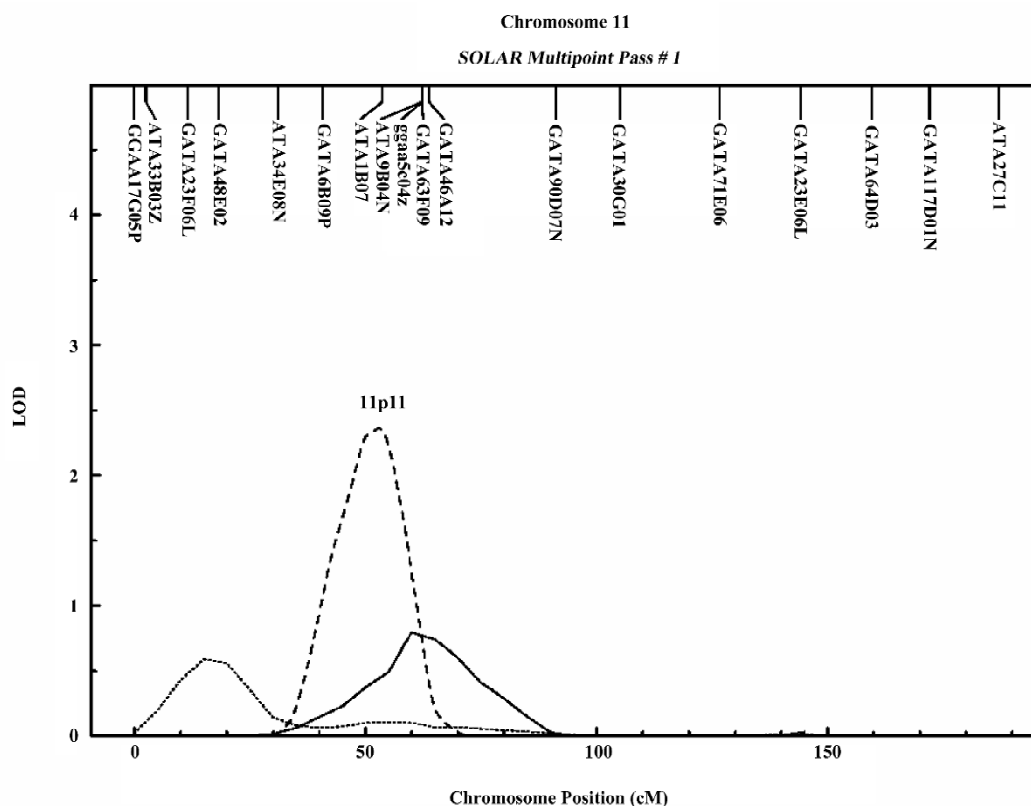


Figure 2. Sex-specific evidence for linkage. Analysis of the male-only data set (dotted line) compared with the complete data set (dashed line) and female-only data set (solid line) on chromosome 11, showing increase in the LOD score.

signal are involved in pathways of bone metabolism, including leptin and interferon regulatory factor 5. Leptin is secreted primarily by adipocytes, with its function to control both bone formation and resorption^[34,35]. Foldes *et al*^[36] reported that femoral BS in obese leptin receptor-deficient (homozygous) rats decreased. A recent study also showed that leptin is involved in genetic variation of hand BS and geometry^[37].

Investigations of sex-specific genetic variants of complex disorders are recommended^[38,39], especially when the disorder exhibits a skewed sex ratio in severity and/or prevalence. The appropriate criteria for assessing genome-wide significance in a sex-specific linkage analysis merit some additional discussion. In our study, we used the rigorous Bonferroni criterion for interpretation of the linkage results. Furthermore, the sex-specific QTL should be interpreted with caution and merit further justification because other factors, such as gene-by-sex interaction, may also lead to sex difference of linkage signals in the subgroup analyses of the 2 sexes.

In the present study, we observed a male-specific QTL on chromosome 11p11. The importance of chromosome 11 was previously reported to show pleiotropic effect on 4r size phenotypes (BS, muscle size, body length, and body weight) in mice^[40]. A prominent candidate gene, exostosin 2 (*EXT2*), underlies the 1-LOD unit drop interval on chromosome 11. The *EXT2* gene encodes an essential component of the glycosyltransferase complex required for the biosynthesis of heparan sulfate, which modulates the signaling involved in bone formation^[41]. Furthermore, mutations or deficiencies in the *EXT2* gene cause multiple bone diseases, including hereditary multiple exostosis^[42] and osteochondromas^[43]. These findings suggest the potential importance of chromosomal 11p11 in bone metabolism.

