CLINICAL INVESTIGATIONS

Quantitative Trait Locus on Chromosome 1q Influences Bone Loss in Young Mexican American Adults

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Abstract Bone loss occurs as early as the third decade and its cumulative effect throughout adulthood may impact risk for osteoporosis in later life, however, the genes and environmental factors influencing early bone loss are largely unknown. We investigated the role of genes in the change in bone mineral density (BMD) in participants in the San Antonio Family Osteoporosis Study. BMD change in 327 Mexican Americans (ages 25–45 years) from 32 extended pedigrees was calculated from DXA measurements at baseline and follow-up (3.5 to 8.9 years later). Family-based likelihood methods were used to estimate heritability (h^2) and perform autosome-wide linkage analysis for BMD change of the proximal femur and forearm and to estimate heritability for BMD change of lumbar spine. BMD change was significantly heritable for total hip, ultradistal radius, and 33% radius ($h^2 = 0.34$, 0.34, and 0.27, respectively; $p < 0.03$ for all), modestly heritable for femoral neck ($h^2 = 0.22$; $p = 0.06$) and not heritable for spine BMD. Covariates associated with BMD change included age, sex, baseline BMD, menopause, body mass index, and interim BMI change, and accounted for 6% to 24% of phenotype variation. A significant quantitative trait locus (LOD = 3.6) for femoral neck BMD change was observed on chromosome 1q23. In conclusion, we observed that change in BMD in young adults is heritable and performed one of the first linkage studies for BMD change. Linkage to chromosome 1q23 suggests that this region may harbor one or more genes involved in regulating early BMD change of the femoral neck.

Keywords Bone mineral density · Bone loss · Genetics · Linkage · Heritability

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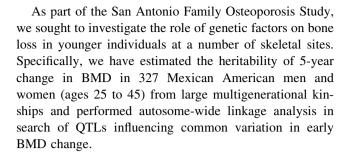University of Maryland School of Medicine, Baltimore, MD,
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Introduction

Peak bone mass and rates of bone loss following menopause in women and later in life in men are commonly acknowledged to be important risk factors for osteoporosis. In fact, Hui et al. suggest that the contributions of peak bone mass acquisition and bone loss after menopause are roughly equal in determining bone mass in women 70 years of age [1]. Over the past three decades, some of the factors that influence peak bone mass in adults, as well as bone loss among older men and women, have been identified [2–8]. In addition to environmental factors, numerous studies have documented that peak bone mineral density (BMD) is highly heritable [9–14] and many quantitative trait loci (QTLs; i.e., implicated chromosomal regions potentially harboring regulatory genes) have been reported [15–34]. More recently, studies have clearly demonstrated that genetic factors regulate BMD change at several skeletal sites in women and older men. Specifically, the heritability of change in BMD at the femoral neck, in premenopausal women [35], lumbar spine, forearm, and whole body (but not hip) in peri- and postmenopausal women [36], and lumbar spine, hip, and forearm (but not whole body) in older men and women (>45 years) [37], has been demonstrated. Studies to identify specific genes influencing peak bone mass and bone loss in older individuals are ongoing.

Although many studies have investigated genetic and environmental factors influencing peak bone mass and bone loss among older individuals, few studies have looked at the factors affecting early change in BMD among young adults, particularly the role of genetics in bone loss [9, 38]. While most cross-sectional studies project that minimal bone loss occurs among individuals <50 years of age [39], more recent longitudinal studies have revealed significant rates of bone loss occurring as early as the third decade in women [35, 40–43] and men [43, 44]. Furthermore, results from cross-sectional and longitudinal studies indicate that the onset of trabecular bone loss may begin in early adulthood, whereas the onset of cortical bone loss is delayed until midlife in women and later in life in men [10, 43]. Thus, bone health later in life may depend on early bone loss, in addition to peak bone mass and advanced age-related bone loss. Furthermore, the importance of early bone loss with regard to later bone health may depend on the trabecular and cortical content of a particular skeletal site. One study has reported that bone loss of the femoral neck is heritable in premenopausal Caucasian women [35], but to date, no study has assessed the genetic contribution to early bone loss at other skeletal sites or in men (<45 years of age). Furthermore, no specific QTLs for longitudinal BMD change have been identified in any population.

