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Bivariate Linkage Study of Proximal Hip Geometry and Body Size Indices: The Framingham Study

D. Karasik,

Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, 1200 Centre Street, Boston, MA, 02131, USA, e-mail: karasik@hrca.harvard.edu

J. Dupuis,

Department of Biostatistics, Boston University School of Public Health, 715 Albany Street, Boston, MA, 02118, USA

L. A. Cupples,

Department of Biostatistics, Boston University School of Public Health, 715 Albany Street, Boston, MA, 02118, USA

T. J. Beck,

Department of Radiology, Johns Hopkins University School of Medicine, 601 N. Caroline St., Suite 4260, Baltimore, MD, 21287-0849, USA

M. C. Mahaney,

Department of Genetics, Southwest Foundation for Biomedical Research, 7620 NW Loop 410, San Antonio, TX, 78227-5301, USA

L. M. Havill,

Department of Genetics, Southwest Foundation for Biomedical Research, 7620 NW Loop 410, San Antonio, TX, 78227-5301, USA

D. P. Kiel, and

Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, 1200 Centre Street, Boston, MA, 02131, USA

S. Demissie

Department of Biostatistics, Boston University School of Public Health, 715 Albany Street, Boston, MA, 02118, USA

Abstract

Femoral geometry and body size are both characterized by substantial heritability. The purpose of this study was to discern whether hip geometry and body size (height and body mass index, BMI) share quantitative trait loci (QTL). Dual-energy X-ray absorptiometric scans of the proximal femur from 1,473 members in 323 pedigrees (ages 31–96 years) from the Framingham Osteoporosis Study were studied. We measured femoral neck length, neck-shaft angle, subperiosteal width (outer diameter), cross-sectional bone area, and section modulus, at the narrowest section of the femoral neck (NN), intertrochanteric (IT), and femoral shaft (S) regions. In variance component analyses, genetic correlations (ρ_G) between hip geometry traits and height ranged 0.30–0.59 and between hip geometry and BMI ranged 0.11–0.47. In a genomewide linkage scan with 636 markers, we obtained nominally suggestive linkages (bivariate LOD scores ≥ 1.9) for geometric traits and either height or BMI at several chromosomes (4, 6, 9, 15, and 21). Two loci, on chr. 2 (80 cM, BMI/shaft section modulus) and chr. X (height/shaft outer diameter), yielded bivariate LOD scores ≥ 3.0 ; although these loci were linked in univariate analyses with a geometric trait, neither was linked with either

height or BMI. In conclusion, substantial genetic correlations were found between the femoral geometric traits, height and BMI. Linkage signals from bivariate linkage analyses of bone geometric indices and body size were similar to those obtained in univariate linkage analyses of femoral geometric traits, suggesting that most of the detected QTL primarily influence geometry of the hip.

Keywords

Proximal femoral geometry; Body size; Heritability; Bivariate linkage analysis; Quantitative trait locus

Osteoporosis is a skeletal disorder characterized by compromised bone strength, predisposing an individual to increased risk of fracture [1]. There are over 1.5 million osteoporotic fractures annually in the United States, with hip fracture remaining the most severe clinical outcome of age-related osteoporosis due to its high prevalence, serious effects on quality of life, and excessive economic costs [2–6]. To advance our understanding of skeletal fragility and improve clinical assessments of fracture risk, knowledge of new mechanisms, risk factors, and predictors of hip fracture is greatly needed.

The strength of bones is determined not only by the amount of bone but also by age-related changes in the spatial distribution of bone tissue, an important determinant of fracture risk that is not captured completely by bone mineral density (BMD). These aspects of bone quality are quantified in terms of structural geometry, which determines the way stresses produced by loading forces are transmitted through the bone. A growing body of evidence indicates that bone geometry contributes substantially to bone strength and fracture risk [7,8]. Compared to those without fractures, women with hip fractures (as well as their daughters [9,10]) have wider femoral necks and longer hip axis lengths, independently of femoral neck BMD, age, and body size [11–17]. Femoral geometry may be assessed noninvasively by dual-energy X-ray absorptiometry (DXA)-based hip structural analysis (HSA). The technique of HSA provides measures of bone size and mineral distribution that allow for indirect evaluation of genetic and environmental influences on bone mechanical properties.

There is substantial evidence suggesting that femoral geometry is largely determined by genes in both humans [18–21] and other animals [22]. Height and body mass index (BMI) are also under genetic influence [23,24]. Genomewide linkage analyses of femoral geometry using plain radiographs [25,26] and DXA-derived traits [27,28] have suggested that the variation in femoral neck geometry is under strong genetic control (heritability of 40–50%). We recently performed a whole-genome linkage analysis of proximal femoral geometric indices in members of 323 pedigrees from the Framingham Heart Study and found evidence of substantial linkage on several autosomes and on chromosome X [29]. We also found that adjustment for height and BMI affected both heritability and the magnitude of linkage signals for some of the traits [29].

