Published in final edited form as:

Bone. 2007 March ; 40(3): 743–750.

Proximal Hip Geometry is Linked to Several Chromosomal Regions: Genome-Wide Linkage Results from the Framingham Osteoporosis Study

S. Demissie¹, J. Dupuis¹, L. A. Cupples¹, T. J. Beck², D. P. Kiel³, and D. Karasik³

1 Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA

2 Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

3 Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, Boston, MA, USA

Abstract

INTRODUCTION—Femoral geometry contributes to bone strength and predicts hip fracture risk. The purpose of this study was to evaluate heritability (h^2) of geometric indices of the proximal hip and to perform whole-genome linkage analyses of these traits, adjusted for body size.

METHODS—DXA scans of the proximal femur from 1473 members of 323 pedigrees (age range 31–96 yrs) from the population-based Framingham Osteoporosis Study were obtained. Using the Hip Structural Analysis program, we measured femoral neck length (FNL, cm) and neck-shaft angle (NSA); subperiosteal width (WID, cm), cross-sectional area (CSA, cm²), and section modulus (Z, cm³) at the narrowest section of the neck (NN), intertrochanteric (IT), and femoral shaft (S) regions. Linkage analyses were performed for the above indices with a set of 615 markers using variance components maximum likelihood method.

RESULTS—Substantial genetic influences were found for all geometric phenotypes, with h^2 values between 0.28 (NSA) and 0.70 (IT_WID). Adjustment for height and BMI did not alter h^2 of NSA and FNL, but decreased h^2 of the cross-sectional indices. We obtained substantial linkage (multipoint LOD>3.0) for S_Z at 2p21 and 21q11, and S_WID at Xq25-q26. Inclusion of height and BMI as covariates resulted in much lower LOD scores for S_Z, whereas linkage signals for S_Z at 4q25, S_CSA at 4q32, and S_CSA and S_Z at 15q21, increased after the adjustment. Linkage of FNL at 1q and 13q, NSA at 2q, and NN_WID at 16q did not change after the adjustment.

CONCLUSION—Suggestive linkages of bone geometric indices were found at 1q, 2p, 4q, 13q, 15q, and Xq. The identification of significant linkage regions after adjustment for BMI and height may point to QTLs influencing femoral bone geometry independent of body size.

Keywords

proximal femur; geometry; heritability; whole-genome linkage; quantitative trait loci; body size and body composition

Mailing address of contact person (reprint requests): David Karasik, Ph.D., Hebrew SeniorLife, 1200 Centre Street, Boston, MA 02131, Fax 617 363-8936, e-mail: karasik@hrca.harvard.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no conflict of interest.

INTRODUCTION

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 a leading cause of morbidity and mortality in the elderly that consumes a disproportionate amount of health care resources, is associated with substantial pain, and significantly decreases active life expectancy[2–4]. Alarming are also the predictions of increasing number of fractures and associated health-care burden as the population ages[5]. To advance our understanding of skeletal fragility and improve clinical assessments of fracture risk, knowledge of mechanisms and predictors of hip fractures is greatly needed.

Strength of osteoporotic bones is mainly compromised by age-related changes in the amount and distribution of the bone tissue within bones. These aspects are quantified by engineers in terms of structural geometry, which determines stresses within bones produced by loading forces. Though low bone mineral density (BMD) has been commonly used as a risk factor for predicting fractures[6], a growing body of evidence indicates that bone geometry also contributes importantly to bone strength and fracture risk[7]. Thus, proximal femora of elderly men and women with any type of osteoporotic fracture have lower estimated bending strength and axial strength, thinner cortices and wider subperiosteal diameters than femora of fracture-free counterparts[8]. Compared to those without fractures, women with hip fractures (as well as their daughters[8,9]) have altered femoral geometry, including wider femoral necks and longer hip axis lengths, independently of femoral neck BMD, age, and body size[10–16]. Currently, femoral geometry is assessed non-invasively by radiogrammetry and/or DXA-based hip structural analysis (HSA), as well as by 3D methods like computed tomography.[17] The technique of HSA describes the bone mass data so that genetic influences on mechanical properties might be indirectly evaluated.

There is a great deal of evidence suggesting that osteoporosis has a substantial genetic component[18,19]. While it is well known that BMD is highly heritable[18,19], bone size and architecture are also under strong genetic influence[20]. Both men and women with a maternal history of osteoporosis have been shown to have thinner femoral cortices[21]. So far, there have been relatively few genetic studies on femoral neck cross-sectional geometry in humans, although several genes have been associated with hip geometric traits[22–24]. Genome-wide linkage analyses of femoral geometry using plain radiographs[25,26] and DXA-derived geometric traits[27,28] suggested that the variation of femoral neck geometry is under strong genetic control.

Therefore, to better understand the genetics underlying hip fractures, it is important to examine heritable determinants of bone geometry. The aim of this study was to determine heritability (h^2) of geometric indices of the proximal hip in men and women members of extended pedigrees from the community based Framingham Osteoporosis Study sample, and to perform whole-genome genetic linkage analyses. Additionally, because there are known effects of body size on size and strength of the bones[29] we evaluated whether or not an adjustment for body size would influence the magnitude of heritability and linkage.

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 an 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 offspring's spouses. Neither the Framingham Original nor Offspring Cohort was selected on the basis of cardiovascular diseases or osteoporosis. Details and descriptions about the Framingham Osteoporosis Study have been reported[30,31]. The study was approved by the Institutional Review Board for Human Subjects Research of Boston University.