As part of the San Antonio Family Osteoporosis Study, we sought to investigate the role of genetic factors on bone loss in younger individuals at a number of skeletal sites. Specifically, we have estimated the heritability of 5-year change in BMD in 327 Mexican American men and women (ages 25 to 45) from large multigenerational kinships and performed autosome-wide linkage analysis in search of QTLs influencing common variation in early BMD change.

Experimental Subjects

Recruitment and data collection for the baseline and follow-up phases of the San Antonio Family Osteoporosis Study have been fully described previously [11, 37]. In brief, participants from 34 multigenerational families were recruited from a low-income neighborhood via a house-to-house recruitment protocol. Probands meeting eligibility criteria (i.e., aged 40 to 60 years and having large families in the San Antonio area) and all first-, second-, and third-degree relatives and spouses were invited to participate irrespective of current health outcomes. Participating families represent a fundamentally unselected, population-based sample of Mexican American kinships, for which longitudinal data are available on 724 individuals. All participants provided informed consent and all research was conducted under approval of respective institutional review boards.

Materials and Methods

Data Collection

Anthropometric, medical, and body composition data were collected during medical examinations at baseline (from 1997 to 2000) and follow-up (3.5 to 8.9 years later; mean = 5.6 years; from 2003 to 2006). Lifestyle, medical history, and reproductive history data were concomitantly assessed at both times via questionnaire. Approximately 81% of the original study participants were re-enrolled for follow-up.

The aim of the present study was to assess environmental and genetic influences on early BMD change; therefore analysis was performed in the subset of participants aged 25 to 45 years at baseline (n = 327). This cohort comprised participants from 32 kinships, including 1434 relative pairs (206 sibling pairs, 96 avuncular relationships, 484 first cousin pairs, and 648 other relationships).

BMD measurements of femoral neck, total hip, total lumbar spine (L1–L4), ultradistal radius, and 33% radius

(measured at 33% of total length from the distal end) were obtained by dual-energy X-ray absorptiometry (DXA) at both baseline and follow-up. Different sites of radius are included because they represent different proportions of trabecular and cortical bone: ultradistal radius is largely trabecular, whereas 33% radius is mostly cortical bone. During the interim between baseline and follow-up clinic visits, DXA equipment was upgraded from the Hologic 1500 W model to the 4500 W model (Hologic Inc., Bedford, MA), along with a software update to ensure comparability of scoring algorithms. Cross-calibration of absorptiometers showed near-perfect agreement on 10 test subjects ($R^2 = 99.9\%$, 99.8%, and 99.9% for spine, total hip, and femoral neck sites, respectively; $p < 10^{-13}$ for all). No mean difference between absorptiometers was detected (paired t-test, $p > 0.1$ for all sites). Precision of the Hologic 1500 W was 0.009 g/cm^2 for spine, 0.007 g/cm^2 for total hip, and 0.002 g/cm^2 for the manufacturer's spine phantom. Precision of the Hologic 4500 W was 0.006 g/cm^2 for spine, 0.007 g/cm^2 for hip, and 0.002 g/cm^2 for radius. For quality control, all DXA readings were performed by the same trained technician, measurement drift was prevented by calibrating equipment daily on the phantom, and baseline and follow-up scans were evaluated by the same reviewer, ensuring comparability of regions of interest.

To prevent any unknown intermachine differences from affecting our assessment of rates of BMD change, measurements of BMD were standardized (mean = 0, SD = 1) independently at baseline and follow-up. Annual change in standardized BMD was calculated as the difference divided by exact elapsed time between standardized baseline and follow-up measurements. This process retains the information of individual measurements relative to the sample, but not of the absolute magnitude of measurements, thus avoiding potential unknown machine differences that could invalidate direct inter-machine comparisons. Rates of BMD change are expressed as percentage of a standard deviation per year (%SD/year). The formula for calculating this metric of BMD change is

$$y_i = \frac{100}{t_i} \cdot \left(\frac{y_{fi} - \bar{y}_f}{\sigma_f} - \frac{y_{bi} - \bar{y}_b}{\sigma_b} \right)$$

where y_i is the rate of BMD change for the ith individual, y_{bi} is the baseline BMD for the ith individual, $\bar{y}_b$ is the mean baseline BMD, σ_b is the standard deviation of baseline BMD, y_{fi} is the follow-up BMD for the ith individual, $\bar{y}_f$ is the mean follow-up BMD, σ_f is the standard deviation of follow-up BMD, and t_i is the elapsed time between baseline and follow-up measurements.

