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Refined QTLs of osteoporosis-related traits by linkage analysis with genome-wide SNPs: Framingham SHARe

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Abstract

Genome-wide association studies (GWAS) using high-density array of single-nucleotide polymorphisms (SNPs) offer an unbiased strategy to identify new candidate genes for osteoporosis.

We used a subset of autosomal SNPs from the Affymetrix 500K+50K SNP GeneChip marker set to examine genetic linkage with multiple highly heritable osteoporosis-related traits, including BMD of the hip and spine, heel ultrasound (attenuation and speed of sound), and geometric indices of the hip, in two generations from the Framingham Osteoporosis Study. Variance component linkage analysis was performed using normalized residuals (adjusted for age, height, BMI, and estrogen status in women).

Multipoint linkage analyses produced LOD scores ≥ 3.0 for BMD on chromosomes (chr.) 9 and 11, and for ultrasound speed of sound on chr. 5. Hip geometric traits were linked with higher LOD scores, such as with Shaft Width on chr. 4 (LOD = 3.9) and chr. 16 (LOD = 3.8), and with Shaft section modulus on chr. 22 (LOD = 4.0). LOD score ≥ 5.0 was obtained for femoral Neck Width on chr. 7.

In conclusion, with a SNP-based linkage approach, we identified several novel potential QTLs and confirmed previously identified chromosomal regions linked to bone mass and geometry. Subsequent focus on the spectrum of genetic polymorphisms in these refined regions may contribute to finding variants predisposing to osteoporosis.

Keywords

quantitative trait loci; BMD; bone geometry; osteoporosis; SNP array

INTRODUCTION

Osteoporotic fractures and their consequences in elderly individuals greatly increase mortality, morbidity, and negatively impact quality of life (49). Osteoporosis is a complex multifactorial

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disorder, with a strong genetic component. The strength of bone is determined not only by the amount but also by the spatial distribution of bone tissue. The most widely used and reliable clinical predictor of an osteoporotic fracture remains areal bone mineral density (BMD)(73), as evaluated by dual-energy x-ray absorptiometry (DXA), probably because it derives from both mineral content as well as bone geometry (intrinsic and extrinsic bone properties, respectively). Whereas low BMD is among the strongest risk factors for fracture(9,44), a number of clinical studies have demonstrated that other measurements, such as quantitative ultrasound (QUS) and bone geometry, are important for fracture prediction and osteoporosis treatment monitoring(14,68,69). QUS of the calcaneus has been associated with hip fracture, sometimes independent of BMD(3,13). Also, a combination of BMD with hip geometry(25) has been suggested to improve risk assessment in clinical practice.

A wealth of studies have documented BMD to be under strong genetic control with 50–70% heritability (21,61). Similarly, QUS(3,16,31) and hip geometry (36,52,60,72) are governed by genetic factors. Numerous biological candidate genes for bone phenotypes have also been proposed, but few have been validated with large-scale evidence (21). Recent meta-analyses of biological candidate genes for osteoporosis (22,35,42,54,67) demonstrated that the polymorphisms in these genes each explain a small percentage of the variation in BMD or fracture. With the completion of the International HapMap Project and rapid improvements in high throughput genotyping technology, the ultimate understanding of the genetic basis of osteoporosis is expected to come from the genome-wide association approach in which the whole human genome is surveyed for common genetic variation in osteoporosis-related heritable quantitative traits such as BMD, QUS, or geometry (72). However, recent GWAS's of complex phenotypes have concluded that a substantial proportion of trait heritability remains to be characterized; this phenomenon, so-called “genetic dark matter”, or “missing heritability”, has been recently reviewed (40,43). Genome-wide linkage studies were used for decades in genetic studies of complex diseases, to identify quantitative trait loci (QTLs) using samples of relatives. Linkage signals may be due to either rare polymorphisms/haplotypes or copy number variations (CNVs), therefore, linkage complements the GWAS signal. However, most GWAS only genotyped or analyzed common, mostly low-effect variants, with minor allele frequency ≥ 0.05 (46).

Therefore, in order to identify the chromosomal regions linked to osteoporosis-related traits, we performed genome-wide linkage analyses using a dense genome scan in members of extended pedigrees from the Framingham Heart Study (FHS). We hypothesized that identity-by-descent using a genome-wide array of SNPs can provide a sufficient resolution of QTLs for osteoporosis-related traits, therefore help fine-mapping chromosomal locations for genes governing osteoporosis.