There are indications, in both humans [30,31] and mice [32], that genetic factors common to femoral dimensions, femoral cross-sectional traits, body weight, and height may act pleiotropically. Both height and body mass appear to affect fracture risk [12], especially risk of hip fracture [33,34]. Some of the reported genetic effects on bone size and mineral distribution may be due in part to genes that are related to growth or to general body size [35]. The objectives of this study were to (1) seek evidence for shared genetic effects on measures of bone size and distribution and on measures of body size (e.g., height and BMI) by evaluating genetic correlations between relevant trait pairs and (2) to perform a genomewide linkage analysis for these pairs of traits to localize observed pleiotropic effects to specific chromosomal regions.

Methods

Sample

The sample used for our analyses was derived from two cohorts of the population-based Framingham Heart Study. 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 with the intent 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 their spouses. Neither the Original nor the Offspring Cohort was selected on the basis of cardiovascular diseases or osteoporosis. Details and descriptions of the Framingham Osteoporosis Study have been reported [36,37]. The study was approved by the Institutional Review Boards for Human Subjects Research of Boston University and Hebrew Rehabilitation Center.

DXA and HSA

The participants underwent bone densitometry by DXA with a Lunar (Madison, WI) DPX-L. The Original Cohort participants underwent bone densitometry during their examination 22 (1992–1993). To maximize the sample size, we used DXA scans from examination 24 (1996–1997) for 31 Original Cohort members who missed DXAs at examination 22. The Offspring Cohort was scanned with the same machine between 1996 and 2001. An interactive computer program [38,39] was used to derive a number of structural variables from the femoral DXA scans. The regions assessed were the narrowest width of the femoral neck (NN), which overlaps or is proximal to the standard Lunar femoral neck region; an intertrochanteric (IT) region located along the bisector of the neck-shaft angle (NSA); and the femoral shaft (S) 1.5 times the minimum neck width distal to the intersection of the neck and shaft axes [see 29 for details].

HSA provided measures of cross-sectional bone area (CSA) and outer diameter at each of the three femoral regions (NN, IT, and S), as well as NSA and femoral neck length (FNL, defined as the distance from the center of the femoral head to the intersection of the neck and shaft axes). CSA in the HSA method is the mechanically relevant surface area of bone tissue in the cross section corresponding to the measured bone mineral content and assuming the mineralization of average adult cortical bone.

Cross-sectional moment of inertia (CSMI) was calculated from the bone mass profile integral as follows:

$$I_x = \frac{x_p}{\rho_m} \sum (y - y_c)^2 m_{bi}$$

where y is the locus of the current pixel, y_c is the locus of the center of mass of the profile, x_p is the pixel spacing along the profile line, and ρ_m is the density of mineral in adult bone tissue. Section modulus was calculated as $Z_x = I_x/d_{\max}$ for maximum bending stress in the image plane, where $d_{\max}$ is the maximum distance from the center of mass to the medial or lateral bone edge, whichever is greater. No assumptions about cross-sectional shape were made in CSA, CSMI, or section modulus. Together with NSA and FNL, there were 11 phenotypes. Coefficients of variation for the different component variables were previously reported to range from 3.3% (NN outer diameter) to 9.1% (FNL) [38].

Measurements of Body Size and Body Composition

Information on age, sex, weight, and height were obtained for each individual at the time of the bone measurement. In brief, in both cohorts, weight (pounds) was measured using a standardized balance beam scale. Height (without shoes) was measured to the nearest one-fourth inch using a standard stadiometer. These measures were converted to kilograms and centimeters, respectively, and BMI was then calculated in kilograms per meter squared.

Genotyping

A genomewide scan was performed in the Framingham Heart Study in two steps. In the first phase, 1,702 individuals in the largest 330 families were genotyped without regard to their clinical characteristics, using 422 polymorphic markers (marker set 9, average heterozygosity 0.77, sex-averaged mean intermarker spacing 8.6 cM; NHLBI Mammalian Genotyping Service in Marshfield, WI [40]). In the second phase, an additional 184 members of the 330 largest pedigrees were genotyped on 382 markers (marker set 13, average heterozygosity 0.76, sex-averaged mean intermarker spacing 8.9 cM). There were 262 markers in common with marker set 9. Also, 94 additional markers genotyped on these 330 largest pedigrees, used to augment the original genome scan, were available and included in the linkage analyses. A total of 636 markers, including 21 markers on chromosome X, were studied, with an average marker spacing of 5.7 cM. Genotype data cleaning, including verification of family relationships and mendelian inconsistencies, have been previously described [41,42].

Out of a total of 1,886 genotyped Framingham participants, HSA measurements were available for 1,473. There were thus 390 phenotyped participants of the original Framingham cohort and 1,083 participants from the Offspring Cohort, members of 323 pedigrees with family sizes ranging from two to 30 genotyped individuals (with ~80% of the sample representing members of two- to six-person pedigrees and a maximum of 30 participants/pedigree). The genotyped sample with bone geometric phenotypes included the following relative pairs: 678 parent-offspring, 1,074 sibling, 659 cousin, and 380 avuncular.

Statistical Methods

Prior to heritability and linkage analyses, multivariable regression analyses were performed to obtain standardized (normalized) residual bone geometry phenotypes. We adjusted for age and age² in each gender and cohort because these variables generally affect height, weight, and bone phenotypic variation significantly.