Based on the precision of our DXA equipment, the least significant change (LSC; detectable with 95% confidence) was ±2.22%SD/year for hip, ±2.54%SD/year for spine,

and ±1.53%SD/year for forearm. Observed rates of change less than the LSC values were not significantly different from zero (i.e., no observed change). Of the 327 participants included in this study, 65%, 73%, and 81% experienced significant rates of change in BMD (i.e., greater than the LSC) at the hip, spine, and radius, respectively.

Covariates included in our analysis were sex, baseline age (years), site-specific baseline BMD (g/cm^2), baseline body mass index (BMI; kg/m^2), yearly interim change in BMI (kg/m^2/year; calculated as difference between baseline and follow-up measurements divided by the exact elapsed time), and interim entrance into menopause (yes or no; defined as surgical menopause or 1 or more years since most recent menstrual cycle). Covariate measurements were collected identically at baseline and follow-up clinic visits, as previously described [11].

Genotypes

Automated genotyping of the San Antonio Family Osteoporosis Study participants has been described previously [19]. DNA from lymphocytes was amplified via polymerase chain reaction using fluorescently tagged primers (MapPairs Human Screening Set Versions 6 and 8; Research Genetics, Huntsville, AL) to detect repeat alleles at highly polymorphic microsatellite markers. Aliquots of amplified DNA were genotyped with Applied Biosystems Model 377 DNA Sequencers and analyzed with GeneScan and Genotyper DNA Fragment Analysis software (Perkin Elmer, Foster City, CA, USA). CRI-MAP [45] was used to assemble 460 microsatellite markers across chromosomes 1 to 22 into a genetic map, for which all marker positions were confirmed by deCODE (deCODE Genetics, Reykjavik, Iceland). Mean intermarker distance was 7.6 cM, ranging from <0.1 to 15.7 cM (Haldane).

Statistical Analyses

Distributions of BMD (at baseline and follow-up) and covariates were assessed, and outliers greater than 4 SD from trait means were excluded (0 to 3 observations removed per trait). Because all traits were relatively normally distributed, no transformations were necessary. Heritability of rate of BMD change was estimated in a variance components framework, which models phenotypic variance as a function of effects due to measured covariates, additive polygenic (based on average allele-sharing between relative pairs), and error components. The general form of this model is $y_i = \mu + \sum_{i=1}^{n} \beta_j X_{ij} + g_i + e_i$, where y_i is the rate of BMD change for the ith individual, μ is the sample mean rate of BMD change, X_{ij} is the jth covariate for the ith individual, β_j is the corresponding

regression coefficient, g_i is the additive polygenic effect, and e_i is the residual error effect. Model parameters were estimated using pedigree-based maximum likelihood methods, from which residual heritability (h_r^2; i.e., the proportion of phenotype variance attributable to the additive genetic component after removing variation due to covariates) was estimated. The significance of covariate and heritable components was tested via the likelihood ratio test, which compares the likelihood of models including and excluding each component. This test follows a chi-square distribution with 1 degree of freedom for testing covariates, and a 50:50 mixture of a point mass at zero and a chi-square distribution with 1 degree of freedom for testing heritability. Only covariates with significant effects at $\alpha = 0.1$ were retained in final models for each skeletal site. The proportion of variance explained by covariates was determined by comparing models including and excluding retained covariates. Models of rates of BMD change for each skeletal site were restricted to participants with data on all retained covariates. Power to detect true heritability of 0.25, 0.35, and 0.45 was 70%, 85%, and 95%, respectively, at a significance threshold of $\alpha = 0.05$.

Multipoint linkage analysis was performed by extending the variance components model described above to include the effect of a theoretical QTL, σ_m^2, as a component of genetic variance. Multipoint identical-by-descent (IBD) probabilities across chromosomes 1 to 22 were estimated from genotype data of relatives via a Markov chain Monte Carlo algorithm implemented by Loki [46, 47]. Maximum likelihood methods were used to estimate σ_m^2 based on the expected covariance due to IBD probabilities between relatives at each locus along the chromosomes. Significance of σ_m^2 was assessed by the likelihood ratio test, which compared the QTL model (i.e., including σ_m^2 as a component of variance) to the polygenic model (i.e., $\sigma_m^2 = 0$), and expressed as a logarithm of the odds (LOD) score ($\log_{10}$ of the likelihood ratio). This test follows a 50:50 mixed distribution of a point mass at zero and a 1-degree-of-freedom chi-square distribution. Empirical LOD score adjustment based on 10,000 simulated unlinked markers was used to guard against inflated LOD scores, which can occur due to deviations of the phenotype distribution from normality [48]. All LOD scores (and corresponding p-values) presented herein are adjusted. Power to detect linkage was low: approximately 25% and 40% at thresholds of LOD = 2.0 and 1.5, respectively, for a QTL explaining 25% of variance in rates of BMD change at any skeletal site.