METHODS

Sample

The sample used for our analyses was derived from the Framingham Heart Study cohorts. The Framingham Study Original Cohort began in 1948 with the primary goal of evaluating risk factors for cardiovascular disease. The Original Cohort participants, initially aged 28–62 years, represented two thirds of the households of the Framingham, MA population and have been examined every 2 years since baseline. In 1971, the Framingham Offspring Cohort Study was initiated to evaluate the role of genetic factors in the etiology of coronary artery disease and was comprised of 71% of all the eligible adult offspring of couples from the Original Cohort and offspring spouses (24). Finally, 4,095 grandchildren (ages 19 to 72 years) of the Original Cohort were recruited in 2002–2005, and comprise the Gen3 Cohort (62). The majority of Framingham participants are Caucasians with origins in Eastern and Western Europe. Neither

the Framingham Original nor Offspring Cohort or Gen3 were selected on the basis of cardiovascular diseases or osteoporosis.

Details and descriptions of the Framingham Osteoporosis Study, a subset of the Framingham Heart Study, have been reported (10,28). To date, only two cohorts, the Original and the Offspring Cohorts, have undergone phenotyping for osteoporosis-related measures; however the Gen3 cohort is currently being evaluated. In total there were from 2,524 to 3,096 subjects in 327 pedigrees available for analyses in this project. The study was approved by the Institutional Review Boards for Human Subjects Research of Boston University and of Hebrew SeniorLife.

Bone measurements

Dual energy x-ray absorptiometry (DXA) and Hip Structural Analysis (HSA)—The participants underwent bone densitometry by DXA with a Lunar DPX-L (Lunar Corp., Madison, WI, USA). The Original Cohort participants underwent bone densitometry during 1992–1993 (examination 22). To maximize the sample size, we used DXA scans from 1996–1997 (examination 24) for 31 Original Cohort members who missed DXAs at examination 22. The Offspring Cohort was scanned using the same machine between 1996 and 2001 (exam 6/7). Femoral DXA scans were analyzed by an interactive computer program (4,33) to derive a number of densitometric and structural variables. The regions assessed were the narrowest width of the femoral neck (NN), which overlaps or is proximal to the standard femoral neck region, and the femoral shaft (S), at a distance of 1.5 times the minimum neck width distal to the intersection of the neck and shaft axes. Subperiosteal outer diameter (width, cm), cross-sectional bone area (CSA, cm²), and section modulus (Z, cm³) at each of the two femoral regions (NN and S) were measured directly from the mass profiles using a principle first described by Martin and Burr (45). In addition, the method measures the femoral neck length (FNL), defined as the distance from the center of femoral head to the intersection of neck and shaft axes, and neck-shaft angle (NSA). Coefficients of variation for the different component variables were previously reported to range from 3.3% (NN outer diameter) to 9.1% (FNL) (33).

Quantitative ultrasound (QUS)—QUS of the right heel was performed to obtain calcaneal broadband ultrasound attenuation (BUA) and speed of sound (SOS) with a Sahara® bone sonometer (Hologic, Inc., Waltham, MA), between 1996–2001. Based on duplicate, same-day measurements on 29 subjects, CVs for BUA and SOS were 5.3% and 0.4%, respectively (48).

Other measurements

Information on age, sex, weight and height was obtained for each individual at the time of the bone scan acquisition. In both Cohorts, weight (in pounds) was measured using a standardized balance beam scale. Height (without shoes) was measured to the nearest ¼ inch using a stadiometer. Body mass index (BMI) was then calculated in kg/m².

For women, estrogen use and menopausal status were recorded. Menopause was defined as having no menstrual period for at least one year. Each woman was assigned to one of the two “estrogenic status” groups: 1) premenopausal or postmenopausal on estrogen (estrogen-replete) or 2) postmenopausal not on estrogen (estrogen-deplete).

Pedigree sample

The Framingham Osteoporosis Study included members of the Original and Offspring Cohorts, 2,211 women and 1,633 men who had DXA and QUS measurements; the majority of whom consented to genetic analyses. Members of 327 pedigrees contributed to the linkage analyses.