In conclusion, we reported a genome-wide linkage analysis for the PC of 7 BS measurements. Our large sample containing more than 150 000 informative relationships makes the results of the linkage analysis powerful and reliable. Evidence of suggestive linkages was observed on the chromosome regions of 7q34 in the overall sample, and 11p11 in the males. Our findings warrant further fine-mapping efforts or focused studies in fine mapping analyses on these identified regions to pinpoint functional mutations of the pleiotropic effects on BS variation at multiple skeletal sites.

Author contributions

Li-jun TAN, Robert R RECKER, and Hong-wen DENG designed research; Peng XIAO and Peng-yuan LIU performed research; Peng XIAO contributed new reagents or analytic

tools; Li-jun TAN, Fang YANG, Zi-hui TANG, and Peng-yuan LIU analyzed data; Li-jun TAN and Yao-zhong LIU wrote the paper.

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References

- 1 Peacock M, Turner CH, Liu G, Manatunga AK, Timmerman L, Johnston CC. Better discrimination of hip fracture using bone density, geometry and architecture. *Osteoporos Int* 1995; 5: 167–73.
- 2 Vega E, Ghiringhelli G, Mautalen C, Rey VG, Scaglia H, Zylberstein C. Bone mineral density and bone size in men with primary osteoporosis and vertebral fractures. *Calcif Tissue Int* 1998; 62: 465–9.
- 3 Seeman E, Duan Y, Fong C, Edmonds J. Fracture site-specific deficits in bone size and volumetric density in men with spine or hip fractures. *J Bone Miner Res* 2001; 16: 120–7.
- 4 Ammann P, Rizzoli R. Bone strength and its determinants. *Osteoporos Int* 2003; 14 Suppl 3: S13–18.
- 5 Stone KL, Seeley DG, Lui LY, Cauley JA, Ensrud K, Browner WS, *et al*. BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. *J Bone Miner Res* 2003; 18: 1947–54.
- 6 Liu YJ, Shen H, Xiao P, Xiong DH, Li LH, Recker RR, *et al*. Molecular genetic studies of gene identification for osteoporosis: a 2004 update. *J Bone Miner Res* 2006; 21: 1511–35.
- 7 Deng HW, Deng XT, Conway T, Xu FH, Heaney R, Recker RR. Determination of bone size of hip, spine, and wrist in human pedigrees by genetic and lifestyle factors. *J Clin Densitom* 2002; 5: 45–56.
- 8 Jian WX, Long JR, Deng HW. High heritability of bone size at the hip and spine in Chinese. *J Hum Genet* 2004; 49: 87–91.
- 9 Long JR, Liu PY, Lu Y, Xiong DH, Zhao LJ, Zhang YY, *et al*. Association between COL1A1 gene polymorphisms and bone size in Caucasians. *Eur J Hum Genet* 2004; 12: 383–8.
- 10 Lei SF, Deng FY, Xiao SM, Chen XD, Deng HW. Association and haplotype analyses of the COL1A2 and ER-alpha gene polymorphisms with bone size and height in Chinese. *Bone* 2005; 36: 533–41.
- 11 Ferrari SL, Deutsch S, Choudhury U, Chevalley T, Bonjour JP, Dermizakis ET, *et al*. Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with variation in vertebral bone mass, vertebral bone size, and stature in whites. *Am J Hum Genet* 2004; 74: 866–75.
- 12 Liu YJ, Liu XH, Lei SF, Li MX, Deng HW. Alpha2-HS glycoprotein gene is associated with bone size at the hip in Chinese. *Yi Chuan Xue Bao* 2005; 32: 1128–35.