Genetic modeling was performed in the set of all participants aged 25 to 45 years ($n = 327$) as well as the subset of age-eligible men and premenopausal women ($n = 292$). To assess whether differences in linkage results obtained from the total sample and premenopausal subset were due to diminished sample size (null hypothesis), or, alternatively, due to inclusion of postmenopausal women, 100 random subsets of 292 individuals (sampled without regard to menopausal status) were used to generate an empirical distribution of the effect of reduced sample size [49]. Genetic analyses were performed using the Sequential Oligogenetic Linkage Analysis Routines (SOLAR) software [50]; data management, summary statistics, outliers, and figures were done in R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the 327 participants included in this study are presented in Table 1. Participants returning for follow-up measurements did not differ in any characteristics from those not available for follow-up. On average, adiposity in this sample was high (BMI > 30) in both men and women, and weight increased (0.82 kg/year in women and 0.45 kg/year in men) over 5.6 years of follow-up (range = 3.5 to 8.9 years). Distributions of rate of BMD change (%SD/year) are shown in Fig. 1. As shown, rate of BMD change (unadjusted for covariate effects) varied widely among young men and women. Change in BMD was calculated as the yearly difference between standardized (mean = 0, SD = 1) BMD measurements at baseline and follow-up to mitigate the effects of differences between baseline and follow-up measurements.

Environmental and genetic influences on rate of BMD change for participants aged 25 to 45 years are listed in Tables 2 and 3. As expected, women who entered menopause during the interim between visits experienced significantly faster bone loss at the femoral neck, total hip, and total spine; however, this relationship was not observed for sites of the forearm (Table 2). Interestingly, women experienced slower rates of bone loss than did men (i.e., female sex was positively correlated with rate of BMD change) for all sites. Bone loss increased with increasing age only at the radius sites. The cumulative amount of variation attributable to measured covariates differed greatly between skeletal sites, ranging from 6% for the 33% radius to 24% for the femoral neck.

Significant residual heritability of rate of BMD change (Table 3) was observed for the total hip ($h_r^2 = 0.34$; $p = 0.01$), ultradistal radius ($h_r^2 = 0.34$; $p = 0.004$), and 33% radius ($h_r^2 = 0.27$; $p = 0.03$). Modest residual heritability was observed for the femoral neck ($h_r^2 = 0.22$; $p = 0.06$). In contrast, rate of BMD change of the total spine was not heritable.

Because menopause is widely acknowledged to have profound effects on bone loss, genetic and environmental

Table 1 Mean (SD) population characteristics

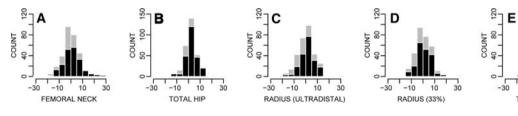| Variable	All	Women	Men
Sample size, n	325	210	115
Follow-up, yr	5.6 (0.7)	5.5 (0.7)	5.6 (0.7)
Age, yr	34.4 (5.9)	34.6 (5.8)	34.2 (6.1)
Anthropometrics			
Height, cm	162.2 (8.8)	157.4 (6.1)	170.8 (6.2)
Weight, kg	80.4 (20.7)	75.9 (19.2)	88.6 (20.8)
BMI, kg/m^2	30.5 (7.0)	30.6 (7.4)	30.3 (6.3)
Annual weight gain, kg/yr	0.69 (1.39)	0.82 (1.40)	0.45 (1.35)
Annual change in BMI, kg/m^2/yr	0.27 (0.52)	0.33 (0.55)	0.15 (0.45)
Medical			
Diabetes, %	10.7	10.4	11.2
Premenopausal, %	–	95.3	–
Oral contraceptives, %	–	20.9	–
Interim entrance into menopause, %	–	9.2	–
Lifestyle			
Alcohol consumption, %	50.8	44.5	62.1
Smoking history, %	20.8	19.4	23.3
Baseline BMD, g/cm^2			
Femoral neck	0.89 (0.13)	0.87 (0.12)	0.92 (0.13)
Total hip	0.99 (0.15)	0.96 (0.13)	1.05 (0.15)
Radius, ultradistal	0.50 (0.07)	0.47 (0.05)	0.55 (0.06)
Radius, 33%	0.72 (0.07)	0.68 (0.04)	0.79 (0.05)
Total spine	1.05 (0.12)	1.05 (0.12)	1.04 (0.13)
Follow-up BMD, g/cm^2			
Femoral neck	0.87 (0.12)	0.86 (0.12)	0.89 (0.13)
Total hip	1.01 (0.14)	0.99 (0.13)	1.06 (0.14)
Radius, ultradistal	0.49 (0.06)	0.47 (0.05)	0.53 (0.07)
Radius, 33%	0.74 (0.07)	0.71 (0.05)	0.80 (0.06)
Total spine	1.03 (0.12)	1.04 (0.11)	1.01 (0.12)