These pedigrees were mostly nuclear (with an average of 2.4 family members and a small proportion of extended families, with 6 to 30 persons). The sample with genotyping and bone phenotypes included the following relative pairs: 1,195 parent-offspring pairs, 1,468 sibling pairs, 779 cousin pairs, and 647 avuncular pairs.

Genome scan and identity-by-descent (IBD) calculation

Genotyping was conducted by the FHS SHARe (SNP Health Association Resource) project, for which 549,781 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array) were genotyped in over 9,274 FHS subjects (41). SNPs included on the Affymetrix SNP chips have been pre-selected primarily on the basis of technical quality and thus represent a quasi-random set of SNPs. We excluded SNPs with call rate < 0.97 (75,673 SNPs; 13.8%); Hardy-Weinberg equilibrium (HWE) test p -value < 0.01 (32,394 SNPs; 5.9%); minor allele frequency (MAF) < 0.2 (226,492 SNPs; 41.2%); with excessive number of Mendelian errors (greater than or equal 1,000 Mendelian errors, - 2 SNPs; $< 0.1\%$), or unknown genomic annotation or not on the autosomes (5,674 SNPs; 1.0%). The MAF cut point of 0.2 was chosen for this analysis to retain sufficient information content of the polymorphisms. We also excluded the participants: 1,170 individuals with an average SNP call rate < 0.90 ($N=203$), excess number of Mendelian errors ($N=51$), individuals who had no relatives with genotypes ($N=860$), and heterozygosity rate more than 5 SD from the mean ($N=56$).

Ultimately, out of the 209,546 SNPs, 4,157 were selected to compute probabilities of identity-by-descent. This subset was selected to minimize linkage disequilibrium (LD) between the SNPs, because current linkage software assumes that markers are in linkage equilibrium, and violation of this assumption has been shown to create spurious linkage evidence in certain contexts (18). We used SNPLINK to select a subset of markers with a low level of LD. The current SNPLINK version can handle up to 1,000 markers per chromosome, thus, each chromosome was divided into multiple smaller windows; we chose to include 800 markers in each window. We used a LD threshold of $D'=0.4$ (a level of tolerable LD found by Huang et al. (18)).

Once a subset of markers with low LD was selected, we further reduced the number of markers used by requiring that markers be spaced at least 0.5 cM apart. This requirement insures convergence of the Markov chain Monte Carlo algorithm used to estimate IBD probabilities in large families. SNPs with > 30 possible double-recombinants were removed using Merlin (1) and replaced whenever possible by SNPs with low LD with other selected SNPs. The final SNP selection consisted of 4,157 SNPs covering the 22 autosomes with an average SNP spacing of 0.84 cM or 669Kb and an average information content of 86%, as measured on families of small to moderate size.

Ultimately, multipoint IBD probabilities were computed using multiple autosomal markers at every 1 cM, in 3,730 men and 4,374 women, members of 922 extended families, with the program LOKI(15) for extended pedigrees and Merlin(1) for moderate sized families. Map distances were interpolated from genetic maps available at the Center for Medical Genetics (<http://research.marshfieldclinic.org/genetics/>), using Marshfield markers with consistent physical and genetic ordering. Marker allele frequencies were estimated from the genotypes of the study participants by simple allele counting; this method yielded allele frequency estimates very similar to those obtained by maximum likelihood estimation in this unascertained, population-based sample.

Statistical methods

Multiple regression analyses were performed stratified by sex and cohort (Original and Offspring) to obtain residual phenotypes, adjusted for age, estrogen status in women, height

and BMI. Combination of height and BMI simultaneously adjusts for body size and body composition (50), and is widely used in genetic epidemiological studies (10). We performed linkage analyses in a sample with sexes combined, as well as stratified by sex.

Variance component analysis (VCA)—VCA for quantitative traits was performed on normalized residuals using the program Sequential Oligogenic Linkage Analysis Routines (SOLAR, SFBR/NIH, San Antonio, TX;

<http://www.sfbr.org/sfbr/public/software/solar/solar.html>). VCA allowed us to estimate heritability (h^2) of each trait, as the proportion of the total trait variance attributable to the additive effects of genes after removing variation due to covariates. Results of heritability analyses for BMD, QUS, and HSA measures were reported by us previously (10,30,31,34).