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 their examination 22 (1992–1993). In order to maximize the sample size, we used DXA scans from examination 24 (in 1996–1997) for 31 Original Cohort members who did not have DXAs at examination 22. The Offspring Cohort was scanned with the same machine between 1996 and 2001. As described previously[32,33], an interactive computer program (Hip Structure Analysis, HSA) 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) - at distance of 1.5 times the minimum neck width, distal to the intersection of the neck and shaft axes.

HSA provided measures of bone cross-sectional area (CSA, cm^2), section modulus (Z, cm^3) and subperiosteal width (WID, cm) at each of the 3 femoral regions (NN, IT, and S). These parameters are measured directly from the mass profiles using a principle first described by Martin and Burr [34]. In addition, the method measures the neck-shaft angle (NSA) and femoral neck length (FNL), defined as distance from the center of femoral head to the intersection of neck and shaft axes (Figure 1). Note that since the geometry measures are derived from the projected mineral mass, they integrate both trabecular and cortical bone and exclude the non-contributing spaces due to trabecular and cortical pores. An important limitation of the method is that the mineral distribution information incorporated into the section modulus is only relevant to bending in the plane of the scan image.

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_WID) to 9.1% (FNL), respectively[32].

Other measurements

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 (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/m^2 .

Genotyping

A genome 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 (markers set 9, average heterozygosity 0.77; sex-averaged mean inter-marker spacing of 8.6 cM, NHLBI Mammalian Genotyping Service in Marshfield, WI[35]). In the second phase, an additional 184 members of the 330 largest pedigrees were genotyped on 382 markers (markers set 13, average heterozygosity 0.76; sex-averaged mean inter-marker spacing of 8.9 cM). There were 262 markers in common with marker set 9. Also, ninety-four additional markers genotyped on these 330 largest pedigrees,

used to augment the original genome scan, were available to us and included in the linkage analyses. A total of 636 markers, including 21 markers on chromosome X were thus 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[36].

Finally, members of the 323 families with family sizes ranging from 2 to 30 genotyped individuals contributed to the linkage analyses (with 66% of the sample representing individuals in pedigrees with 2 to 6 person). Out of a total of 1702 genotyped Framingham participants, 1473 had HSA measurements. There were 390 members of the Original Framingham cohort and 1083 participants from the Offspring Cohort. The sample with genotyping and bone geometric phenotypes included the following relative pairs: 678 parent-offspring pairs, 1074 sibling pairs, 659 cousin pairs, and 380 avuncular pairs.

Statistical methods

Prior to heritability and linkage analyses, multivariate regression analysis was performed in each sex (men and women) and Cohort (Original and Offspring) in order to obtain normalized residual bone geometry phenotypes, adjusted for age only (Model 1) and for height and BMI in addition to age (Model 2). Combination of height and BMI simultaneously adjusts for body size and body composition[37], and is widely used in epidemiological studies.

A variance component analysis (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 as listed above for Models 1 and 2.

In the linkage VCA, models incorporating genetic data at a putative quantitative trait locus – in the form of probabilities of sharing 0, 1 or 2 alleles identical-by-descent (IBD) by pairs of related individuals – were compared with models incorporating only polygenic effects (i.e. without genetic marker data).

For the autosomes, single-point probabilities of IBD between relative pairs were computed using SOLAR and the multipoint (using multiple markers) probabilities of IBD were approximated at every 1 cM with the program LOKI[38]. For the chromosome X, IBD probabilities were computed using the *minx* subroutine of MERLIN [39], 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 pedigree, 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://research.marshfieldclinic.org/genetics/>) whenever available or estimated otherwise; map distances for the X chromosomes were obtained from DeCODE[40]. 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.

Linkage analyses were performed in SOLAR, at every marker (single-point) and at every 1 cM (multipoint). Multipoint linkage analysis has been shown to be more powerful than the single-point analyses, since the former contains information from adjacent markers, and potentially provides an unbiased estimate of QTL location[41] and fewer false positive observations than the single-point analysis[42]. A LOD score was computed as the log base

10 of the likelihood ratio of the locus-specific model to the polygenic model. We tested the null hypothesis of no linkage to a particular genome location, using the likelihood ratio test. Under the null hypothesis of no linkage, for normally distributed traits, twice the log of the likelihood ratio statistic at a putative QTL location is asymptotically distributed as a 50:50 mixture of a χ^2_1 and a point mass at zero. We do not provide estimates of the QTL effects since these estimates are considered biased (inflated)[43].

We used the procedure “*lodadj*” implemented in SOLAR [42] to assess the significance of the observed LOD scores. We estimated the empirical *p* values for observed LOD scores by simulating an unlinked fully-informative marker and evaluating evidence for linkage. For each phenotype we simulated 10,000 tests and calculated empirical *p*-values as the proportions of simulated LOD scores greater than an observed LOD score.

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 (including unrelated members of the Original Cohort). In each Cohort, men and women were of similar age. As expected, male participants were heavier, taller and in general had greater average geometric measures at all femur sites compared to females.