Univariate Linkage Analysis—Univariate variance component analyses for all traits were performed on normalized residuals using the computer package SOLAR (version 2.0; SFBR/NIH, San Antonio, TX) [43], available online (<http://www.sfbr.org/sfbr/public/software/solar/solar.html>). Heritability (h^2) of each trait was estimated as a proportion of the residual trait variance (i.e., that remaining after accounting for the mean effects of covariates) attributable to the additive effects of genes. Subsequent linkage analyses were also conducted using SOLAR. This method, described in detail elsewhere [43], entails specification of the genetic covariance among arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus and models the covariance matrix for a pedigree as the sum of the additive genetic covariance attributable to the quantitative trait locus (QTL), the additive genetic covariance due to the effects of loci other than the QTL, and the variance due to unmeasured environmental factors.

We tested the hypothesis of linkage by comparing the likelihood of a restricted model in which variance due to the QTL equaled zero (no linkage) to that of a model in which it did not equal zero (i.e., was estimated). The logarithm of odds (LOD) score of classical linkage analysis was obtained as the quotient of the difference between the two *log* likelihoods divided by *log* 10.

IBD Computations—For autosomes, multipoint probabilities of IBD were approximated at every 1 cM using the Markov chain Monte Carlo approach implemented in the computer program Loki [44]. For chromosome X, IBD probabilities were computed using the *minx* subroutine of MERLIN [45], which can perform multipoint linkage analysis on chromosome X but unfortunately is not able to handle large pedigrees. Therefore, large pedigrees were broken down into smaller ones by splitting families and/or deleting family members while keeping as many members with genotypes as possible. We thus computed IBD for 342 “derivative” pedigrees (including 251 intact pedigrees, 51 reduced pedigrees, and 40 newly derived pedigrees) for chromosome X multipoint analysis. For the autosomal markers, map distances were obtained from the Center for Medical Genetics (<http://www.research.marshfieldclinic.org/genetics/>) whenever available or estimated otherwise; map distances for the X chromosomes were obtained from DeCODE [46]. 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.

Bivariate Linkage Analysis—To test the hypothesis that QTL jointly influence variation in measures of hip geometry at the three femoral sites and the body size measures, we performed genomewide bivariate linkage analyses for all pairs of traits. The bivariate method that we employed is a simple extension of the univariate maximum likelihood-based variance components statistical genetics approach described above. In this approach, we simultaneously model the mean effects of covariates plus the contributions of genes (both the QTL and the residual polygenes) and unmeasured environmental factors on two traits. The bivariate model differs from the univariate one in that it also estimates the portions for the residual phenotypic correlation (ρ_P) between trait pairs that are due to shared, additive effects of genetic variation at the QTL (a QTL-specific genetic correlation, ρ_Q), shared additive effects of genes other than those at the QTL (a residual additive genetic correlation, ρ_G), and shared effects of unmeasured environment (residual environmental correlation, ρ_E , including nonadditive genetic factors). These components of the correlation are additive, so that $\rho_P = \rho_Q h_{1Q1} h_{1Q1} + \rho_G h_1 h_2 + \rho_E (1 - h_1) (1 - h_2)$; where h_{1Q1} and h_{2Q1} are the square roots of the QTL-specific heritabilities, respectively, for traits 1 and 2 in a trait pair and h_1 and h_2 are the square roots of the residual heritability estimates for the same traits [47].

We interpret a genetic correlation that is significantly different from 0 to be consistent with the detection of a pleiotropic effect of the genes in that component on more than one trait. Rejection of the hypothesis that $\rho_Q = 0$ suggests that genetic variation at the QTL influences variation in both phenotypes. We interpret a failure to reject the hypothesis that $\rho_Q = 0$ as an indication of coincident linkage between two independent QTL, each influencing one of the two traits in the pair. Similarly, rejection of $\rho_G = 0$ is consistent with the detection of shared effects of genes other than those at the QTL on a pair of traits. Failure to reject the hypothesis of $\rho_G = 0$ means that we did not detect any evidence for pleiotropy at loci other than the QTL. Rejection of $|\rho_Q| = 1$ or $|\rho_G| = 1$ is consistent with complete pleiotropy, i.e., the case in which all the genetic effect of that component (QTL or residual genetic) is shared between the two traits. Note that this is analogous to interpretations of bivariate correlations in regression analyses; i.e., we interpret the square of the genetic correlation as an estimate of the proportion of the additive *genetic* variance in each trait in the pair that is shared with the other trait. In the case of complete pleiotropy, 100% of the additive genetic effects of the QTL or genes other than the QTL on two traits is due to the same genes, $(\rho_Q)^2 = 1$ or $(\rho_G)^2 = 1$. If, for example, $\rho_G = 0.50$, we would conclude that 25% of the additive genetic variance in two traits may be due to the effects of the same gene or genes.

We determined the significance of these correlations using likelihood ratio tests in which we compared the likelihood of a more general model in which the correlations were estimated to

a model in which a parameter of interest (e.g., ρ_Q or ρ_G) was constrained to 0 (or 1, for the test of complete pleiotropy). More extensive details regarding the development, implementation, and power of bi- and multivariate extensions to linkage analyses have been published elsewhere [47–49].