- 13 Schuit SC, van Meurs JB, Bergink AP, van der KM, Fang Y, Leusink G, *et al*. Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. *J Clin Endocrinol Metab* 2004; 89: 303–9.
- 14 Zmuda JM, Cauley JA, Kuller LH, Ferrell RE. A common promoter variant in the cytochrome P450c17alpha (CYP17) gene is associated with bioavailability testosterone levels and bone size in men. *J Bone Miner Res* 2001; 16: 911–7.
- 15 Huang QY, Xu FH, Shen H, Deng HY, Conway T, Liu YJ, *et al*. Genome scan for QTLs underlying bone size variation at 10 refined skeletal sites: genetic heterogeneity and the significance of phenotype refinement. *Physiol Genomics* 2004; 17: 326–31.
- 16 Deng HW, Shen H, Xu FH, Deng H, Conway T, Liu YJ, *et al*. Several genomic regions potentially containing QTLs for bone size variation were identified in a whole-genome linkage scan. *Am J Med Genet A* 2003; 119: 121–31.
- 17 Shen H, Long JR, Xiong DH, Guo YF, Xiao P, Liu YZ, *et al*. A genomewide scan for quantitative trait loci underlying areal bone size variation in 451 Caucasian families. *J Med Genet* 2006; 43: 873–80.
- 18 Tommasini SM, Nasser P, Jepsen KJ. Sexual dimorphism affects tibia size and shape but not tissue-level mechanical properties. *Bone* 2007; 40: 498–505.
- 19 Beck TJ, Ruff CB, Shaffer RA, Betsinger K, Trone DW, Brodine SK. Stress fracture in military recruits: gender differences in muscle and bone susceptibility factors. *Bone* 2000; 27: 437–44.
- 20 Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. *Lancet* 2002; 359: 1761–7.
- 21 Kellie SE, Brody JA. Sex-specific and race-specific hip fracture rates. *Am J Public Health* 1990; 80: 326–8.
- 22 Deng HW, Deng H, Liu YJ, Liu YZ, Xu FH, Shen H, *et al*. A genomewide linkage scan for quantitative-trait loci for obesity phenotypes. *Am J Hum Genet* 2002; 70: 1138–51.
- 23 Deng HW, Chen WM, Conway T, Zhou Y, Davies KM, Stegman MR, *et al*. Determination of bone mineral density of the hip and spine in human pedigrees by genetic and life-style factors. *Genet Epidemiol* 2000; 19: 160–77.
- 24 Li JL, Deng H, Lai DB, Xu F, Chen J, Gao G, *et al*. Toward high-throughput genotyping: dynamic and automatic software for manipulating large-scale genotype data using fluorescently labeled dinucleotide markers. *Genome Res* 2001; 11: 1304–14.
- 25 O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998; 63: 259–66.
- 26 Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002; 30: 97–101.
- 27 Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998; 62: 1198–211.
- 28 Yang YJ, Dvornyk V, Jian WX, Xiao SM, Deng HW. Genetic and environmental correlations between bone phenotypes and anthropometric indices in Chinese. *Osteoporos Int* 2005; 16: 1134–40.
- 29 Karasik D, Cupples LA, Hannan MT, Kiel DP. Genome screen for a combined bone phenotype using principal component analysis: the Framingham study. *Bone* 2004; 34: 547–56.
- 30 Blangero J, Williams JT, Almasy L. Robust LOD scores for variance component-based linkage analysis. *Genet Epidemiol* 2000; 19 Suppl 1: S8–14.
- 31 Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; 11: 241–7.
- 32 Koller DL, White KE, Liu G, Hui SL, Conneally PM, Johnston CC, *et al*. Linkage of structure at the proximal femur to chromosomes 3, 7, 8, and 19. *J Bone Miner Res* 2003; 18: 1057–65.
- 33 Kato R, Kishibayashi J, Shimokawa O, Harada N, Niikawa N, Matsumoto N. Congenital glaucoma and Silver-Russell phenotype associated with partial trisomy 7q and monosomy 15q. *Am J Med Genet* 2001; 104: 319–22.
- 34 Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, *et al*. Leptin inhibits osteoclast generation. *J Bone Miner Res* 2002; 17: 200–9.
- 35 Livshits G, Patsulaia I, Trofimov S, Kobylansky E. Genetic variation of circulating leptin is involved in genetic variation of hand bone size and geometry. *Osteoporos Int* 2003; 14: 476–83.
- 36 Foldes J, Shih MS, Levy J. Bone structure and calcium metabolism in obese Zucker rats. *Int J Obes Relat Metab Disord* 1992; 16: 95–102.
- 37 Eleftheriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, *et al*. Serum leptin level is a regulator of bone mass. *Proc Natl Acad Sci USA* 2004; 101: 3258–63.
- 38 Suresh R, Ambrose N, Roe C, Pluzhnikov A, Wittke-Thompson JK, Ng MC, *et al*. New complexities in the genetics of stuttering: significant sex-specific linkage signals. *Am J Hum Genet* 2006; 78: 554–63.
- 39 Weiss LA, Pan L, Abney M, Ober C. The sex-specific genetic architecture of quantitative traits in humans. *Nat Genet* 2006; 38: 218–22.
- 40 Masinde GL, Wergedal J, Davidson H, Mohan S, Li R, Li X, *et al*. Quantitative trait loci for periosteal circumference (PC): identification of single loci and epistatic effects in F2 MRL/SJL mice. *Bone* 2003; 32: 554–60.
- 41 Morimoto K, Shimizu T, Furukawa K, Morio H, Kurosawa H, Shirasawa T. Transgenic expression of the EXT2 gene in developing chondrocytes enhances the synthesis of heparan sulfate and bone formation in mice. *Biochem Biophys Res Commun* 2002; 292: 999–1009.
- 42 Stickens D, Zak BM, Rougier N, Esko JD, Werb Z. Mice deficient in Ext2 lack heparan sulfate and develop exostoses. *Development* 2005; 132: 5055–68.
- 43 Signori E, Massi E, Matera MG, Poscente M, Gravina C, Falcone G, *et al*. A combined analytical approach reveals novel EXT1/2 gene mutations in a large cohort of Italian multiple osteochondromas patients. *Genes Chromosomes Cancer* 2007; 46: 470–7.