Table 2 provides a matrix of correlations among the studied geometric traits, height and BMI. A majority of the correlations are statistically significant with $p < 0.0001$ (except NSA and NN_WID with BMI, and NSA with FNL). Strong correlations were observed among the cross-sectional measurements (CSA and Z) at all 3 sites ($r = 0.94$ – 0.95 for each skeletal site); between the cross-sectional measurements and FNL ($r = 0.31$ – 0.52); and between most of the HSA indices and height ($r = 0.49$ – 0.75 , except for NSA, $r = 0.17$). Correlations between HSA indices and BMI were moderate ($r = 0.10$ – 0.42) and in the case of NSA and NN_WID, non-significant. Of note, correlations between NSA and the rest of the HSA indices, height and BMI were low ($r < 0.19$).

Variance component analysis estimates of heritability revealed a moderate to strong additive genetic component for all HSA measures, with h^2 values between 0.28 (NSA) and 0.70 (IT_WID)(Table 3). Adjustment for both height and BMI (Model 2) reduced the h^2 estimates for a majority of the traits (up to 20% lower h^2 estimates, especially in the cases of WID and Z indices); however adjustment did not change heritability of NSA and FNL, since the body size variables had little contribution to variation in these phenotypes (Table 2).

Selected results of linkage analyses (suggestive or significant linkage signals, multipoint LOD ≥ 1.9 in model 1 or 2) are displayed in Table 4. For majority of our traits, LOD score of 1.9 corresponded to an empiric *p*-value of 0.0015–.0020, and LOD score of 3.0 corresponded *p*-value of 0.0002–.0003. Multipoint LOD scores > 3.0 were obtained (adjusted for age) for S_Z at 2p21 (80 cM) and 21q11 (3 cM), and S_WID at chromosome Xq25–q26. In addition, suggestive linkages were observed for NSA, NN_CSA, NN_Z, IT_Z, IT_CSA, S_WID, FNL, NN_WID, IT_WID, S_CSA, S_Z. The CSA and Z (indices of resistance to axial and bending loads, respectively) appeared to be linked to the same chromosomal loci. On the other hand, the NSA and FNL did not share linkage loci with subperiosteal widths and cross-sectional geometric indices at NN, IT, and shaft sites (except at one locus on chromosome 15, 43 cM).

After adjustment for height and BMI, linkage results revealed notable differences in the maximum multipoint LODs. In most instances, adjustment for height and BMI resulted in much

lower LOD scores (e.g., multipoint LODs for S_Z on 2p21 and 21q11 were 1.01 and 1.00 in Model 2 vs. 4.14 and 3.35 in Model 1); this effect was evident for linkages of several traits on 2p, 5q, 6q, 9q, 12p, and X. On the other hand, linkage peaks became enhanced for S_Z at 4q25 and S_CSA at 4q32 after adjustment for height and BMI. Similarly, linkage signals became somewhat higher for S_CSA and S_Z on 15q21.

Multipoint LOD scores, both height and BMI-adjusted and non-adjusted, are depicted in Figure 2 for NN_WID, S_CSA and S_Z. In Figure 3, both single-point and multipoint LOD scores are presented for S_WID on chromosome X. In models unadjusted for body size, we observed suggestive linkage for S_WID on Xq25-q26 (maximal LOD=3.77). This linkage peak was weakened after adjustment for height and BMI (maximal LOD became 2.10).

DISCUSSION

In the sample of extended pedigrees from the Framingham Study, we evaluated the genetic components of HSA variables and whether there are chromosomal regions of linkage with these variables. We confirmed that proximal femoral bone geometric indices are heritable: all 11 indices of proximal femoral geometry were under moderate to strong genetic influence (heritability values ranging between 0.28 and 0.70), which justified performing the genetic linkage analysis. Several chromosomal regions were further identified with strong evidence for linkage (multipoint LOD scores greater than 3.0). These were 2p21 (with S_Z), 4q25 and 4q32 (with NN_Z and S_CSA), 21q11 (with S_Z), and Xq25-Xq26 (with S_WID). The NSA and FNL, in general, did not share loci of linkage with subperiosteal widths and cross-sectional geometric indices. Notable was an overlap between the “synonymous” indices of different femoral sites, such as IT_Z and S_Z at 2p21, NN_CSA and IT_CSA at 9q, and IT_WID and S_WID at Xq, which is expected given the high correlation between these variables. Stronger signals for linkage obtained with the narrow neck (NN) and femoral shaft (S) sites than with the intertrochanteric (IT) site may imply that these loci have greater influence on regions which contain greater amounts of cortical bone. The narrow neck site is clinically important (fractures occur here), while the femoral shaft cross-section is more axially symmetric and is nearly all consisting from cortical bone.

We also specifically investigated the question as to whether body size measured by a combination of height and body mass index, altered results of our heritability and linkage analyses. Adjustment for height and BMI did not change heritability estimates of NSA and FNL, since these traits had lower correlations with the anthropometrics. In most cases, however, heritability estimates from height and BMI adjusted models were lower, suggesting the presence of common genetic factors influencing HSA and body size and composition indices that were perhaps adjusted out (removed) in Model 2. While the effect of height and BMI adjustment on heritability estimates was generally consistent (lowering the estimates), its effect on linkage signals was rather variable. Adjustment for height and BMI reduced the LOD scores for S_Z at 2p21 and 21q11 while it enhanced linkage signals for NN_Z at 4q25 and S_CSA at 4q32, as well as for S_CSA and S_Z on 15q21. This result is probably important, since it highlights the need to take into account height and body mass index in order to reveal QTLs that otherwise might be overlooked. Additionally, by eliminating the contribution of as many covariates as possible, we decreased variability of the trait and thus increased the genetic signal to noise ratio. In other words, our analysis focuses on contribution of loci governing bone geometry *per se*, instead of general robustness of the skeleton due to overall body size and mass. There are also indications, in both humans[44,45] and mice[29] that common genetic factors contribute to femoral dimensions and cross-sectional traits, body weight and height. In future studies we plan to explore the presence of the genetic correlation between femoral geometric parameters, body size and body composition, in the same sample.

