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Autosome-Wide Linkage Analysis of Hip Structural Phenotypes in the Old Order Amish

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Abstract

Introduction—Fracture risk is associated with bone mineral density (BMD) and with other indices of bone strength, including hip geometry. While the heritability and associated fracture risk of BMD are well described, less is known about genetic influences of bone geometry. We derived hip structural phenotypes using the Hip Structural Analysis Program (HSA) and performed autosome-wide linkage analysis of hip geometric structural phenotypes.

Materials and Methods—The Amish Family Osteoporosis Study was designed to identify genes affecting bone health. BMD was measured at the hip using dual x-ray absorptiometry (DXA) in 879 participants (mean age ± SD = 49.8 ± 16.1 yrs, range 18–91 yrs) from large multigenerational families. From DXA scans, we computed structural measures of hip geometry at the femoral neck (NN) and shaft (S) by HSA, including cross sectional area (CSA), endocortical or inner diameter (ID), outer diameter (OD) buckling ratio (BR) and section modulus (Z). Genotyping of 731 highly polymorphic microsatellite markers (average spacing of 5.4 cM) and autosome-wide multipoint linkage analysis was performed.

Results—The heritability of HSA-derived hip phenotypes ranged from 40 to 84%. In the group as a whole, autosome-wide linkage analysis suggested evidence of linkage for QTLs related to NN_Z on chromosome 1p36 (LOD=2.36). In sub-group analysis, ten additional suggestive regions of linkage were found on chromosomes 1, 2, 5, 6, 11, 12, 14, 15 and 17, all with LOD ≥ 2.3 except for our linkage at 17q11.2–13 for men and women age 50 and under for NN_CSA, which had a lower LOD of 2.16, but confirmed a previous linkage report.

Conclusions—We found HSA-derived measures of hip structure to be highly heritable independent of BMD. No strong evidence of linkage was found for any phenotype. Confirmatory evidence of linkage was found on chromosome 17q11.2–12 for NN_CSA. Modest evidence was found for genes affecting hip structural phenotypes at ten other chromosomal locations.

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Keywords

hip structural phenotype; bone geometry; heritability; genetics; autosome-wide scan

INTRODUCTION

Hip fractures cause significant morbidity and mortality in the elderly, and place a major economic burden on the health care system [1,2]. The individual and societal burdens from hip fracture are increasing as the population ages. In fact, the number of hip fractures is expected to increase by 50% in the U.S. from 2005 to 2025, if more aggressive efforts are not taken to treat individuals at risk [3]. The factors contributing to fracture risk are incompletely understood, but since osteoporosis is fundamentally a condition characterized by bones that are reduced in mechanical strength and thus susceptible to fracture, the risk factors must act by degrading mechanical strength. Bone mineral density (BMD) is commonly used in the clinical setting as a strength surrogate due to its strong statistical links with fracture risk, but this measure is structurally ambiguous in that any given BMD value can be produced in a range of different and realistic bone dimensions with different mechanical properties. This makes it difficult to identify from BMD the mechanical differences that might lead to reduced strength. Mechanical properties that are related to bone strength include structural stability, axial bone strength and bending strength, represented by the measurable phenotypes buckling ratio (BR), cross sectional area (CSA) and section modulus (Z) respectively (4). One recent approach, implemented in the Hip Structural Analysis (HSA) program, infers geometric properties from two-dimensional images of bone, and derives structural/mechanical properties from these inferred three dimensional projections.

The HSA program was developed specifically to express the mineral mass from a dual-energy x-ray absorptiometry (DXA) scan of the proximal femur in a structural geometry format that can be interpreted using conventional engineering methods [4–6]. HSA used in previous studies has shown reduced hip geometry measures of bone strength (lower bending strength, thinner cortical width), in women with hip fracture compared to those without fracture [7]. In addition, aging is known to be associated not only with a reduction in BMD but also with decreases in HSA-derived cortical thickness, femur width and bending strength (section modulus) [8]. Previous studies have shown that hip geometry-derived measures of bone strength are associated with fracture risk and that this association is at least partly independent of differences in BMD [9–13).