In our bivariate linkage screens, we computed a bivariate LOD score as the *log* likelihood ratio of the locus-specific model to the polygenic model. The analytical software SOLAR implements an automatic conversion of the two-degrees of freedom bivariate LOD score to a one-degree of freedom effective LOD score [50,51]. In addition to the bivariate LOD score comparisons, the additive genetic variance at the QTL (i.e., the QTL- or locus-specific heritability, h_Q^2) was used to estimate the magnitude of the effect of the specific QTL on the residual trait variance. This value is expressed as the proportion of residual trait variance explained by the specific QTL. These estimates are generally considered to be biased (inflated) when obtained from analysis of data in relatively small, simple pedigrees such as those in this study [52], so it is important to note that although we present these estimates (along with the ratio of h_Q^2 and the trait's h^2) for descriptive purposes, we did not base our judgment about the presence of shared QTL on the h_Q^2 values. Instead, QTL pleiotropy was formally tested as proposed by Almasy et al. [47] and described above.

Correction for False-positive Rate and Multiple Testing—To control for the overall false-positive rate in our genomewide linkage screens of a single phenotype, we employed an approach for quantitative traits based on the work of Feingold et al. [53] and implemented in Gauss 6.0.17 (Aptech Systems, Maple Valley, WA). This approach takes into account the finite marker density in the linkage map utilized in the multipoint QTL screens and the mean recombination rate for the pedigree population studied. Using the criteria discussed in detail by Lander and Kruglyak [54], nominal “significant” evidence (i.e., genomewide $P = 0.05$) for a bone-related QTL in analyses of data from these Framingham Heart Study families is obtained at LOD = 3.01 and “suggestive” evidence (genomewide $P = 0.1$) is obtained at LOD = 1.76. There are indications that threshold for significance for the X chromosome is higher [55].

Correction for multiple testing was performed using a modification of the methods described by Camp and Farnham [56]. The total number of bivariate tests performed was 22 (11 geometric traits and height, BMI; Table 2), which corresponds to the estimated number of 8.1 effectively independent genomewide linkage analyses. For “significant” and “suggestive” thresholds with LOD = 3.01 and LOD = 1.76, the corresponding corrected thresholds were thus ~4.58 and ~3.20.

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 geometric or related traits.

Results

Table 1 displays descriptive statistics of the Framingham osteoporosis study participants by sex and cohort. Participants of the Original Cohort (parental generation) were on average 20–21 years older than the offspring and had lower height and BMI. In each cohort, men and women were of similar age; male participants were heavier and taller than females. In both cohorts, males had in general greater average bone geometry values at all skeletal sites compared to females.

Variance component analyses revealed significant additive genetic components (h^2) for all geometric measures ranging from 0.28 (NSA) and 0.70 (IT outer diameter) (Table 2).

Heritability of height was high, 0.89, perhaps reflecting assortative mating. Heritability of BMI was also substantial (0.49). As follows from Table 2, there exist moderate to high phenotypic correlations between most geometric traits and the two measures of body size. Except for NSA, height was significantly ($P < 0.0001$) correlated with geometric traits ($\rho_P = 0.18$ – 0.47). Similar phenotypic correlations were also observed with BMI (except for NSA and neck length as well as a low inverse correlation between BMI and NN outer diameter). Table 2 also provides evidence for shared genetic and environmental influences between geometric traits and either height or BMI. Genetic correlations were substantial between the majority of geometric traits and height; all except for NSA ranged 0.30–0.59. Genetic correlations between geometric traits and BMI ranged 0.11–0.47 (except for NN outer diameter). There were also substantial environmental correlations between the majority of geometric traits and BMI but lower ones between geometric traits and height (e.g., maximal ρ_E was 0.63 between BMI and S CSA but only 0.16 between height and S CSA).

Results from bivariate and univariate linkage analyses are displayed in Table 3. At least nominally suggestive evidence for bivariate linkage (LOD scores ≥ 1.76) for geometric traits and either height or BMI was found at the following chromosomal regions: chr. 2 (80 cM), chr. 4 (124 and 185 cM), chr. 6 (159 and 181 cM), chr. 9 (108 cM), chr. 15 (43 cM), chr. 21 (3 cM), and chr. X. Two of the above loci yielded LOD scores ≥ 3.01 (the value required for a nominal genomewide significance at $P = 0.05$), namely chr. 2 (80 cM, BMI/S section modulus) and chr. X (height/S outer diameter). In univariate analysis (Table 3), these loci were linked mostly with a corresponding geometric trait and less with either height or BMI, with the exception of chr. 9 (suggestively linked to BMI), chr. 6 (at 181 cM, modest linkage with BMI), and chr. 21 (modest linkage with height). Additionally, locus-specific heritability (h^2_Q) estimates indicate that, for most of the chromosomal regions, the effect of a specific QTL in bivariate analysis was stronger on the geometric trait than on the body size trait. For example, a locus on chr. 2 (at 80 cM) resulted in a substantial h^2_Q for S CSA that ranged 0.28–0.32, while h^2_Q for either height or BMI was much lower, 0.09 and 0.19, respectively. Overall, h^2_Q ranged 0.16–0.38 for geometric traits, 0–0.14 for height, and 0–0.24 for BMI. When testing for colinkage vs. pleiotropy of the loci, the hypothesis for coincident linkage ($\rho_q = 0$) could not be rejected ($P > 0.1$) for all QTL in Table 3. Only a few QTL provided some putative indication in favor of pleiotropy: these were QTL on chr. 9 (S CSA with height) and chr. 21 (S section modulus with both height and BMI). For these QTL, the hypothesis for pleiotropy ($H_0: \rho_q = 1$) could not be rejected ($P = 0.06, 0.08$, and 0.06 , respectively), although this significance is borderline. Thus, 31% of the residual phenotypic variance in S section modulus and 14% of phenotypic variance in height is attributable to shared genetic effects at chr. 21 QTL. The maximum multipoint LOD score from the univariate analysis of height in this region was only 1.02. The locus on chr. 9 was shared between cross-sectional area and BMI, with $h^2_Q = 0.32$ for NN CSA and 0.19 for BMI (similarly, 0.33 for S CSA and 0.24 for BMI).