Several candidate genes reside in proximity to the linkage peaks identified above. Thus, aromatase (CYP19) is on 15q21, where femoral neck length and shaft cross-section strength indices yielded an indication of suggestive linkage. This gene is known to be involved in height regulation[46] and bone maintenance [47–49], although its contribution to the geometric properties is less evident[50–52]. Epidermal growth factor (EGF) resides on 4q25, in close proximity to our linkages for NN_Z. Also, there is a promising overlap between regions identified in this study and femoral cross-sectional area in a large genetically heterogeneous population of mice[53]. In particular, Klein et al.[53] found linkages on mouse chromosome 8 (syntenic with human 4q31) and chromosome X (syntenic with Xq26-Xq27), where S_CSA and S_WID, respectively, were linked in our sample.

Our linkages on chromosome X deserve special attention. Our linkage peaks, a modestone around 44,769,700 bp (76 cM) linked with IT_WID and one spanning from 120,600,000 to 128,801,000 bp (133 cM) with S_WID, corresponded to femoral geometry linkage findings of other two groups, namely femoral neck cortical thickness (at 127 cM [27] and 144 cM[28]). This convergence of findings from three groups (this study, Indiana[26] and Nebraska[27, 28]) that used different designs (brother pairs and pedigrees), variety of age groups, and different phenotype definitions (radiogrammetry, Lunar and Hologic DXA), is noteworthy and may strongly suggest that genes regulating proximal femoral geometry are residing on chromosome X. This X-linkage may account for the well-recognized differences between men and women in the majority of bone geometric traits (even when adjusted for body size[54]). There are some potential candidate genes of interest in the identified chromosomal region, namely NF-kappa B-repressing factor (NRF) and fibroblast growth factor 13 (FGF13)[55], but other yet unknown genes may reside here.

The results of this study should be interpreted in light of several methodological considerations. First, the HSA method is based upon two-dimensional projections (planar densitometry) of complex 3-D anatomy and employs a few assumptions, such as circular shape of the femoral neck and shaft regions, ellipsoid intertrochanteric region; fixed proportions of cortical to trabecular bone for each region; an algorithm for converting distance between peaks in the mass profile into endosteal diameter. The cross-sectional indices we studied are applicable only to a plane of a DXA image. However, proximal hip geometry obtained by the above method is widely used to understand femoral structure and strength[56,57] and has been shown to predict osteoporotic fragility in several epidemiological studies[8,58,59].

Second, current linkage analysis approaches in the sample of pedigrees similar to ours are underpowered for loci with modest effects. Studies in other populations measured with standardized techniques (such as DXA-based HSA) are thus warranted, as well as meta-analysis of genome scans for hip geometry, with larger samples sizes corresponding to an increased power, especially for the sex- and gender-specific linkage analyses. Third, a secular trend may be apparent in hip geometry. Studies reveal[60,61], on the basis of archived films, that femoral neck length to femoral neck diameter ratios were smaller in previous generations of women, and it was suggested that an increasing FNL/femoral neck diameter ratio (“gracile” bones) may explain increasing fracture rates in the last decades. We thus adjusted for a number of covariates, including age, generation, and body mass index that take inter-individual variation in account.

In summary, this study represents an important step toward identifying genes contributing to geometric aspects of bone strength, especially to the indices of resistance to axial and bending loads. Use of hip geometry traits may help clarify the genetic contributions to bone strength and osteoporotic fragility in general population. Current predictors of the osteoporotic fractures include BMD or the combination of BMD and bone width[62]. In the future, genetic information will be incorporated along with the traditional clinical diagnostic tests in order to

identify persons at risk for hip fracture earlier in their life for ensuing prophylaxis and treatment. Another important observation is that genetic factors on bone geometry may be shared with the body size (height and ponderosity), suggesting that body size measures have to be taken into account when dissecting the genetics of bone geometry.

Acknowledgements

From the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195), the National Institute of Arthritis, Musculoskeletal and Skin Diseases and the National Institute on Aging (grants No. R01-AR050066 and R01-AR/AG 41398).

We gratefully acknowledge the Framingham Study members who participated in this study, as well as the study coordinators, who contributed to the success of this work. We would like also to express our thanks to Drs L. Havill, M. Mahaney, and M. Bouxsein for their useful suggestions in performing the analysis for this manuscript and Ms Sophia Menn and Mei Lu for their help with data preparation and figures.