The significant contributions of genetic factors to osteoporosis and bone mineral density are well accepted [14–16] though the geometric differences underlying the BMD effect are unclear. There is growing evidence to support the importance of heredity on geometric components of bone strength, including suggestive linkages to quantitative trait loci influencing proximal femur geometry in reports of genome-wide linkage analysis from three different research groups [17–21,32]. In the most recent of these reports, the same HSA methods were used as in the current report [21,22]. In aggregate, these linkage studies suggest that genes distinct from those that influence BMD are determinants of hip geometry traits. In this study, we report heritability and autosome-wide linkage analysis using HSA-derived measures of hip geometric strength in a large family-based cohort of Old Order Amish.

MATERIALS AND METHODS

The Amish Family Osteoporosis Study (AFOS)

The AFOS was started in 1997 to identify genetic determinants of osteoporosis [23,24].The Old Order Amish (OOA) population is an attractive one for genetic studies of complex traits

because it is a closed Caucasian founder population with large families who live in close proximity and have a relatively homogeneous lifestyle. OOA study subjects believed to be at risk for osteoporosis by virtue of fracture history or prior BMD measurements were recruited. The diagnosis of osteoporosis was verified by measurement of BMD by DXA. Individuals with a T score of ≤ -2.5 were identified as "probands". The spouses of probands as well as first-degree relatives aged ≥ 20 years were asked to participate. This report is restricted to 879 participants of AFOS who received DXA scans and for whom hip structural analysis was performed. Height and weight were taken in standard Amish dress without shoes. Height was measured using a stadiometer. The studies were conducted at the Amish Research Clinic in Strasburg, PA. The protocol was approved by the IRB of the University of Maryland School of Medicine. Informed consent was obtained from all participants.

Dual energy x-ray absorptiometry (DXA)

DXA scans of the hip and whole body were performed by a registered nurse certified in bone densitometry on a Hologic 4500W scanner (Hologic Inc., Bedford, MA). Hip scans were exported for analysis by the Hip Structure Analysis (HSA) software at the Johns Hopkins University [4]. The coefficient of variation for the total hip BMD scans, determined by 3 sequential measures on one day for each of 15 individuals, was 0.90% and 0.71% for total hip and total body, respectively.

Hip structural analysis (HSA)

The HSA program uses a principle first described by Martin and Burr [6] that mineral mass profiles across the bone axis in a bone mass image are projections of the corresponding cross-section and can partially reveal the geometry of the cross-section. The principle was later modified by Beck [4,5], to derive cross-sectional geometry from femur images acquired from DXA scans of the hip. The proximal femur cross-sections used in this report include the narrow neck (NN), defined as the narrowest point across the femoral neck, and the shaft (S) region located a distance of 1.5 times the width of the femoral neck distal to the intersection of the neck and shaft axes. The structural parameters derived by HSA at the NN and S regions of the hip included bone cross-sectional areas (CSA, cm^3), section modulus (Z, cm^3), outer diameter (OD), estimates of the endocortical or inner diameter (ID) and buckling ratio (BR). Outer diameter is measured as the blur corrected width of the bone mass profile. The bone CSA is the area of bone in the cross-section occupied by bone tissue and was measured from the integral of bone mineral in the profile assuming the average tissue mineralization of adult cortex. The section modulus was derived from the cross-sectional moment of inertia measured from the mass profile, divided by d_{max}. D_{max} is the maximum distance from the profile center of mass to the medial or lateral cortical margin. Resistance to stresses due to axial and bending loads is inversely related to the CSA and section modulus, respectively. Although as measured from DXA data, the section modulus only reflects bending resistance in the plane of the DXA image. The buckling ratio, an index of cortical stability was computed as the ratio of d_{max} to average cortical thickness. Average cortical thickness was estimated by assuming that the cross-section is a circular annulus with 60% and 100% of the bone CSA in the cortical shell for the narrow-neck and shaft respectively [8]. The endocortical diameter is an estimation of the inside diameter of the cortex derived from the annulus model.