Linkage analyses were performed in SOLAR at every 1 cM (multipoint). Multipoint linkage analysis has been shown to be more powerful than the single-point analyses because the former incorporates information from adjacent markers in the computation (2,6). In the linkage VCA, models incorporating genotype data at a putative quantitative trait locus – in the form of probabilities of sharing 0, 1 or 2 alleles IBD by pairs of related individuals – were compared with models incorporating only polygenic effects (i.e. without genetic marker data). A LOD score was computed as the $\log_{10}$ of the likelihood ratio of the locus-specific model to the polygenic model. 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 phenotypes or related traits.

Correction for false positive rate and multiple testing—To control for the overall false positive rate in our genome-wide linkage screens of a single phenotype, we used the approximation provided by Tang and Siegmund (66). Using the criteria of Lander and Kruglyak (37), nominal significant evidence (i.e., genome-wide $p=0.05$) for a bone-related QTL in analyses of data from our Framingham Heart Study families is obtained at $\text{LOD}=3.4$ and “suggestive” evidence (LOD score value expected to occur one time at random in a genome scan) is obtained at $\text{LOD}=2.1$.

Correction for multiple testing was performed using a modification of methods described in Camp and Farnham (7). The total number of tests performed was 30 (10 traits in three samples, - men, women, and both sexes; Table 1), which corresponds to the estimated number of 22 effectively independent genome-wide linkage analyses. The corresponding multiple-test corrected thresholds for “significant” and “suggestive” are ~ 4.8 and ~ 3.5 .

RESULTS

Table 1 displays descriptive statistics of the study participants by cohort and sex. In each cohort, men and women were of similar age. As expected, male participants were heavier, taller and in general had greater BMI, BMD, QUS and geometric measures than females. Of the total 2,211 women, 784 (35.5%) were estrogen-replete (mostly from the Offspring Cohort).

Results from our multipoint linkage analyses for the bone phenotypes adjusted for covariates are displayed in Table 2 for bone mass traits (nominally suggestive evidence for linkage, with LOD scores ≥ 2.1 , unadjusted for the multiple traits/models tested; 1-LOD drop interval is also provided, in cM). Multiple-test corrected suggestive evidence of linkage for bone density was found on chr. 9 (81 Mbp) with lumbar spine (LS) BMD ($\text{LOD}=3.64$), in a combined-sexes sample, and at a proximal region of chr. 11 with trochanter (TR) BMD in women ($\text{LOD}=3.19$).

There were several regions with suggestive LOD scores (multiple-test corrected) linked to hip geometric traits, such as on chr. 4 (at 40 Mbp) and 16 (88 Mbp) with Shaft Width ($\text{LOD} \sim 3.9$

and 3.8, respectively), and on chr. 22 (42–43 Mbp) with Shaft section modulus (LOD = 4.01). Notably, LOD score ≥ 5.0 was obtained for femoral Neck Width on chr. 7 (134.8 Mbp, or 140.7 cM, LOD = 5.24) in the whole sample. Sex-specific QTLs were observed for geometry traits on chr. 20 (107.9 cM) with femoral Shaft Width in men (LOD=4.27) but not women or in the sexes-combined (both LOD scores < 1.0). However, several suggestive QTLs, for example on chr. 22, were also observed in the combined-sexes sample, with LOD scores even stronger than in the sex-specific analyses. QTL on chr. 7 was also found to be linked with the NSA in men only. Figure 1 illustrates sex-specific and combined-sexes results for selected geometric traits.

Interestingly, we and others have described most of the QTLs identified in this analysis, and/or were suggested by a recent meta-analysis of BMD linkage (Table 3). For example, there was a linkage signal for bone density on chr. 9 (81 Mbp) and on chr. 11 (84 Mbp) in women, which coincides with the recent meta-analysis of BMD (21), as well as linkage with SOS on chr. 5 (25 Mbp), which confirms our previous work (31). However, there are several novel regions with a suggestive evidence of linkage, such as one on chr. 11 (20 Mbp) with TR BMD in women. Similarly, some of the novel QTLs for hip geometric traits were identified on chr. 4 (40 Mbp), chr. 5 (75 Mbp), chr. 16 (88 Mbp), and chr. 20 (62.4 Mbp). In addition, other QTLs have been previously reported by us (chr. 20pter and 22 with hip geometry (34)) and by others (chr. 7 with hip BMD (64)).