Fig. 1 Distributions of change in BMD (%SD/year) for **a** femoral neck, **b** total hip, **c** ultradistal radius, **d** 33% radius, and **e** total spine. Gray bars represent the total sample (women + men); black bars represent women

influences on rate of BMD change were also assessed after excluding 35 women who reported having undergone menopause during the years of follow-up. Residual heritability estimates in this subset were greater than those of the total group for the femoral neck ($h_r^2 = 0.29$) and total hip ($h_r^2 = 0.49$) and similar for the ultradistal radius ($h_r^2 = 0.34$), 33% radius ($h_r^2 = 0.25$), and spine ($h_r^2 = 0.0$). Standard errors and cumulative variance due to covariates (excluding the effect of menopause) were essentially unchanged.

Figure 2 depicts autosome-wide multipoint linkage scans for rate of BMD change of the femoral neck, total hip, ultradistal radius, and 33% radius (but not the spine because change at this site was not heritable). Significant evidence for linkage was observed on chromosome 1q23 at 151 cM for rate of femoral neck BMD change (LOD = 3.6, adjusted $p = 0.00004$) (Fig. 3). No other chromosomal regions exhibited evidence of linkage at LOD > 1.5 in the entire sample. In the subset of men and premenopausal women, linkage was diminished for the

Table 2 Relationship between rate of early BMD change and covariates: β coefficients (p-Values)

	Femoral neck	Total hip	Radius (ultradistal)	Radius (33%)	Total spine
N	300	300	313	313	295
R^2	0.24	0.16	0.14	0.06	0.14
Gender	1.9[a] (0.023)	2.2 (<0.001)	5.1 (<0.001)	2.5 (0.001)	3.8 (<0.001)
Age	–[b] (0.738)	– (0.284)	−0.2 (0.002)	−1.6 (0.012)	– (0.489)
Meno[c]	−4.4 (0.008)	−3.3 (0.009)	– (0.699)	– (0.336)	−5.5 (0.001)
BMI	5.1 (<0.001)	0.2 (<0.001)	−0.1 (0.097)	– (0.343)	−0.1 (0.045)
ΔBMI	0.6 (<0.001)	3.0 (<0.001)	1.4 (0.064)	−1.6 (0.03)	– (0.642)
BMD	−15.9 (<0.001)	– (0.663)	14.9 (0.045)	– (0.164)	−10.6 (0.002)

Note: R^2, proportion of variance due to covariates; ΔBMI, yearly change in BMI during interim (kg/m²/year); BMD, site-specific baseline BMD (g/cm²). β coefficients are interpreted as %SD/year per unit of covariate

[a] Effect of female sex with respect to male sex

[b] Only covariates with significant effects at $\alpha = 0.1$ are included in genetic models

[c] Meno: entrance into menopause during interim between baseline and follow-up

Table 3 Residual heritability of early BMD change

Trait	n	h_r^2	SE	p-Value
Femoral neck	300	0.22	0.16	0.06
Total hip	300	0.34	0.17	0.01
Radius (ultradistal)	313	0.34	0.16	0.004
Radius (33%)	313	0.27	0.16	0.03
Total spine	295	0.00	–	0.50

Note: h_r^2: residual heritability, while simultaneously accounting for variation due to covariates enumerated in Table 2. Only covariates with significant effects at $\alpha = 0.1$ are included in genetic models

femoral neck QTL (LOD = 2.9 on chromosome 1) and an additional suggestive QTL was identified for ultradistal radius (LOD = 2.9 on chromosome 6q26–27 at 189 cM; adjusted $p = 0.0003$) (results not shown).