The coefficient of variation of HSA geometry using the Hologic QDR4500 has been reported for the narrow neck (NN) to be 3.0% for BMD, 3.0% for CSA, 4.0% for Z, 2.6% for endocortical diameter, and 4.6% for buckling ratio; for shaft (S), 2.2%, 2.3%, 2.5%, 2.8%, 3.5%, respectively [24].

To assess the effects of age and sex on hip geometry phenotypes, we initially computed mean values of each geometry-derived phenotype for men and women aged 20–50 years and aged

50 years and over. These two age groups were selected because individuals in the younger group are typically at peak bone mass, while those in the older group typically experience some degree of bone loss. The effects of age and sex were assessed using analysis of variance. Pearsonian correlations were computed as a measure of the correlations among phenotypes.

Heritability calculation

Genetic effects were estimated simultaneously along with the environmental effects using a pedigree-based likelihood approach [26,27]. Only additive polygenic effects were estimated so that we defined heritability as the proportion of the total trait variance (σ^2_T) attributable to the additive effects of genes (σ^2_A) (i.e., "narrow sense" heritability; $h^2 = \sigma^2_A/\sigma^2_T$). Estimation of the additive genetic heritability follows basic quantitative genetic theory, which models the phenotypic covariances (conditional upon covariate effects) between two individuals in a pedigree as a function of their degree of biologic relatedness. Maximum likelihood methods were used to estimate the values of the parameters (including the heritability) that resulted in highest likelihood obtained across all of the pedigrees. Significance testing was performed by likelihood ratio tests (LRT). This test compares the likelihood of a full model (all covariates and additive genetic effects) with that of a nested model in which the covariate being tested is removed from the model. The likelihood ratio statistic is distributed asymptotically as a χ^2 statistic with one degree of freedom (*df*). All genetic analyses were conducted using the SOLAR software program [28]. Initially , all analyses were adjusted for the effects of sex, age, sex × age, age^2, and sex × age^2. Additional analyses were then carried out with height and weight as covariates to determine if genes influencing variation in hip geometry phenotypes were independent of those influencing variation in body size, and again with BMD as a covariate to determine if genes influencing variation in hip geometry phenotypes were independent of those influencing variation in BMD.

Autosome-wide scan

DNA was isolated from leukocytes using Qiagen Maxipreps (Santa Clarita, CA) for polymerase chain reaction and semi-automated genotyping. A genome scan was performed at the Marshfield Medical Research Foundation (Marshfield, WI) of all 22 autosomes, using 731 highly polymorphic microsatellite markers (Marshfield marker sets 51 and 11, http://research.marshfieldclinic.org/genetics/sets/combo.html) with average spacing of 5.4 cM. For genotyping quality control we used PedCheck [29] to identify genotypes causing Mendel errors and SimWalk2 [30] to identify genotypes causing double recombinants. These genotypes were then removed from further analyses. We estimated genetic distances between markers in our dataset using Crimap [31].

The primary aim of the current analyses was to identify quantitative trait loci (QTLs) that contribute to variation in HSA-derived hip geometry phenotypes in the total AFOS sample (n = 879 individuals). We further hypothesized that QTLs might exist that have greater effects on BMD in men or women, or in younger or older age groups. Thus, secondary analyses were performed in restricted datasets of men only (n = 339), women only (n = 540), participants ≤ 50 yrs old (n = 500) and participants over age 50 (n = 379).