DISCUSSION

In this study, we performed genome-wide linkage analyses of osteoporosis-related risk factors, using identity-by-descent probabilities obtained from 4,157 single-nucleotide polymorphisms genome-wide. Several thousand family members from two generations of the Framingham Osteoporosis Study contributed their bone phenotypes. We identified several novel potential QTLs, as well as confirmed chromosomal regions (initially identified using microsatellites) to be linked to genetic variants that presumably regulate bone mass and geometry. Some of the QTLs were linked to more than one phenotype, which is expected, given high genetic correlations among osteoporosis-related traits (29).

Osteoporosis is a complex disease with a strong genetic component. To date, dozens of genome-wide linkage scans across multiple populations have been performed to find osteoporosis susceptibility genes, focusing mostly on bone mineral density (see (38) and (19) for review), but also on hip geometry (10,52,63), heel ultrasound (31), and other fracture-related traits (32). Some QTLs for BMD have been replicated in large consortia (21); however, most of the variance in the heritability of the osteoporosis remains unaccounted for (71) and the identification of key candidate genes even within the replicated regions is challenging. Ongoing work continues to resolve the QTLs to a gene responsible for the signal, both in humans (20) and animal models (12). Positional candidates in the QTL may include many genes known to affect bone growth or maintenance and genes that have not yet been associated with bone mineralization, structure, or body size but potentially influence these traits. Therefore, further refining of the chromosomal intervals responsible for the osteoporosis susceptibility, as well as finding QTL variants, is warranted.

One of the main findings of this study was narrowing of the region producing the genetic signal as well as increased strength of the signal for some osteoporosis traits, in comparison to previous microsatellite-based linkage analyses of the same traits. For example, the QTL on chromosome 7q33-q34 was recently identified by Streeten et al. (64) for FN BMD (LOD=3.09) as well as by us for metacarpal traits (LOD ~ 2.2)(32). In this study, a LOD score of 5.24 was obtained in the same location for femoral neck width. Genes such as pleiotrophin (*PTN*), heparin binding growth factor 8/neurite growth-promoting factor 1), solute carrier family 23, member 4 (*SLC23A4*), and myotrophin (*MTPN*), reside there. A novel region suggested in this study

for trochanter BMD in women, on 11p15.1 (22.20–27.29 cM, LOD=3.79), is also of interest since it contains genes such as solute carrier family 6, member 5 (*SLC6A5*, or neurotransmitter transporter-glycine), neural epidermal growth factor-like (*NELL1*), which is expressed in cranial bone, as well as *SOX6*, which is active during endochondral ossification (75). This latter gene was found to be significantly associated with femoral neck BMD in a recent study of 19,195 adults from five Caucasian populations(56); also, the same gene was picked up by the bivariate association with hip BMD/BMI, in males ((39), though here the signals are at either side of a recombination hotspot). We therefore performed a conditional analysis, by choosing 4 SNPs in the *SOX6* region (rs1503436, rs1354329, rs7927191, and rs297335), with minor allele frequency ranging from 0.10 to 0.31 and p-values for association with TR BMD ranging from 0.00049 to 0.05. LD between the SNPs is minimal: r^2 ranging from 0.002 and 0.123. Linkage analysis adjusted for these SNPs is provided in Figure 2. As follows from these results, SNPs with nominally significant association in the region do not explain the evidence of linkage. These findings suggest that other than common SNPs variants should be considered, or “secondary” signals do exist in the 11p15.1 region, not necessary in the *SOX6* gene.

It should be noted that when examining a given QTL region for candidate genes, we should not simply rely on selecting an obvious positional candidate under a linkage peak, but to comprehensively screen all genes in the vicinity for association with the bone trait (58). There may be multiple functional variants, multiple quantitative trait nucleotides, in each QTL (58). Also, since there might be non-additive relationships between the genes in the QTL region, epistatic effects need to be studied. Other variants, such as copy number variants, inversions, and translocations, insertion/deletions etc. might be responsible for the signal (8,17).

This study has noteworthy strengths. Some of the changes in the LOD scores between our published results and this study can be attributed to the increase in sample size with much larger number of relative pairs: for example, for femoral geometric traits, our published results were limited to ~1,450 individuals compared to 2,844 family members included in our current study. This increase in sample size corresponds to better power and thus improved gene mapping ability; it also eliminates spurious loci of linkage with a greater confidence. For example, in our previous studies, multipoint LOD scores >3.0 were obtained (adjusted for age) for Shaft section modulus at 2p21 (80 cM) and 21q11 (3 cM)(10). In the present analysis, the LOD score on 2p21 was reduced (LOD <2.0), and on chr. 21, the signal practically disappeared (LOD <1.0).

