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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 an 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.

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 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) [72], 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 [14,67,68]. 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 [21,60]. Similarly, QUS [3,16,30] and hip geometry [36,52,59,71] 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 [22,35,42,54,66] 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 [71]. However, recent GWASs 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

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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 helping in 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. Finally, 4095 grandchildren (ages 19 to 72 years) of the original cohort were recruited in 2002–2005 and comprise the Gen3 cohort [61]. The majority of Framingham participants are Caucasians with origins in Eastern and Western Europe. Neither the Framingham original nor the 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 [10,27]. 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 2524 to 3096 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 to 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 (examination 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, cm^2), and section modulus (Z, cm^3) 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 and 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 1/4 inch using a stadiometer. Body mass index (BMI) was then calculated in kg/m^2.

For women, estrogen use and menopausal status were recorded. Menopause was defined as having no menstrual period for at least 1 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, 2211 women and 1633 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: 1195 parent–offspring pairs, 1468 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 9274 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%), and minor allele frequency (MAF) <0.2 (226,492 SNPs; 41.2%), with excessive number of Mendelian errors (greater than or equal 1000 Mendelian errors—2 SNPs; <0.1%) or unknown genomic annotation or not on the autosomes (5674 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: 1170 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, 4157 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