Multipoint genome scans were performed using a variance components method that has been extended for use on multigenerational pedigrees as implemented in SOLAR [26]. Briefly, maximum likelihood methods were used to estimate the genetic variance attributable to the region around a specific genetic marker (σ^2_m) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. Sex, age, sex × age, age^2, and sex × age^2 were included as covariates. We compared the likelihood of the restricted model of no linkage, where the variance due the marker,σ^2_m, is

constrained to 0, to the likelihood of the unconstrained model, where the variance σ^2_m is estimated, to maximize the likelihood. 'True' multipoint IBD probabilities were computed using the Markov Chain Monte Carlo algorithm implemented in Loki [32]. To assess the significance of the multipoint LOD scores for all traits we generated empirical null distributions of nominal LOD scores for each phenotype by simulating 5,000 unlinked markers and then evaluating evidence for linkage to each marker. All LOD scores given in the text are empirically adjusted LOD scores. None of the LOD scores presented are adjusted for the multiple traits (10 hip geometry phenotypes) analyzed.

We estimated the power of our sample to detect QTL effects given our family structures. These results, obtained by simulating QTLs of known effect size and dropping them down through the pedigrees, revealed that we would have 55% power and 71% power to detect QTLs that accounted for 15% and 20% of the phenotypic variation, respectively, at a LOD > 2.

RESULTS

Clinical characteristics

The mean age (±SD) of the 879 subjects included in this analysis was 49.6 (± 15.8) yrs in men and 49.9 (±16.3) yrs in women. On average, women had slightly higher (though not statistically significant) body mass indices (BMI) than men (27.9± 5.9 kg/m^2 vs 26.5 ±4.2 kg/m^2). Approximately 46% of women were post-menopausal. BMD and hip geometry phenotypes obtained from HSA are shown for the neck and shaft regions according to sex and age in Table 1. BMD and geometric measures were higher in men than women, as expected. Mean Z-scores for total hip from DXA were +0.09 ± 0.83 and +0.33 ± 1.11 for men and women, respectively (data not shown). There were significant differences for all hip geometry phenotypes between men and women (Table 1). With increasing age, there were significant changes in hip geometry phenotypes in both sexes, with a progressive decrease in CSA, and a progressive increase in OD and BR in both the narrow neck and shaft regions (p= <0.001 for all) (Table 1). For section modulus, values decreased with increasing age at the narrow neck for men and women. At the shaft, section modulus increased with age for men and decreased for women. The correlations between pairs of hip geometry phenotypes was significant for most comparisons as shown in Table 2. These are unadjusted Pearson correlations.

Heritability of hip geometry phenotypes and hip BMD

The number of relative pairs used for the heritability in linkage analyses is shown in Table 3. Among these relative pairs were 538 parent-offspring pairs, 1,385 sibling pairs, 1,633 avuncular pairs, and 1,095 first cousin pairs. Heritability of BMD and the hip geometry phenotypes are shown in Table 4. After accounting for age and sex, heritabilities for hip geometry phenotypes for NN ranged from 0.40 to 0.63 and for S from 0.49 to 0.94. Additional adjustment for the effects of height and weight altered these estimates very little. With the exception of buckling ratio (BR), heritability estimates were also not substantially altered after further adjustment for the effects of BMD.

Autosome-wide linkage analysis

No strong evidence for linkage (LOD>3.0) was detected in the autosome-wide linkage analysis of the study population as a whole. The highest LOD score was 2.36 for NN_Z on chromosome 1p36 at 43 cM; this region also demonstrated linkage to NN_CSA (LOD=1.88). Two other regions achieved LOD scores > 2.0, both for S_Z on chromosome 1, (LOD=2.07 at 22 cM) and chromosome 9 (LOD=2.08 at 160 cM).

We performed secondary linkage analyses in AFOS subjects stratified by sex and age. Although no strong evidence for linkage was found, these analyses revealed 11 regions with suggestive

linkages with bone geometry phenotypes as shown in Table 5. Of these, 10 linkages had LOD ≥ 2.3 and the eleventh linkage for men and women ≤ 50 years on chromosome 17q11.2-12 for NN_CSA (LOD=2.16) confirmed a previously reported linkage performed in a population similar to ours composed of Caucasian nuclear families [20].