To verify the robustness of our chromosome 1 QTL and determine whether the decrease in LOD score in the subset of men and premenopausal women was due to reduced sample size, or due to heterogeneity specific to postmenopausal women, we generated an empirical distribution for 100 subsets in which 35 (10.7%) individuals were excluded at random. The mean maximum LOD score across all leave-35-out subsets was 3.0 (at position 151 cM), which is
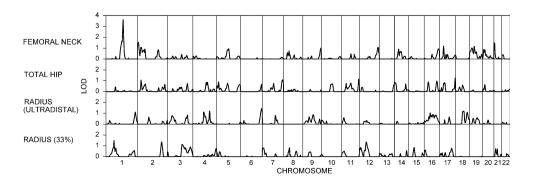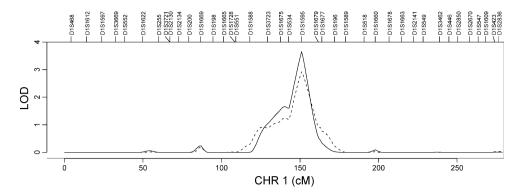
not significantly different ($p = 0.45$) from our observed LOD = 2.9 in the subset of individuals that included only men and premenopausal women. Thus, the observed decrease in evidence of linkage in the subset is indistinguishable from the LOD-score decrease due to a reduction in sample size alone, and therefore we have no evidence that heterogeneity is contributing to our linkage signal.

Discussion

Cross-sectional and longitudinal studies have shown that BMD declines in young men and women of European [41, 43, 44] and African ancestry [10], and that most of this change results from loss of trabecular bone density. One method by which to increase our understanding of the mechanisms influencing early bone loss is to determine whether such longitudinal loss is heritable and, if so, to identify the specific genes involved.

In this study we show substantial variation in the individual rates of early areal BMD change at the femoral neck, total hip, ultradistal radius, and 33% radius, and estimate that genetic factors are responsible for up to 35% of this variation. In contrast, we found that early bone loss

Fig. 2 Autosome-wide multipoint linkage scans

Fig. 3 Multipoint LOD score profile for femoral neck BMD change on chromosome 1 in total sample (solid line) and subset of men and premenopausal women (dashed line)

at the lumbar spine was not heritable, though the reason for this is unclear. Both baseline and follow-up spine BMD are heritable in young Mexican Americans ($h_r^2 = 0.78$ and 0.71, respectively), and the rate of change in BMC and area does not differ qualitatively from the other skeletal sites. Although our results include both men and women, they are consistent with those of Hui et al. [35], who reported heritability of change in femoral neck BMD in premenopausal women only. Our estimates of heritability for early BMD change are also similar, with a few exceptions, to those for bone loss among older individuals at several skeletal sites [36, 37]. Together, this body of evidence suggests that rates of BMD loss are heritable for many skeletal sites, in both men and women, young and old.

Because BMD is calculated from the mineral content (g) over the two-dimensional projected area (cm^2) of a bone, change in BMD may be reflective of either change in bone mineral content (BMC), change in bone area, or some combination of the two. In this sample, both BMC and area were changing, on average, in the same direction ($r = 0.53$ to 0.78), though the rates of percentage change differed between BMC and area. For example, femoral neck BMD (mean change = -0.7% per year) was decreasing faster than area (-0.5% per year; $p = 0.001$). Percentage change in BMC was also less than percentage change in area for the ultradistal radius and lumbar spine ($p < 0.001$ for both). In contrast, percentage change in BMC was greater than percentage change in area for the total hip (1.2% versus 0.7% per year) and 33% radius ($p < 0.001$ for both). These observations suggest that genes affecting the interplay between BMC and bone area, rather than one or the other, are responsible for the observed heritability of BMD change; the specific genetic relationships among these traits within skeletal sites remain to be resolved.