Sex-specific QTLs are known to contribute to variability in BMD(21,27) and bone geometry (11,26). Indeed, sex-specific QTLs were observed for geometry traits, most obviously on chr. 20 (107.9 cM) with femoral Shaft Width in men and chr. 11 (25.8 cM) with TR BMD in women. However, several suggestive QTLs, for example on chr. 22, were also observed in the combined-sexes sample, with LOD scores even stronger than in the sex-specific analyses. This finding can be attributed to increased power with the combined sample if the directionality of the signal is same in men and women. A limitation of this study is our inability to additionally stratify the family members by age within sex, due to low power in such subsamples. For example, the FAMOS study from UK (53) had a large sample size (3691 individuals from 715 families) and thus was sufficiently powered to allow stratification by age in each sex; this study thus identified a unique QTL for BMD in men on chromosome 10q21. Absence of stratification by age may explain why results of published linkage analyses from different groups may vary even in homogeneous ethnic samples (59).

In recent years, several genome-wide association studies have been performed for osteoporosis-related traits (55,56,65); however, they mostly focused on BMD. Here we report also results for other proxies for osteoporosis risk, such as QUS and hip and metacarpal geometry. In the absence of meta-analysis of GWAS results for bone geometry phenotypes, it

is imprudent to speculate whether the association results in the vicinity of the QTLs reported here will confirm our linkage findings. It is well established that most individual cohorts with GWAS are underpowered; therefore large homogenous samples are required to be meta-analyzed and/or used for replication (47,51). Scanning the genome for association between markers and complex diseases typically requires testing hundreds of thousands of genetic polymorphisms. Testing such a large number of hypotheses exacerbates the trade-off between power to detect meaningful associations and the chance of making false discoveries (57). Even before the full genome is scanned, investigators often favor certain regions on the basis of the results of prior investigations, such as previous linkage scans. One example is a QTL on chromosome 1p36, which was recently thoroughly perused for candidate genes by Devoto et al. (74) and Kung et al. (19). A gene on 1p36, *ZBTB40*, was found to be significantly associated with lumbar spine and femoral neck BMD in the large GWAS meta-analysis (56). Our data additionally suggest that results of genome-wide SNP-based linkage may help localize or prioritize the genes. Roeder et al. (57) presented a method for using linkage data to weight the association P values, by up-weighting the association results in regions with prior evidence for linkage with the weighting factor depending on the strength of the linkage signal. Linkage results can enhance the power of GWAS scans by relaxing P-value thresholds in the few instances in which suggestive findings overlap but are not definitive (57).

There are other advantages of using family-based approach in GWAS, including the capability to perform combined linkage and association analyses for fine-mapping and testing for locus heterogeneity (5). Indeed, in a recent study, Johansson et al. (23) identified an additional novel signal influencing height by combining linkage and genome-wide association analyses. Family-based study designs offer the possibility of using the new generation of genome sequencing machines to carry out deep sequencing of linked loci to determine the genetic basis of linkage peaks (70); evaluate rare variants (heritable CNVs). Notable, however, “new” CNVs might not be captured by the SNP arrays thus do not contribute to GWAS signals.

In conclusion, through SNP-based identity-by-descent approach, we identified several novel potential QTLs, as well as confirmed chromosomal regions that appear to regulate the variability of the bone mass and geometry in a sample of healthy adults. Subsequent focus on the variety of variants in genes in these refined regions may contribute to further discovery of the genetic sources of osteoporosis.

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Figure 1. Linkage results on chromosome 7 (Neck Width) and 22 (ShaftZ), in men, women, and the combined sample. Multipoint LOD scores are displayed.

Figure 2. Linkage results of trochanter BMD on chromosome 11, in women: not conditional on SNPs in *SOX6* (triangles) and conditional (crosses). Multipoint LOD scores are displayed.

Table 1

Characteristics of the studied sample, by Cohort and gender.