DISCUSSION

Our study found that hip geometric phenotypes derived from hip structural analysis (HSA) are highly heritable, with genes accounting for 40–84% of the residual variation in these phenotypes after accounting for the effects of age and sex. These heritabilities were found to be largely independent of BMD suggesting distinct genetic influences on these fracture risk factor traits. We found suggestive evidence of linkage to chromosome 17q11.2–12 for NN_CSA for men and women age 50 years and below, confirming a previous linkage reported by Xiong (20) for cortical thickness, a trait that they found highly correlated (0.9) to CSA. A candidate gene within this region is sclerostin (*SOST*), the gene that is abnormal in the sclerosing bone disorder sclerosteosis [33].

In addition, suggestive evidence for linkage (LOD ≥ 2.3) was found at 10 other sites on chromosomes 1, 2, 5, 6, 11, 12, 14 and 15. Although none of these suggestive linkages confirmed previous reports, five linkages (at 1p35.5, 1p36, 2p11.2, 4q11 and 6q22–23) were reported in the Framingham population with different but related phenotypes [21,22]. For example, at 1p35.5, our linkage was with NN_ID whereas the previous report was for NN_OD. Although not identical, these phenotypes are related in that NN_OD is composed of NN_ID plus 2 times cortical thickness [21]. Similarly, our linkage at 4q11 was for S_Z, whereas the previous report was for NN_Z [21], both related to bending strength but at different areas of the proximal femur. Among our suggestive linkages reported here, those at chromosomes 1p36 and 14q23 were previously reported by us as being suggestive regions of linkage for femoral neck BMD [24]. However, in our previous report, the 1p36 linkage was found only in women, whereas in the current study, our findings are for men. Our suggestive linkage at 14q23 to NN_Z was found for men but previously was found for BMD for men and women age 50 years and under [4]. The paucity of confirmatory linkages among studies may reflect the relatively low power in individual studies, differences in study design or genetic heterogeneity [34].

Linkage analysis in our population as a whole yielded 2 suggestive linkages with LOD scores ≥ 2.3 (at 1p36 and 4q11). In subgroup analysis, 8 additional suggestive linkages were found, 2 for women only (6q22–23, 11q22.1), 2 for those > 50 (2p11.2, 12q24.2) and 4 for those ≤ 50 (1p35.5, 1q23–23, 2p25, 5q24–30). Finding different linkages in subgroups suggests that different genes may affect hip geometric phenotypes in men vs women and at peak bone mass vs those in the bone loss phase of life. Evidence for sex-specific QTLs related to hip geometric phenotypes has been found in other studies as well, but at different locations than we found. In linkage studies on sister pairs and brother pairs from Indiana, three out of seven suggestive autosomal linkages to hip structural phenotypes (at 2p, 5p and 4p) were male-specific (14). In addition, in a large population of nuclear families of European descent living in Nebraska, suggestive or significant evidence for QTLs related to hip structural phenotypes was found to be male-specific at one site (7q21) and female-specific at 4 sites (2p14, 3q26, 15q21, 3q26,) (20). Of linkage studies reported to date on hip structural phenotypes, we are aware of only one that has reported linkages that reached genome-wide significance, for regions of 20q12 and Xq25 (for cortical thickness, LODs 4.28 and 3.90 respectively) (20). It is notable that this study included a huge number (3998) of participants (20). Since none of our linkages reached genome-wide significance, confirmation will be required to determine their importance.

We found the greatest similarities with our linkage results when compared to results reported from the Framingham study, as described above. One reason for this could be that the

Framingham hip geometric phenotypes were obtained using the same HSA program that we used. Two additional groups have generated hip geometry phenotypes using methods different from HSA, hip radiographs in one (17) and DXA-derived phenotypes but not the same HSA analysis as used here in the other (19). There was no overlap between linkages detected from these populations and those detected in ours. Methodological differences are one possible explanation for the differences in linkage results.