References

1. <http://www.surgeongeneral.gov/library/bonehealth/>
2. Melton LJ 3rd. Epidemiology of hip fractures: implications of the exponential increase with age. *Bone* 1996;18(3 Suppl):121S–125S. [PubMed: 8777076]
3. Melton LJ 3rd, Thamer M, Ray NF, Chan JK, Chesnut CH 3rd, Einhorn TA, Johnston CC, Raisz LG, Silverman SL, Siris ES. Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation. *J Bone Miner Res* 1997;12(1):16–23. [PubMed: 9240721]
4. Oden A, Dawson A, Dere W, Johnell O, Jonsson B, Kanis JA. Lifetime risk of hip fractures is underestimated. *Osteoporos Int* 1998;8(6):599–603. [PubMed: 10326067]
5. Melton LJ 3rd. Adverse outcomes of osteoporotic fractures in the general population. *J Bone Miner Res* 2003;18(6):1139–41. [PubMed: 12817771]
6. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures [see comments]. *Bmj* 1996;312(7041):1254–9. [PubMed: 8634613]
7. Faulkner KG, Cummings SR, Black D, Palermo L, Gluer CC, Genant HK. Simple measurement of femoral geometry predicts hip fracture: the study of osteoporotic fractures. *J Bone Miner Res* 1993;8(10):1211–7. [PubMed: 8256658]
8. Filardi S, Roger M, Zebaze D, Duan Y, Edmonds J, Beck T, Seeman E. Femoral neck fragility in women has its structural and biomechanical basis established by periosteal modeling during growth and endocortical remodeling during aging. *Osteoporos Int* 2004;15:103–107. [PubMed: 14605802]
9. Tabensky A, Duan Y, Edmonds J, Seeman E. The contribution of reduced peak accrual of bone and age-related bone loss to osteoporosis at the spine and hip: insights from the daughters of women with vertebral or hip fractures. *J Bone Miner Res* 2001;16(6):1101–7. [PubMed: 11393787]
10. Peacock M, Turner CH, Liu G, Manatunga AK, Timmerman L, Johnston CC Jr. Better discrimination of hip fracture using bone density, geometry and architecture. *Osteoporos Int* 1995;5(3):167–73. [PubMed: 7655177]
11. Michelotti J, Clark J. Femoral neck length and hip fracture risk. *J Bone Miner Res* 1999;14(10):1714–20. [PubMed: 10491219]
12. Karlsson KM, Sernbo I, Obrant KJ, Redlund-Johnell I, Johnell O. Femoral neck geometry and radiographic signs of osteoporosis as predictors of hip fracture. *Bone* 1996;18(4):327–30. [PubMed: 8726389]
13. Goulding A, Gold E, Cannan R, Williams S, Lewis-Barned NJ. Changing femoral geometry in growing girls: a cross-sectional DEXA study. *Bone* 1996;19(6):645–9. [PubMed: 8968032]
14. Cheng XG, Lowet G, Boonen S, Nicholson PH, Brys P, Nijs J, Dequeker J. Assessment of the strength of proximal femur in vitro: relationship to femoral bone mineral density and femoral geometry. *Bone* 1997;20(3):213–8. [PubMed: 9071471]
15. Faulkner KG, McClung M, Cummings SR. Automated evaluation of hip axis length for predicting hip fracture. *J Bone Miner Res* 1994;9(7):1065–70. [PubMed: 7942153]

16. Boonen S, Koutri R, Dequeker J, Aerssens J, Lowet G, Nijs J, Verbeke G, Lesaffre E, Geusens P. Measurement of femoral geometry in type I and type II osteoporosis: differences in hip axis length consistent with heterogeneity in the pathogenesis of osteoporotic fractures. *J Bone Miner Res* 1995;10(12):1908–12. [PubMed: 8619371]
17. Cheng X, Li J, Lu Y, Keyak J, Lang T. Proximal femoral density and geometry measurements by quantitative computed tomography: Association with hip fracture. *Bone*. 2006
18. Eisman JA. Genetics of osteoporosis. *Endocr Rev* 1999;20(6):788–804. [PubMed: 10605626]
19. Ralston SH. Genetic control of susceptibility to osteoporosis. *J Clin Endocrinol Metab* 2002;87(6):2460–6. [PubMed: 12050200]
20. Karasik D, Ginsburg E, Livshits G, Pavlovsky O, Kobylansky E. Evidence on major gene control of cortical bone loss in human population. *Genet Epidemiol* 2000;19:410–421.
21. Looker AC, Beck TJ. Maternal History of Osteoporosis Is Related to Femur Bone Density and Geometry in Men and Women. *J Bone Mineral Research* 2002;17(1):S200–S200.
22. Qureshi A, McGuigan F, Seymour D, Hutchison J, Reid D, Ralston S. Association between COLIA1 Sp1 alleles and femoral neck geometry. *Calcified Tissue International* 2001;69(2):67–72. [PubMed: 11683425]
23. Rivadeneira F, Houwing-Duistermaat JJ, Beck TJ, Janssen JA, Hofman A, Pols HA, Van Duijn CM, Uitterlinden AG. The Influence of an Insulin-Like Growth Factor I Gene Promoter Polymorphism on Hip Bone Geometry and the Risk of Nonvertebral Fracture in the Elderly: The Rotterdam Study. *J Bone Miner Res* 2004;19(8):1280–1290. [PubMed: 15231015]
24. van Meurs JB, Rivadeneira F, Jhamai M, Hugens W, Hofman A, van Leeuwen JP, Pols HA, Uitterlinden AG. Common genetic variation of the low-density lipoprotein receptor-related protein 5 and 6 genes determines fracture risk in elderly white men. *J Bone Miner Res* 2006;21(1):141–50. [PubMed: 16355283]
25. Koller DL, Liu G, Econs MJ, Hui SL, Morin PA, Joslyn G, Rodriguez LA, Conneally PM, Christian JC, Johnston CC, Foroud T, Peacock M. Genome screen for quantitative trait loci underlying normal variation in femoral structure. *Journal of Bone and Mineral Research* 2001;16(6):985–91. [PubMed: 11393795]
26. Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ. Sex-specific quantitative trait loci contribute to normal variation in bone structure at the proximal femur in men. *Bone* 2005;37(4):467–73. [PubMed: 16046210]
27. Shen H, Long JR, Xiong DH, Liu YJ, Liu YZ, Xiao P, Zhao LJ, Dvornyk V, Zhang YY, Rocha-Sanchez S, Liu PY, Li JL, Deng HW. Mapping quantitative trait loci for cross-sectional geometry at the femoral neck. *J Bone Miner Res* 2005;20(11):1973–82. [PubMed: 16234971]
28. Xiong DH, Shen H, Xiao P, Guo YF, Long JR, Zhao LJ, Liu YZ, Deng HY, Li JL, Recker RR, Deng HW. Genome-wide scan identified QTLs underlying femoral neck cross-sectional geometry that are novel studied risk factors of osteoporosis. *J Bone Miner Res* 2006;21(3):424–37. [PubMed: 16491291]
29. Lang DH, Sharkey NA, Mack HA, Vogler GP, Vandenberg DJ, Blizard DA, Stout JT, McClearn GE. Quantitative Trait Loci Analysis of Structural and Material Skeletal Phenotypes in C57BL/6J and DBA/2 Second-Generation and Recombinant Inbred Mice. *J Bone Miner Res* 2005;20(1):88–99. [PubMed: 15619674]
30. Demissie S, Cupples L, Myers R, Aliabadi P, Levy D, Felson D. Genome Scan for Quantity of Hand Osteoarthritis: The Framingham Study. *Arthritis Rheumat* 2002;46(4):946–952.
31. Karasik D, Cupples LA, Hannan MT, Kiel DP. Genome screen for a combined bone phenotype using principal component analysis: the Framingham study. *Bone* 2004;34(3):547–56. [PubMed: 15003802]
32. Khoo BC, Beck TJ, Qiao QH, Parakh P, Semanick L, Prince RL, Singer KP, Price RI. In vivo short-term precision of hip structure analysis variables in comparison with bone mineral density using paired dual-energy X-ray absorptiometry scans from multi-center clinical trials. *Bone* 2005;37(1):112–21. [PubMed: 15869917]
33. Beck TJ, Ruff CB, Scott WW Jr, Plato CC, Tobin JD, Quan CA. Sex differences in geometry of the femoral neck with aging: a structural analysis of bone mineral data. *Calcif Tissue Int* 1992;50(1):24–9. [PubMed: 1739866]