	Original (ex.22) Cohort		Offspring (ex.6–7) Cohort	
	Males	Females	Males	Females
Variable	Mean $\pm$ S.D	Mean $\pm$ S.D	Mean $\pm$ S.D	Mean $\pm$ S.D
N*	318	554	1315	1657
Age (yrs)	78.0 $\pm$ 4.0	78.5 $\pm$ 4.4	60.9 $\pm$ 9.4	59.8 $\pm$ 9.3
Height (meter)	1.70 $\pm$ 0.07	1.55 $\pm$ 0.07	1.75 $\pm$ 0.07	1.61 $\pm$ 0.06
Weight (kg)	77.9 $\pm$ 12.6	64.4 $\pm$ 12.9	88.2 $\pm$ 15.1	71.4 $\pm$ 15.2
BMI (kg/m ²)	27.0 $\pm$ 3.8	26.6 $\pm$ 5.0	28.8 $\pm$ 4.4	27.7 $\pm$ 6.1
Premenopausal or Currently on Estrogen	--	6.15%	--	45.28%
BMD (g/cm²)				
1. Femoral neck	0.875 $\pm$ 0.134	0.727 $\pm$ 0.111	0.996 $\pm$ 0.139	0.886 $\pm$ 0.145
2. Trochanter	0.863 $\pm$ 0.141	0.634 $\pm$ 0.118	0.904 $\pm$ 0.139	0.732 $\pm$ 0.138
3. Lumbar spine	1.353 $\pm$ 0.218	1.077 $\pm$ 0.192	1.318 $\pm$ 0.194	1.162 $\pm$ 0.193
Quantitative Ultrasound				
4. BUA (dB/MHz)	74.02 $\pm$ 21.53	50.40 $\pm$ 17.61	80.55 $\pm$ 17.16	70.04 $\pm$ 16.99
5. SOS (m/s)	1539.40 $\pm$ 35.85	1510.11 $\pm$ 31.60	1555.13 $\pm$ 31.27	1547.34 $\pm$ 33.10
Bone geometry				
6. NSA (degrees)	131.4 $\pm$ 6.6	128.0 $\pm$ 6.0	130.0 $\pm$ 5.1	127.7 $\pm$ 5.4
7. FNL (cm)	5.4 $\pm$ 0.8	4.6 $\pm$ 0.7	5.6 $\pm$ 0.7	4.7 $\pm$ 0.6
8. Narrow Neck Outer diameter (cm)	3.4 $\pm$ 0.3	2.9 $\pm$ 0.3	3.3 $\pm$ 0.3	2.8 $\pm$ 0.2
9. Shaft Outer diameter (cm)	3.3 $\pm$ 0.3	3.0 $\pm$ 0.2	3.1 $\pm$ 0.2	2.9 $\pm$ 0.2
10. Shaft Section modulus (cm ³)	2.5 $\pm$ 0.5	1.5 $\pm$ 0.4	2.7 $\pm$ 0.5	1.8 $\pm$ 0.4

* number of subjects for all available participants with both genotypes and phenotypes; it can be less for some of the traits due to missing values.

Table 2

Linkage results for osteoporosis-related traits

(multipoint LOD score higher than 2.1).

Chr	Position (Mbp)	cM	Confidence Interval (cM)	Trait	Sample	maxLOD
bone mass						
5	24,957	38.7	37.5 – 45.0	SOS	female	3.06
6	134,185	133.8	130.5 – 140.0	lumbar spine BMD	female	2.79
6	150,079	150.7	148.5 – 154.5	SOS	both sexes	2.75
8	29,322	58.3	55.0 – 66.0	Trochanter BMD	both sexes	2.11
9	81,048	80.3	77.0 – 83.0	lumbar spine BMD	both sexes	3.64
9	118,808	125.9	121.5 – 130.5	femoral neck BMD	both sexes	2.24
11	20,051	25.8	21.5 – 28.5	Trochanter BMD	female	3.19
11	84,003	87.2	85.0 – 95.0	femoral neck BMD	female	2.96
12	65,683	77.3	75.0 – 80.0	BUA	female	2.34
17	46,758	71.3	64.5 – 82.0	BUA	both sexes	2.53
17	52,577	79.2	68.0 – 84.5	BUA	male	2.00
19	54,178	75.3	69.5 – 81.0	Trochanter BMD	male	2.65
bone geometry						
1	98,968	131.7	130.5 – 138.0	Neck Length	both sexes	3.15
1	114,358	148.6	131.5 – 157.0	Neck Length	female	2.51
1	155.0	206.0	194.5 – 216.5	ShaftZ	male	2.85
1	220.0	234.0	228.0 – 241.5	ShaftZ	female	3.09
2	235,509	246.0	245.5 – 251.0	NSA	both sexes	2.70
4	40.0	52.0	50.5 – 64.5	Shaft Width	male	3.87
5	75.0	82.0	80.0 – 84.5	Shaft Width	male	3.48
6	159,064	162.6	161.0 – 168.0	ShaftZ	female	1.95
7	134,847	140.7	135.5 – 143.5	Neck Width	both sexes	5.24*
7	136,463	142.7	135.0 – 149.0	NSA	male	1.83*
8	129,299	142.5	142.0 – 145.5	ShaftZ	female	3.59
10	130.0	174.0	169.5 – 174.0	Neck Width	both sexes	2.87
12	11,507	22.8	18.0 – 27.5	Neck Width	female	3.20