Data derived from Hip Structural Analysis (HSA) provide different information about hip strength than BMD and could help to explain why some patients with low trauma hip fractures have normal BMD. Previous studies have shown that hip structural phenotypes vary with race, age, gender, body weight and activity and that these changes are not necessarily concordant with changes in BMD [7,22,35–38]. As found in other studies, men in our study had higher values for bone geometry measures than women except for buckling ratio which was higher in women, consistent with greater strength in male femurs. In addition, with age in both men and women, BR increased with age whereas Z, CSA, ID and OD decreased in the narrow neck. The effects of a change in geometry phenotype with age can be to decrease the deleterious reduction in BMD. For example, the relatively small reductions in section modulus with age, compared to larger BMD decreases, show that hip strength is more stable with age than BMD alone would indicate [8]. This, in turn, may partly explain why hip fracture is less universal in the elderly than reductions in BMD. Thus, variability in HSA phenotypes with age may explain some of the fracture risk that cannot be attributed to low BMD.

Previous studies have shown that hip geometry-derived measures of bone strength are associated with fracture risk and that this association is at least partly independent of differences in BMD [9,10,13]. Our data showed in the Amish support this. Previous data from HSA have shown that military recruits who suffered tibial stress fractures during basic training had reductions in section modulus and bone cross-sectional areas (CSA), relative to body mass, compared to those without fractures [4]. Our data revealing that section modulus and CSA are highly heritable could help to explain in part why these measures were lower in some recruits than in others.

We have previously observed the hip fracture rate to be lower in the Amish compared to non-Amish Caucasians [23]. It is possible that the Amish, perhaps by virtue of their active lifestyle, might also have a more favorable profile for other geometry-derived parameters of hip strength than the non-Amish. However, comparisons of these hip strength parameters across studies are difficult since HSA analysis is sensitive to the procedures used to measure BMD by DXA. A previous report has shown, however, that physical exercise increases hip CSA and section modulus [39]. We speculate that beneficial effects from high physical activity on geometric measures of hip strength in the Amish could be a contributing factor to their reduced risk of hip fracture.

In summary, we have found high heritability of hip geometry measured by hip structural analysis in the Amish and found suggestive evidence for QTLs affecting proximal femur geometric measures of strength in 11 different chromosomal locations. Our linkage on chromosome 17q, confirms a previous report. The 10 additional linkages showed very modest evidence for QTLs affecting geometric hip strength measures on chromosomes 1, 2, 5, 6, 11, 12, 14 and 15. Continuing to study the genetics of these geometric phenotypes related to bone strength might further our understanding of the heredity of osteoporosis and hip fracture.

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Table 1

Mean values (± s.e.) of BMD and hip geometry variables according to age and sex.