34. Martin RB, Burr DB. Non-invasive measurement of long bone cross-sectional moment of inertia by photon absorptiometry. *J Biomech* 1984;17(3):195–201. [PubMed: 6736056]
35. Yuan B, Vaske D, Weber JL, Beck J, Sheffield VC. Improved set of short-tandem-repeat polymorphisms for screening the human genome [letter]. *Am J Hum Genet* 1997;60(2):459–60. [PubMed: 9012420]
36. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. *Hypertension* 2000;36(4):477–83. [PubMed: 11040222]
37. Michels K, Greenland S, Rosner B. Does body mass index adequately capture the relation of body composition and body size to health outcomes? *Am J Epidemiol* 1998;147:167–172.
38. Heath SC. Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. *Am J Hum Genet* 1997;61(3):748–60. [PubMed: 9326339]
39. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002;30(1):97–101. [PubMed: 11731797]
40. Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. *Nat Genet* 2002;31(3):241–7. [PubMed: 12053178]
41. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998;62(5):1198–211. [PubMed: 9545414]
42. Blangero J, Williams JT, Almasy L. Robust LOD scores for variance component-based linkage analysis. *Genet Epidemiol* 2000;19(Suppl 1):S8–14. [PubMed: 11055364]
43. Goring HH, Terwilliger JD, Blangero J. Large upward bias in estimation of locus-specific effects from genomewide scans. *Am J Hum Genet* 2001;69(6):1357–69. [PubMed: 11593451]
44. Yang YJ, Dvornyk V, Jian WX, Xiao SM, Deng HW. Genetic and environmental correlations between bone phenotypes and anthropometric indices in Chinese. *Osteoporos Int* 2005;16(9):1134–40. [PubMed: 15711779]
45. Malkin I, Ermakov S, Kobylansky E, Livshits G. Strong association between polymorphisms in ANKH locus and skeletal size traits. *Hum Genet* 2006;120(1):42–51. [PubMed: 16724232]
46. Ellis JA, Stebbing M, Harrap SB. Significant population variation in adult male height associated with the Y chromosome and the aromatase gene. *J Clin Endocrinol Metab* 2001;86(9):4147–50. [PubMed: 11549641]
47. Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, Albagha O, Ralston SH, Tsatsoulis A. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis. *J Bone Miner Res* 2002;17(11):2048–60. [PubMed: 12412813]
48. Livshits G. Genetic epidemiology of skeletal system aging in apparently healthy human population. *Mech Ageing Dev* 2005;126(2):269–79. [PubMed: 15621207]
49. Van Pottelbergh I, Goemaere S, Kaufman JM. Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. *J Clin Endocrinol Metab* 2003;88(7):3075–81. [PubMed: 12843146]
50. Albagha OM, Pettersson U, Stewart A, McGuigan FE, MacDonald HM, Reid DM, Ralston SH. Association of oestrogen receptor alpha gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone. *J Med Genet* 2005;42(3):240–6. [PubMed: 15744038]
51. Lei SF, Deng FY, Xiao SM, Chen XD, Deng HW. Association and haplotype analyses of the COL1A2 and ER-alpha gene polymorphisms with bone size and height in Chinese. *Bone* 2005;36(3):533–41. [PubMed: 15777651]
52. Masi L, Becherini L, Gennari L, Amedei A, Colli E, Falchetti A, Farci M, Silvestri S, Gonnelli S, Brandi ML. Polymorphism of the aromatase gene in postmenopausal Italian women: distribution and correlation with bone mass and fracture risk. *J Clin Endocrinol Metab* 2001;86(5):2263–9. [PubMed: 11344237]