In Fig. 1, multipoint LOD scores are presented for chromosomes 2, 9, and X, revealing that the linkage for the combination of height and/or BMI with a geometric trait followed the same pattern as the univariate linkage for the corresponding geometric trait on chr. 2 and X (Fig. 1A, C). Bivariate LODs for chr. 9 followed the pattern of the univariate linkage peaks for both NN CSA and BMI (Fig. 1B), corresponding to the above finding of probability of a pleiotropic effect at this locus.

Discussion

In this sample of extended pedigrees from the Framingham Study, we evaluated evidence for common genetic effects that are shared between measures of proximal femur geometry and

two measures of body size, height and BMI. We also examined whether there exist chromosomal regions that jointly contribute to variation in these traits. Presence of substantial phenotypic correlations between geometric traits and anthropometrics (up to 0.47 for height and up to 0.54 for BMI) and strong effects of adjusting for body size on linkage peaks in our previous study [29] motivated our joint heritability and linkage analysis of these traits. Our findings suggest that proximal femoral bone geometric indices and body size are influenced by shared genetic and environmental factors. Thus, genetic correlations between the majority of hip geometric traits and height ranged 0.30–0.59, indicating that 12–40% of the residual variance in hip geometry and 27–53% of the residual variance in height is due to the same gene or suite of genes. Genetic correlations between the majority of hip geometric traits and BMI ranged 0.11–0.47, indicating that 5–26% of the residual variance in hip geometry and 6–23% of the residual variance in BMI is due to the same gene(s). The latter results are similar to those reported in a Chinese study, in whom genetic correlations between the hip geometric traits and BMI ranged 0.33–0.45 [31].

It should be noted that NSA was not correlated with body size (phenotypic, genetic, and environmental correlations all around 0). Similarly, genetic correlations between BMI and FNL were virtually absent. This is an important observation because of the ongoing debate as to whether or not hip geometric traits are key predictors of hip fracture and to what extent they are correlated with body size. Larger femoral neck diameter as well as narrower NSA [57–59] were found to be protective against hip fractures in some studies [60,61]. Height, in turn, has been shown in several large prospective studies in women and men to be related to hip fracture [33,34,62], although its significance as a risk factor is uncertain [63]. Our study suggests that since both phenotypic and genetic correlations between NSA and FNL and body size are negligible, these traits seemingly relate to hip fracture independently, and alleles predisposing to fractures are expected to differ among these traits.

There were also considerable environmental correlations between the majority of femur geometric traits and BMI. Environmental correlations between femur geometry and BMI were generally higher than with height. This finding is important since it points out the existence of common environmental factors that may jointly contribute to both bone geometry and body composition. Some of these factors, such as nutrition and exercise, are of a modifiable nature and thus deserve more emphasis in future studies aimed at prevention of bone fragility.

In a univariate linkage analysis, several chromosomal regions provided strong evidence for linkage with femur geometric indices [reported in 29]. These were QTL on chromosomes 2, 4, 6, 13, 15, 16, 21, and X. Substantial genetic correlations between the majority of femur geometric traits and anthropometrics motivated us to search for evidence of QTL being shared between the geometric traits and anthropometrics by means of bivariate linkage analysis. In some situations, bivariate linkage analysis has been shown to increase power to detect linkage of related traits to a common QTL [64].

To test whether this was true in our sample, we performed the following simulation analyses to estimate the statistical power to detect a QTL in both univariate and bivariate statistical genetic analyses. Utilizing the pedigree structure and phenotype data from more than 1,400 Framingham participants, we simulated a linked marker with ten alleles through the pedigrees. Our simulations used a residual heritability for each geometric trait ($h^2 = 0.28–0.70$). After the analysis of 1,000 univariate and bivariate replicates, we observed the following. In univariate linkage analyses, we had 80% power to detect a QTL responsible for 39% of the residual phenotypic variance in the trait at $LOD = 4.58$ (the threshold for a genomewide significance corrected for the multiple testing) and 30% at $LOD = 3.20$ (the threshold for a genomewide suggestive linkage corrected for the multiple testing). At this same level of significance, bivariate linkage analyses (assuming complete QTL pleiotropy and residual genetic correlation

$\rho_G = 0.50$) improved this situation to the following degree: we had 80% power to detect a QTL accounting for 30% and 23% of the residual phenotypic variance at LOD = 4.58 and 3.20, respectively. These power calculations demonstrate increased power to detect linkage with pleiotropic QTL for traits having high residual genetic correlation between them.