	Men		Women			
	20–50 (n = 199)	**> 50 (n = 140)**	**20–50 (n = 301)**	**>50 (n = 239)**	**Sex adjusted p-value[1]**	**Ageadjusted p-value[1]**
Age	38.3 ± 0.5	65.7 ± 0.8	37.6 ± 0.5	65.4 ± 0.6		
Height	67.6 ± 0.2	66.2 ± 0.2	63.5 ± 0.1	61.7 ± 0.2	< 0.001	< 0.001
Weight	168.4 ± 2.0	170.1 ± 2.6	155.7 ± 1.9	155.9 ± 2.3	0.46	< 0.001
Neck						
BMD (g/cm^2)	0.833 ± 0.008	0.729 ±0.011	0.843 ±0.007	0.678 ± 0.010	< 0.001	0.005
CSA	2.744 ± 0.027	2.466 ±0.033	2.345 ±0.020	1.948 ±0.026	< 0.001	< 0.001
Z	1.522 ± 0.018	1.374 ±0.023	1.105 ±0.011	0.915 ±0.014	<0.001	< 0.001
BR	11.49 ± 0.16	13.94 ± 0.27	9.56 ± 0.12	13.09 ± 0.25	< 0.001	< 0.001
ID	3.151 ± 0.019	3.294 ± 0.023	2.607 ±0.014	2.778 ± 0.016	< 0.001	< 0.001
OD	3.469 ± 0.018	3.569 ± 0.021	2.925 ±0.012	3.037 ± 0.014	< 0.001	< 0.001
Shaft						
BMD (g/cm^2)	1.369 ± 0.011	1.336 ± 0.019	1.170 ±0.008	1.059 ± 0.012	< 0.001	< 0.001
CSA	4.184 ± 0.034	4.215 ± 0.055	3.191 ±0.024	2.993 ±0.034	0.48	< 0.001
Z	2.443 ± 0.028	2.563 ± 0.036	1.684 ±0.015	1.694 ±0.018	<0.001	< 0.001
BR	3.414 ± 0.056	3.663 ± 0.093	3.580 ±0.035	4.354 ±0.080	< 0.001	< 0.001
ID	2.233 ± 0.025	2.391 ±0.054	2.027 ±0.013	2.243 ±0.020	< 0.001	< 0.001
OD	3.218 ± 0.019	3.345 ± 0.045	2.864 ±0.010	2.982 ±0.013	< 0.001	< 0.001

[1] Age effect; 2 Sex effect; BMD = bone mineral density; CSA = bone cross-sectional area; Z = section modulus; BR = buckling ratio; ID = inner diameter; OD = outer diameter;

Table 2

Pearsonian correlations (and p-values) among hip geometry phenotypes

	NN_BMD	NN_CSA	NN_Z	NN_BR	NN_ID	NN_OD	S_BMD	S_CSA	S_Z	S_BR	S_ID	S_OD
NN_BMD	1	0.84 <.0001	0.60 <.0001	−0.87 <.0001	−0.36 <.0001	−0.20 <.0001	0.61 <.0001	0.47 <.0001	0.23 <.0001	−0.58 <.0001	−0.41 <.0001	−0.13 0.0005
NN_CSA		1	0.91 <.0001	−0.55 <.0001	0.20 <.0001	0.35 <.0001	0.78 <.0001	0.76 <.0001	0.59 <.0001	−0.55 <.0001	−0.21 <.0001	0.19 <.0001
NN_Z			1	−0.28 <.0001	0.47 <.0001	0.60 <.0001	0.75 <.0001	0.81 <.0001	0.69 <.0001	−0.44 <.0001	−0.06 0.09	0.34 <.0001
NN_BR				1	0.65 <.0001	0.54 <.0001	−0.45 <.0001	−0.24 <.0001	−00.02 0.62	0.63 <.0001	0.51 <.0001	0.33 <.0001
NN_ID					1	0.99 <.0001	0.22 <.0001	0.46 <.0001	0.61 <.0001	0.11 <.0001	0.42 <.0001	0.59 <.0001
NN_OD						1	0.34 <.0001	0.57 <.0001	0.68 <.0001	0.04 0.22	0.38 <.0001	0.60 <.0001
S_BMD							1	0.90 <.0001	0.61 <.0001	−0.79 <.0001	−0.44 <.0001	0.06 0.11
S_CSA								1	0.89 <.0001	−0.51 <.0001	−0.002 0.95	0.48 <.0001
S_Z									1	−0.14 0.0001	0.43 <.0001	0.81 <.0001
S_BR										1	0.76 <.0001	0.43 <.0001
S_ID											1	0.87 <.0001
S_OD												1

BMD = bone mineral density; CSA = bone cross-sectional area; Z = section modulus; BR = buckling ratio; ID = inner diameter; OD = outer diameter;