53. Klein RF, Turner RJ, Skinner LD, Vartanian KA, Serang M, Carlos AS, Shea M, Belknap JK, Orwoll ES. Mapping quantitative trait loci that influence femoral cross-sectional area in mice. *J Bone Miner Res* 2002;17(10):1752–60. [PubMed: 12369778]
54. Kaptoge S, Dalzell N, Loveridge N, Beck TJ, Khaw KT, Reeve J. Effects of gender, anthropometric variables, and aging on the evolution of hip strength in men and women aged over 65. *Bone* 2003;32(5):561–70. [PubMed: 12753873]
55. Lovec H, Hartung H, Verdier AS, Mattei MG, Birnbaum D, Goldfarb M, Coulier F. Assignment of FGF13 to human chromosome band Xq21 by in situ hybridization. *Cytogenet Cell Genet* 1997;76(3–4):183–4. [PubMed: 9186518]
56. Greenspan SL, Beck TJ, Resnick NM, Bhattacharya R, Parker RA. Effect of hormone replacement, alendronate, or combination therapy on hip structural geometry: a 3-year, double-blind, placebo-controlled clinical trial. *J Bone Miner Res* 2005;20(9):1525–32. [PubMed: 16059624]
57. Uusi-Rasi K, Beck TJ, Semanick LM, Daphtary MM, Crans GG, Desai D, Harper KD. Structural effects of raloxifene on the proximal femur: results from the multiple outcomes of raloxifene evaluation trial. *Osteoporos Int* 2006;17(4):575–86. [PubMed: 16392026]
58. Duan Y, Beck TJ, Wang XF, Seeman E. Structural and biomechanical basis of sexual dimorphism in femoral neck fragility has its origins in growth and aging. *J Bone Miner Res* 2003;18(10):1766–74. [PubMed: 14584886]
59. Melton LJ 3rd, Beck TJ, Amin S, Khosla S, Achenbach SJ, Oberg AL, Riggs BL. Contributions of bone density and structure to fracture risk assessment in men and women. *Osteoporos Int* 2005;16(5):460–7. [PubMed: 15688123]
60. O'Neill TW, Grazio S, Spector TD, Silman AJ. Geometric measurements of the proximal femur in UK women: secular increase between the late 1950s and early 1990s. *Osteoporos Int* 1996;6(2):136–40. [PubMed: 8704352]
61. Reid IR, Chin K, Evans MC, Jones JG. Relation between increase in length of hip axis in older women between 1950s and 1990s and increase in age specific rates of hip fracture. *Bmj* 1994;309(6953):508–9. [PubMed: 8086904]
62. Szulc P, Duboeuf F, Schott AM, Dargent-Molina P, Meunier PJ, Delmas PD. Structural determinants of hip fracture in elderly women: re-analysis of the data from the EPIDOS study. *Osteoporos Int* 2006;17(2):231–6. [PubMed: 15983728]

LIST OF ABBREVIATIONS

BMI	Body mass index
BMD	bone mineral density
CSA	cross-sectional area
DXA	dual x-ray absorptiometry
FNL	femoral neck length
h²	heritability
HAS	hip structural analysis
IBD	identical-by-descent

IT	intertrochanteric
NN	narrowest neck
NSA	neck-shaft angle
QTL	quantitative trait locus
S	femoral shaft
VCA	variance component analysis
WID	subperiosteal width
Z	section modulus

Figure 1.

Hip image from a DXA scanner showing positions of thin analysis regions across the femur at the neck, intertrochanteric and shaft regions. On the left are shown typical bone mass profiles from a single line of pixels across the bone. Geometry measurements are averaged over 5 parallel lines ~1 mm apart at each region.

Figure 2. Multipoint linkage results

A. S_Z, Chromosome 2

B. S_CSA, Chromosome 4

C. NN_WID, Chromosome 16.

Model 1 (solid line) and Model 2 (dashed line).

Figure 3. Single-point and multipoint linkage results for S_WID on chromosome X
 Diamonds: Single-point LOD scores.
 Multipoint results shown for Model 1 (solid line) and Model 2 (dashed line).

Tab. 1

Descriptive statistics by sex.