This study (to our knowledge, the first bivariate linkage analysis of hip geometry and body size) generated an important finding: we observed that the above QTL are linked to femur geometric traits largely independently of anthropometrics. In general, we were unable to accept a hypothesis of pleiotropy for the majority of QTL identified here. Only a few QTL provided some putative indication in favor of pleiotropy, such as loci on chromosome 9 (S CSA with height) and chromosome 21 (S section modulus with both height and BMI). For example, the locus on chromosome 9 was shared between CSA (both NN and shaft regions) and BMI (Fig. 1B). However, despite the fact that this QTL appears to be shared by femur geometric indices and BMI (with 32–33% of variation in CSA and up to 24% in BMI explained by the locus), the test for pleiotropy also could not reject a competing hypothesis of coincidental linkage. The wide region on chromosome 9 (75–120 cM) was one of the few that showed consistently stronger linkage of BMI in women than in men in a recent study of Atwood et al. [23]. We thus conclude either that the singular QTL characterized by substantial correlations with both traits in a pair (ρ_Q) represents, in reality, the composite effects of several tightly linked polymorphic loci or that we are underpowered to discern a real pleiotropic effect of the same locus from a tight linkage of two or more loci.

Bivariate linkage analysis thus suggested that most of the above QTL are specific for femur geometric indices rather than being shared with the anthropometric traits. For example, the majority of the QTL explained much more heritability of the geometric trait than of its anthropometric counterpart; thus, the QTL at 2p21, where section modulus of the shaft and BMI were linked, explained 38% of variation in section modulus and only 18% in BMI. Similarly, the QTL at Xq25-Xq26, where shaft diameter and height were linked, explained 34% of shaft diameter and only 7% of height. This heritability of 7% due to the QTL effect is relatively small given the high overall heritability of height in our sample (89%). High heritability of adult height, ranging 0.75–0.90, has also been reported by other genetic studies; however, it probably reflects an effect of assortative mating. The above suggests that QTL detected here may influence femur geometric traits by contributing to them directly and, to a lesser degree, via the indirect effects of body height and/or body mass.

Animal models have suggested that the effect of body size on bone size is complex, is modified by body dimensions, and can interact with physical activity in creating skeletal loading effects [32]. There is evidence, in both humans [30] and mice [32], that common genetic factors contribute to femoral dimensions and cross-sectional traits as well as to body weight and height. In mice, Lang et al. [32] performed linkage analyses of bone width, CSA, cortical thickness, CSMI, and bone length. They found that the QTL for these bone phenotypes, weight and body length, were often mapped to the same regions in C57BL/6J and DBA/2 second-generation (F_2) and recombinant inbred mice.

Several genomewide linkage studies in humans and mouse models [65–67] revealed a convergence with the QTL reported by us. Several biologically plausible candidate genes are located within the most promising chromosomal regions in our analysis (candidate gene locations were obtained from the UCSC Genome Bioinformatics Web site [68]). Thus, in a region at 80 cM on 2p16, where S CSA was linked, several biological candidate genes are mapped, including calmodulin 2 (*CALM2*), follicle-stimulating hormone receptor (*FSHR*), and transforming growth factor α (*TGF α*). Of note, in older Amish, Streeten and colleagues (personal communication) found a linkage with NN CSA at 104 cM on chromosome 2. Under the peak of NN section modulus linkage at 4q27 (124 cM), interleukin 2 (*IL2*) is mapped. This

region is syntenic with the one identified by Klein et al. [66] on mouse chromosome 8 to influence femoral CSA in mice. In Amish, Streeten and colleagues (personal communication) also found a linkage of S section modulus at 4q (147 cM) with LOD >2.0. Finally, on chromosome 6, the linkage peak for IT outer diameter is located at 6q25, where the estrogen receptor α gene (*ESR1*) is mapped. Insulin-like growth factor 2 receptor (*IGF2R*) is located within the 6q27 region, where S CSA and S section modulus were linked in our sample. Streeten and colleagues (personal communication) found linkage at this location for NN CSA in Amish women (LOD >2.0). The syntenic region in mice was associated with vertebral trabecular bone mass and microarchitecture [67].

Thus, there is a promising overlap between regions identified in this study and findings in animal models, including femoral CSA in a large, genetically heterogeneous population of mice [66]. In particular, Klein et al. [66] found linkages on mouse chromosome X (syntenic with Xq26-Xq27), where shaft CSA and outer diameter, respectively, were linked in our sample. Similarly, Masinde et al. [65] found linkage of murine periosteal circumference with DXMit208, which again corresponds to Xq26 in humans. These two chromosomal regions thus deserve more attention for follow-up (fine-mapping) studies. Given that our analyses do not take the possible X inactivation into consideration, we interpret the results on chromosome X with caution.