Chr	Position (Mbp)	cM	Confidence Interval (cM)	Trait	Sample	maxLOD
12	124,594	145.7	141.0 – 151.0	Shaft Width	female	2.89
16	88,021	131.8	130.0 – 136.0	Shaft Width	both sexes	<u>3.82</u>
20	0,485	2.0	0 – 5.0	Neck Width	male	3.02
20	62,377	107.9	106.0 – 109.0	Shaft Width	male	<u>4.27</u>
22	42,816	48.5	45.0 – 50.5	ShaftZ	male	3.42
22	43,166	49.4	48.0 – 51.0	ShaftZ	both sexes	<u>4.01</u>

Bold font: multiple-test corrected “significant” LOD score (>4.8); underlined: multiple-test corrected “suggestive” LOD score (>3.5).

* chromosomal region with possible pleiotropy

ShaftZ = Shaft Section modulus

Table 3

Published QTLs for osteoporosis-related traits in the above regions of linkage.

a) bone mass				
Our QTL		Published QTL		
Chr	cM	Marker (Mbp) or Confidence Interval (cM)	Trait (sex[*])	Ref.
5	38.7	D5S817 (11,568,538 Mbp)	SOS	[27,30]
6	133.8	131.1- 164.8 cM	lumbar spine BMD (men)	[21]
6	150.6	131.1- 164.8 cM	lumbar spine BMD (men)	[21]
8	58.3	-- 48 cM	femoral neck BMD (men)	[24]
9	80.3	84.9- 109.9 cM	femoral neck BMD	[21]
9	125.9	109.9- 136.5 cM	femoral neck BMD	[21]
11	25.8	-- --	no	--
11	87.2	72.8- 99.0 cM	lumbar spine BMD	[21]
12	77.3	53.3- 82.1 cM	femoral neck BMD (men)	[21]
17	71.3	rs8081154 (46,196,038 Mbp)	BUA	[34]
17	79.2	25.1- 63.6 cM	femoral neck BMD	[21]
19	75.3	-- --	no	--

b) bone geometry				
Our QTL		Published QTL		
Chr	cM	Marker (Mbp) or Confidence Interval (cM)	Trait	Ref.
1	131.7	96 cM	ShaftBR	[62]
	148.6	112,663,861 Mbp	Neck Length	[34]
	206.0	142.2 -170.8 cM	lumbar spine BMD	[21]
	234.0	D1S2800 (255 cM)	lumbar spine BMD	[32]
2	246.0	286 cM	NSA	[10]
		2q33-37	VOS (BUA)	[70]
4	52.0	--	no	--
5	82.0	--	no	--
6	162.6	159.0 cM	IT WID	[10]
7	140.7	146.0 cM	hip BMD	[63]
	142.7	149.0 cM	MCT, MCI	[31]
8	142.5	164.5 cM	Trochanter BMD	[27]
10	174.0	130,314,425 Mbp	Neck Width	[34]
12	22.8	20.0 cM	Neck Width	[10]
	145.7	12q24.31-qter	lumbar spine BMD	[21]
16	131.8	--	no	--

b) bone geometry				
Our QTL		Published QTL		
Chr	cM	Marker (Mbp) or Confidence Interval (cM)	Trait	Ref.
20	2.0	649,631 Mbp	Neck Width	[34]
	107.9	--	no	--
22	49.4	44,039,280 Mbp	ShaftZ	[34]

*
if sex-specific QTL

**
in cM