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 (ys)	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
NSA (degree)	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
NN WID (cm)	3.4 $\pm$ 0.3	2.9 $\pm$ 0.3	3.3 $\pm$ 0.3	2.8 $\pm$ 0.2
NN CSA (cm ²)	2.4 $\pm$ 0.5	1.8 $\pm$ 0.4	2.8 $\pm$ 0.5	2.2 $\pm$ 0.4
NN Z (cm ⁻³)	1.4 $\pm$ 0.3	0.9 $\pm$ 0.2	1.6 $\pm$ 0.3	1.1 $\pm$ 0.3
IT WID (cm)	5.9 $\pm$ 0.5	5.2 $\pm$ 0.4	5.8 $\pm$ 0.4	5.1 $\pm$ 0.4
IT CSA (cm ²)	4.5 $\pm$ 0.9	3.0 $\pm$ 0.7	5.0 $\pm$ 0.9	3.7 $\pm$ 0.8
IT Z (cm ³)	4.7 $\pm$ 1.1	2.7 $\pm$ 0.8	5.0 $\pm$ 1.1	3.1 $\pm$ 0.8
S WID (mm)	3.3 $\pm$ 0.3	3.0 $\pm$ 0.2	3.1 $\pm$ 0.2	2.9 $\pm$ 0.2
S CSA (cm ²)	4.2 $\pm$ 0.7	2.7 $\pm$ 0.6	4.6 $\pm$ 0.7	3.4 $\pm$ 0.6
S Z (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 can be less for some of the traits due to missing values.

NSA, neck-shaft angle; FNL, femoral neck length; WID, subperiosteal width; CSA, cross-sectional area; Z, section modulus in the proximal hip narrowest neck (NN), intertrochanteric (IT), or shaft (S)

Table 2

Pearson correlation coefficients (r) between the proximal femoral geometric indices, height and BMI

	NSA	FNL	NN_WID	NN_CSA	NN_Z	IT_WID	IT_CSA	IT_Z	S_WID	S_CSA	S_Z
Height	0.171	0.493	0.571	0.690	0.704	0.622	0.673	0.708	0.527	0.719	0.754
BMI	0.003*	0.098	-0.042*	0.290	0.254	0.16264	0.388	0.368	0.142	0.419	0.411
NSA		-0.024*	0.152	0.092	0.104	0.192	0.091	0.151	0.137	0.128	0.160
FNL			0.367	0.380	0.392	0.357	0.509	0.506	0.521	0.518	0.518
NN_WID				0.390	0.530	0.72977	0.348	0.476	0.524	0.440	0.509
NN_CSA					0.941	0.42979	0.890	0.824	0.272	0.866	0.801
NN_Z						0.548	0.841	0.842	0.368	0.842	0.821
IT_WID							0.502	0.645	0.671	0.555	0.668
IT_CSA								0.940	0.313	0.921	0.871
IT_Z									0.448	0.892	0.904
S_WID										0.624	0.624
S_CSA										0.395	0.947

* p-values >0.05, all other p<0.0001

Correlation between height and BMI < 0.10.

r>0.8 are in bold

Table 3

Heritability of the proximal geometric indices (adjusted for covariates)*

Trait	Model 1	Model 2
NSA	0.28±0.06**	0.28±0.06
FNL	0.40±0.05	0.37±0.05
NN_WID	0.49±0.06	0.37±0.06
NN_CSA	0.42±0.06	0.39±0.06
NN_Z	0.40±0.06	0.31±0.06
IT_WID	0.70±0.05	0.52±0.06
IT_CSA	0.44±0.06	0.42±0.05
IT_Z	0.45±0.06	0.35±0.06
S_WID	0.63±0.05	0.47±0.06
S_CSA	0.44±0.06	0.41±0.06
S_Z	0.56±0.06	0.39±0.06

*

Adjustment for covariates:

Model 1: age, Cohort, sex

Model 2: Model 1 + height and BMI

**

standard error of heritability estimate

All p values are less than 0.001.

Table 4Selected linkage results (multipoint LOD scores ≥ 1.9 in Model 1 or Model 2).

Chromosome	Location	Femoral geometry trait	Model 1 [*]	Model 2
1	(66 cM)	NN_WID	2.09 ^{a**}	1.26
	(155 cM)	FNL	1.97 ^a	2.33 ^a
2	(80 cM)	IT_Z	1.94 ^a	<1.0
		S_CSA	2.37 ^b	<1.0
		S_Z	4.14 ^c	1.01
4	(124 cM)	NSA	2.40 ^a	2.22 ^a
		NN_Z	2.90 ^b	1.65 ^a
		S_Z	<1.0	1.94 ^a
5	(185 cM)	S_CSA	<1.0	2.50 ^b
		S_WID	1.81 ^b	1.55 ^a
		IT_WID	2.38 ^b	1.38
6	(159 cM)	IT_WID	2.38 ^b	1.38
		S_CSA	2.80 ^b	<1.0
		S_Z	3.00 ^b	<1.0
9	(119 cM)	IT_CSA	1.92 ^a	1.17
		NN_CSA	2.49 ^b	<1.0
		NN_WID	2.51 ^a	<1.0
12	(20 cM)	NN_WID	2.51 ^a	<1.0
13	(120 cM)	FNL	1.89 ^a	2.01 ^a
15	(43 cM)	FNL	2.41 ^a	1.81 ^a
		S_CSA	1.47	2.20 ^a
		S_Z	1.28	2.10 ^a
16	(47 cM)	NN_WID	2.43 ^a	2.48 ^a
21	(3 cM)	S_CSA	1.86 ^a	<1.0
		S_Z	3.35 ^c	1.00
X	(76 cM)	IT_WID	2.00 ^a	0.99
		S_WID	3.77 ^c	2.05 ^a

* Adjustment for covariates:

Model 1: age, Cohort, sex

Model 2: Model 1 + height and BMI

** empiric significance:

^a p-value<0.005;^b p-value<0.001;^c p-value<0.0001. The rest of the LOD scores non-significant (p > 0.05).