There are several limitations to our study. First, the HSA method employs two-dimensional projections of complex three-dimensional anatomy, and the section modulus is relevant for bending resistance only in the plane of the image; thus, out-of-plane differences in geometry may be unrecognized. The method assumes that bone tissue mineralization is fixed so that any differences in mineral quantity or distribution are expressed geometrically. Conceivably, some genetic factors may influence average tissue mineralization, which confounds geometry measured by this method. Despite these limitations, noninvasive measurements of bone geometry by some imaging method are the only practical approach since genetic studies of bone fragility (using biomechanical tests) in a large sample of humans are infeasible. For this reason, linkage studies followed by experiments in animal models are of paramount importance for the genetics of femoral strength [22,32].

Second, BMI is a surrogate measure of fat and lean body mass. It is known that up to 45–55% of body weight is due to lean mass, while lean mass is predominantly muscle. Muscle forces dominate the forces on bones in normal activities and, hence, the loads to which the bones adapt. In mice, body size can interact with physical activity in creating skeletal loading effects, resulting in bone geometric phenotypes [32]. However, physical activity and muscular mass, both of which also play a role in the skeletal loading environment [32], were not studied here. As for physical activity, it was subjected to a regression diagnostics analysis and was not chosen as an important covariate with the HSA-derived geometric traits (not shown here). It may be argued, however, that physical activity should be measured throughout the adult life span instead of the time point proximal to the hip geometry exam. Evaluation of lean mass specifically was beyond the scope of this report and is a focus of our ongoing studies. Finally, gender effects – which are known to contribute to variability in hip bone geometry [69,70] as well as to confound genetics of hip geometry [26] – were not studied here. In the current analysis, we focused on the combined gender sample; however, sex-specific effects on genetic correlations between hip geometry and body size are explored in our ongoing studies.

In summary, this study represents an important step toward identifying genes that contribute to geometric aspects of bone strength and provides a number of chromosomal locations to pursue. In our sample from a general population, proximal femoral geometric indices seem to have genetic contributions common with and independent of the anthropometric traits. A large part of the QTL effects on hip geometry appears to be independent of body size and weight,

as suggested by our tests for pleiotropy. These findings are important to the design and interpretation of genetic studies of skeletal geometric traits and merit further molecular genetic study using combinations of bone geometry with fat and lean mass measures in humans, as well as a study of pleiotropic effects of candidate genes for body size on hip geometric properties.

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Fig. 1. Univariate and bivariate linkage results (multipoint LOD scores). *Horizontal line*, corrected for multiple testing “suggestive” linkage threshold. **(A)** Shaft section modulus (S Z), height, BMI, and combinations of shaft section modulus/height and shaft section modulus/BMI, chromosome 2. **(B)** Narrow neck cross-sectional bone area (NN CSA), height, BMI, NN CSA/height, and NN CSA/BMI, chromosome 9. **(C)** Shaft cross-sectional bone area (S CSA), height, BMI, S CSA/height, and S CSA/BMI, chromosome X

Table 1

Descriptive statistics by sex and Cohort

Variable	Original		Offspring	
	Males Mean $\pm$ S.D	Females Mean $\pm$ S.D	Males Mean $\pm$ S.D	Females Mean $\pm$ S.D
N*	346	592	490	597
Age (yrs)	78.4 $\pm$ 4.4	79.9 $\pm$ 4.8	58.2 $\pm$ 9.4	58.4 $\pm$ 9.8
Height (cm)	169.9 $\pm$ 7.0	155.1 $\pm$ 6.5	175.1 $\pm$ 7.1	161.1 $\pm$ 6.8
Weight (kg)	78.0 $\pm$ 12.9	64.3 $\pm$ 12.8	88.6 $\pm$ 14.8	71.9 $\pm$ 16.4
BMI (kg/m ²)	27.0 $\pm$ 3.9	26.6 $\pm$ 5.0	28.8 $\pm$ 4.4	27.7 $\pm$ 6.1
NSA (degrees)	131.4 $\pm$ 6.5	128.1 $\pm$ 6.1	130.6 $\pm$ 5.7	127.9 $\pm$ 5.9
FNL (cm)	5.4 $\pm$ 0.8	4.6 $\pm$ 0.7	5.6 $\pm$ 0.7	4.7 $\pm$ 0.6
Narrow Neck (NN)				
Outer diameter (cm)	3.4 $\pm$ 0.3	2.9 $\pm$ 0.3	3.3 $\pm$ 0.3	2.8 $\pm$ 0.2
CSA (cm ²)	2.4 $\pm$ 0.5	1.8 $\pm$ 0.4	2.8 $\pm$ 0.5	2.2 $\pm$ 0.4
Section modulus (cm ³)	1.4 $\pm$ 0.3	0.9 $\pm$ 0.2	1.6 $\pm$ 0.3	1.1 $\pm$ 0.3
Intertrochanteric				
Outer diameter (cm)	5.9 $\pm$ 0.5	5.2 $\pm$ 0.4	5.8 $\pm$ 0.4	5.1 $\pm$ 0.4
CSA (cm ²)	4.5 $\pm$ 0.9	3.0 $\pm$ 0.7	5.0 $\pm$ 0.9	3.7 $\pm$ 0.8
Section modulus (cm ³)	4.7 $\pm$ 1.1	2.7 $\pm$ 0.8	5.0 $\pm$ 1.1	3.1 $\pm$ 0.8
Shaft				
Outer diameter (cm)	3.3 $\pm$ 0.3	3.0 $\pm$ 0.2	3.1 $\pm$ 0.2	2.9 $\pm$ 0.2
CSA (cm ²)	4.2 $\pm$ 0.7	2.7 $\pm$ 0.6	4.6 $\pm$ 0.7	3.4 $\pm$ 0.6
Section modulus (cm ³)	2.5 $\pm$ 0.5	1.5 $\pm$ 0.4	2.7 $\pm$ 0.5	1.8 $\pm$ 0.4