Table 3

Number of relative pairs in study sample

5491	Unrelated
879	Self
538	Parent-offspring
1385	Siblings
57	Grandparent-grandchild
1633	Avuncular
118	Grand avuncular
1095	1st cousins
185	1st cousins, 1 rem
1	2nd cousins
44	Double 1st cousins

Table 4

Heritability of hip geometry phenotypes (mean ± s.e.), unadjusted and adjusted for BMD

	Heritability*	Heritability(+ BMD-adjusted)
Narrow Neck (N)		
BMD	0.62 ± 0.07	—
CSA	0.50 ± 0.07	0.54 ± 0.07
Z	0.40 ± 0.07	0.45 ± 0.08
BR	0.58 ± 0.06	0.37 ± 0.08
ID	0.63 ± 0.06	0.57 ± 0.07
OD	0.59 ± 0.07	0.54 ± 0.07
Shaft (S)		
BMD	0.56 ± 0.08	—
CSA	0.59 ± 0.07	0.74 ± 0.08
Z	0.61 ± 0.07	0.64 ± 0.05
BR	0.49 ± 0.08	0.54 ± 0.09
ID	0.84 ± 0.07	0.92 ± 0.05
OD	0.94 ± 0.05	0.92 ± 0.05

* adjusted for the effects of sex, age, age^2, age × sex, and age^2 × sex.

BMD = bone mineral density; CSA = bone cross-sectional area; Z = section modulus; BR = buckling ratio; ID =inner diameter (endocortical diameter); OD = outer diameter

Table 5

All LOD scores of 2.3 and above obtained from entire group and subgroup analysis by sex (n = 336 men, 546 women) and age (n = 498 ≤ 50 yrs, n = 384 > 50 yrs) and LOD scores <2.3 at sites reported previously.

Chrom Location	Distance (cM)	Geom Pheno.	Peak LOD In AFOS	AFOS population	Flanking Marshfield Marker	Supporting data			Candidate genes
						Population	Distance	Ref	
1p35.5	46	NN_ID	2.57	≤ 50	GATA29A05 ATA008				*ALKPL*
		NN_OD				M F	66	21	
1p36	43	NN_Z	2.36	ALL	GATA27E01 TTTA063				*BMND 3, MTHFR*
			2.43	M					
		NN_OD				M, F	43	32	
1q23-24	96	S_BR	2.65	≤ 50	TATC028 AGAT118				*SAC, Hypercalciuria*
2p11.2	104	NN_CSA	1.92	> 50	GATA181G08 GATA69E12				
		S_CSA				M, F	80	32	
		S_Z				M, F		32	
2p25	17	NN_ID	2.37	≤ 50	GATA116B01 GAAT1A5				
4q11	147	S_Z	1.94	ALL	ATA26B08 GATA135C03				
		NN_Z				M, F	124	21	
5q24–30	151	S_ID	2.59	≤ 50	AGAT129 GATA51D11				*LOX*
6q22–23	170	NN_CSA	2.46	F	GATA184A08 TATC050Z				*COL10A1, ENPP-1, WISP3*
		S_CSA				M,F	181	32	
11q22.1	104	S_CSA	2.3	F	TCTA025 GATA35				
12q24.2	96	S_BR	2.63	> 50	GATA26D02 AGAT114				
14q23	68	NN_Z	2.5	M	ATA069 GATA169E06				
17q11.2-12	59	NN_CSA	2.16	≤ 50	GGAA9D03 GATA169F02	M, F	55	20	*SOST*

ALKPL (alkaline phosphatase), *BMND3* (bone mineral density quantitative locus), *MTHFR* (methylene tetrahydrofolate reductase), *SAC* (soluble adenyl cyclase), *LOX* (lysyl oxidase), *COL10A1*(type X collagen alpha polypeptide), *ENPP-1* (ectonucleotide pyrophosphatase/phosphodiesterase 1, also called alkaline phosphodiesterase 1), *WISP3* (wnt-1 inducibel signaling pathway protein 3), *SOST* (sclerostin)