Our linkage scan yielded a putative QTL influencing bone loss in young adults. The QTL for rate of change in femoral neck BMD on chromosome 1q23 (LOD = 3.6) has been reported to influence variation in peak spine BMD in several [17, 21, 27, 30], but not all [51], previous cross-sectional studies; specifically, this QTL was not observed in our Mexican American sample for either peak femoral

neck BMD [19] or femoral neck BMD loss in older individuals [37]. These possibly contradictory results could be explained by a gene or genes influencing BMD change, rather than peak BMD, in an age-specific manner. We know that trabecular and cortical bone loss varies by age and skeletal site [10, 43]. Thus, linkage studies for peak BMD [17, 21] and BMD change in young individuals are well suited to detect a genetic regulator of early BMD change, whereas linkage [19] and exclusion [51] analysis for BMD in mixed-aged samples and linkage analysis for BMD change in older samples [37] are not.

The 1-LOD unit support interval for the femoral neck QTL on chromosome 1q23 ranges from 146 to 155 cM ($\sim$133 to 157 Mbp) in a gene-rich region containing 429 known or hypothetical genes. In particular, this region includes BGLAP/osteocalcin, which has well-known effects on bone formation [52, 53], and IL6R, for which previous studies have shown genetic association to BMD [54] and linkage to BMD and osteopenia [55]. Additionally, a zinc/iron transporter (SLC39A1; mouse homologue expressed in osteoblasts of developing bone) [56] and 17 calcium binding proteins (S100A1-A7, -A7.1, -A7.2-A14, -A16) are contained in the support interval. One or more of these genes, or other linked loci, may contribute to our observed linkage signal.

We also obtained suggestive evidence for a QTL influencing early BMD change at the 33% ulna on chromosome 11p14–15 (LOD = 2.5). Due to its position, this QTL is likely distinct from those detected in previous studies for peak BMD of the spine at 11q12–13, the *LRP5* locus [18, 22], or 11q23 [26]. The 1-LOD-unit support interval for our suggestive QTL ranges from 22 to 42 cM ($\sim$14 to 25 Mbp), covering 88 genes. Notable genes in this region are calcitonin α (*CALCA*), calcitonin β (*CALCB*), and calcitonin pseudogene (*CALCP*). Calcitonin is a thyroid hormone regulating serum calcium levels and has known inhibitory effects on the resorptive activity of osteoclasts [57]. Calcitonin could also be involved in early BMD change and is a reasonable positional candidate gene for BMD change.

We speculate that because the femoral neck is comprised of both cortical and trabecular bone, and because

growing evidence suggests that early bone loss occurs primarily in the latter [10, 43], the observed linkage to chromosome 1q23 may be due to one or more (possibly site-specific) genes affecting femoral neck trabecular bone loss. Though direct measurements of trabecular and cortical content are not available in this study, this hypothesis reflects recent findings in other populations [10, 43] and is consistent with the largely uncorrelated linkage scans for different skeletal sites.

A major strength of the current study is the combined longitudinal and family-based study design. Our assessment of 5-year BMD change is better suited to elucidate the genetic effects of early bone loss than cross-sectional linkage scans, and the inclusion of many types of relatives pairs within our extended families allows us to better model truly genetic effects on BMD change (as opposed to familial nongenetic effects, such household effects) than do studies using twins or sibships. These strengths notwithstanding, several limitations of the present study also need to be addressed. First, while statistical power to detect heritability of rate of BMD change was adequate, power to detect linkage at genome-wide significance was poor, and our sample size precluded testing for gene × sex or gene × environment interactions. Also, densitometry equipment was upgraded during the follow-up interim, which may have introduced measurement bias; however, due to the independent standardization of baseline and follow-up measurements and excellent agreement observed in our cross-calibration experiment, any unknown effects of our equipment upgrade are probably minimal. Finally, DXA technology is inherently limited in its ability to detect BMD change because areal projection may not adequately account for the size (depth) of bone or possible change in bone size over time, and cannot measure trabecular and cortical content separately.

In conclusion, this study reports that rate of early change in BMD at several skeletal sites is heritable and similar in magnitude to the heritability of late bone loss [35, 37]. The QTL for rate of early BMD change that we detected on chromosome 1q23 is among the first loci implicated in longitudinally assessed BMD change and may act as a candidate region for additional investigations into the genetic determinants of early bone loss. Identification of specific genes or pathways influencing early BMD change may lead to better understanding of bone health and possible early therapeutic interventions for individuals at greater risk of developing osteoporosis.

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