* including unrelated members of both cohorts; number of subjects can be less for some of the traits due to missing values

NSA, neck-shaft angle; FNL, femoral neck length; CSA, cross-sectional area of bone surface

Table 2

The heritabilities of the studied phenotypes and phenotypic, genetic, and environmental correlations between body height and mass and bone geometric parameters

Variable*	h ² Correlations	(h ² = 0.89)		BMI	(h ² = 0.49)	
		Height	ρ_G		ρ_G	ρ_E
NSA	0.28	-0.004	0.00	-0.005	0.106 ± 0.121	-0.073 ± 0.067
FNL	0.40	0.184 [‡]	0.302 ± 0.071 [‡]	0.066 [‡]	0.155 ± 0.099	0.00
Narrow-neck (NN)						
Outer diameter	0.49	0.360 [‡]	0.575 ± 0.058 [‡]	-0.100 [‡]	-0.049 ± 0.093	-0.145 ± 0.072 [‡]
CSA	0.42	0.355 [‡]	0.528 ± 0.062 [‡]	0.298 [‡]	0.123 ± 0.098	0.453 ± 0.060 [‡]
Section modulus	0.40	0.380 [‡]	0.592 ± 0.064 [‡]	0.267 [‡]	0.157 ± 0.099	0.361 ± 0.063 [‡]
Intertrochanteric (IT)						
Outer diameter	0.70	0.443 [‡]	0.567 ± 0.047 [‡]	0.186 [‡]	0.227 ± 0.078 [‡]	0.142 ± 0.089
CSA	0.44	0.268 [‡]	0.402 ± 0.066 [‡]	0.460 [‡]	0.326 ± 0.084 [‡]	0.591 ± 0.048 [‡]
Section modulus	0.45	0.350 [‡]	0.550 ± 0.063 [‡]	0.471 [‡]	0.362 ± 0.081 [‡]	0.583 ± 0.049 [‡]
Shaft (S)						
Outer diameter	0.63	0.471 [‡]	0.587 ± 0.047 [‡]	0.155 [‡]	0.290 ± 0.083 [‡]	-0.015 ± 0.082
CSA	0.44	0.296 [‡]	0.404 ± 0.068 [‡]	0.535 [‡]	0.438 ± 0.077 [‡]	0.632 ± 0.047 [‡]
Section modulus	0.56	0.432 [‡]	0.580 ± 0.054 [‡]	0.531 [‡]	0.472 ± 0.069 [‡]	0.606 ± 0.054 [‡]

h² - heritability, ρP - phenotypic correlations, ρG - genetic correlations, and ρE - environmental correlations

* adjusted for age, Cohort, sex

** Significance levels for correlation coefficients:

[‡] p < 0.0001

[†] 0.0001 < p < 0.05

the rest, non-significant (p > 0.05)

Table 3
Bivariate linkage of geometric properties measured by HSA with height and BMI. LOD scores ≥ 3.01 are in bold

Chr. #	Position	Geometric Trait	Univariate LOD Geom. Trait	Bivariate with Height		Univariate LOD for Height	Bivariate with BMI		Univariate LOD for BMI
				LOD	$h^2_{q, \text{geom. trait}}$		LOD	$h^2_{q, \text{geom. trait}}$	
2	(80 cM)	S CSA	2.37	1.56	0.28	<1.0	2.23	0.32	1.09
4	(124 cM)	NN section modulus	2.90	2.05	0.31	<1.0	2.17	0.34	0
	(185 cM)	S CSA	<1.0	<1.0	0.16	<1.0	2.06	0.20	<1.0
6	(159 cM)	IT Outer diameter	2.38	2.13	0.26	<1.0	2.22	0.24	1.28
	(181 cM)	S CSA	2.80	2.34	0.30	<1.0	2.66	0.29	1.28
		S section modulus	3.00	2.08	0.26	<1.0	2.66	0.27	1.28
9	(109 cM)	NN CSA	1.10	1.04	0.19	0	2.50	0.32	2.80
		S CSA	1.36	1.73	0.26	0	2.44	0.33	2.80
15	(43 cM)	FNL	2.41	1.57	0.28	0	2.12	0.29	0
21	(3 cM)	S section modulus	3.35	2.45	0.31	1.02	2.73	0.33	<1.0
X	(133 cM)	S Outer diameter	3.77	3.28	0.34	0	2.97	0.33	0

Bold font: LOD scores "suggestive" for linkage (corrected for multiple testing LODs > 3.2)