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A Genome Wide Linkage Scan of Metacarpal Size and Geometry in the Framingham Study

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Abstract

Bone geometry is a significant component of bone strength, and has a clinical utility in predicting fractures and quantifying bone loss. Bone geometry is known to have a substantial genetic component. We performed linkage analysis to identify chromosomal regions governing metacarpal bone geometry.

A genome-wide scan (with a set of 615 markers with spacing of ~5.7 cM) was performed on 1,702 individuals from 330 extended families of the Framingham study. Midshaft width was measured and several indices calculated, namely Metacarpal Cortical Thickness (MCT), Cortical Index (MCI), and Section Modulus (MZ), using digitized x-rays of 1,380 participants (men, n= 666, mean age 55.2 yrs, women, n=714, 55.5 yrs). Metacarpals 2, 3 and 4 were averaged.

Heritability was significant for all indices, ranging from 0.51 to 0.72. Linkage analysis of indices adjusted for age, age², and estrogen status in women, identified chromosomal regions 6p21, 9p21, 11q21-q22, and Xq26-Xq27, with LOD scores >2.0. Additional adjustment for smoking, height, and BMI, generally reduced the LOD scores. Finally, bivariate linkage analysis confirmed that a QTL on chr. 6 (51 cM) was shared by midshaft width and MZ (LOD = 2.40, adjusted for all covariates). Neither MCT nor MCI shared linkage loci with width or MZ.

In conclusion, we have identified chromosomal regions potentially linked to bone geometry. Genes in these regions may regulate bone geometry via effects on body size. Identification and subsequent characterization of loci for bone geometry can further elucidate the genetic contributions to bone's resistance to stress.

Introduction

Osteoporotic fractures and their consequences in the elderly population greatly increase mortality, morbidity, and negatively impact the quality of life (Melton and others 1997). Fractures occur due to compromised bone strength, which becomes insufficient over time to withstand forces on the bone. Evidence for bone geometry's contribution to bone strength has been shown in numerous studies. For example, metacarpal cortical index (MCI), a bone geometry measure, has been utilized in epidemiological research as well as in primary care to identify patients with osteoporosis (Haara and others 2003). MCI and other measures of bone

geometry have contributed to the understanding of various disease risks (Fox and others 1986; Livshits and others 1998; Plato and others 1982; Plato and Purifoy 1982; Zhang and others 1997). Radiogrammetry of the metacarpals, particularly the second metacarpal, has long been used as a standard to obtain measures of bone geometry to assess skeletal health in both clinical and anthropological realms (Barnett and Nordin 1960; Lazenby 1998). It is widely accessible and inexpensive in comparison to other more sophisticated bone assessment techniques.

The demonstration that metacarpal bone geometry predicts fracture along with the availability of hand radiographs in large epidemiologic samples makes it an attractive bone phenotype of genetic studies. Several studies have provided evidence of a heritable component of bone geometry using metacarpal radiographic indices (Duren and others 2007; Gong and others 1999; Karasik and others 2000b; Skaric-Juric and Rudan 1997).

In order to objectively pursue the genetic components of bone geometry, we have examined the quantitative geometric indices of metacarpal bones 2 to 4. The aim of this project was to determine heritability of these components followed by linkage analysis to identify physical chromosomal locations of genes governing metacarpal bone dimensions and cross-sectional indices. By utilizing the large cohorts of the Framingham population, we may effectively be able to narrow down chromosomal regions in which genes governing bone geometry may reside.

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 (Demissie and others 2002; Karasik and others 2004).

In the Framingham Original Cohort hand radiographs were obtained in 1967–1969. As part of a study investigating the heritability of osteoarthritis, participants of the Framingham Offspring Cohort who had at least one parent (member of the Original Cohort) with hand radiographs, were radiographed using the same techniques in 1993–1995 (Demissie and others 2002; Felson and others 1998). This study utilized the right-hand radiographs from both the Original and Offspring Cohorts.

The study was approved by the Institutional Review Boards for Human Subjects Research of Boston University and of Hebrew Rehabilitation Center for Aged.

Metacarpal Measurements (Radiogrammetry)—Hand radiographs were digitized at 450dpi resolution using a *COBRAscan* 612 SL Digitizer and Windows' Imaging Software to acquire an image with a pixel size of 0.12 mm and 1024 gray levels. For future scale reference a label with a pre-printed millimeter scaled ruler was placed on a transparent portion of each hand radiograph prior to digitization. A custom-written script developed for *ImageTool* software (UTHSCSA, San Antonio, TX) was used to semi-automatically obtain metacarpal

measurements from the digitized images. Measurements of the second, third and fourth metacarpals (Met2, Met3 and Met4, respectively), were obtained by outlining the contour of the digitized metacarpal bones of interest on a flat-screen monitor with the use of a computer mouse. The algorithm implemented in the software then determined coordinates of outlined bone, generated a mass profile, and converted the data into the cortical macrostructure, calculating, among several geometric properties, metacarpal length (L), width in the middle of bone diaphysis/midshaft (or periosteal width, D50), and midshaft medullary cavity width (endosteal width, d50) (Livshits and others 2003). It should be noted, that for Met2, D50 and d50 were calculated as an average of widths at 40 and 60 percent of bone length, due to permanent dark markings on the original x-rays at the middiaphysis, as a result of measuring the same radiographic images with digital calipers in previous studies (Kiel and others 2001).

Most of the measurements in each cohort were generated by one reader, after ensuring intra- and inter-reader reliability. Coefficients of variation (calculated as root-mean-square average (Gluer and others 1995) ranged from 1.0 % (Met3 length) to 1.95 (Met2 D50) to 7.0 % (Met2 d50); the latter most probably being a result of extrapolating d50 as an average of d40 and d60. Geometric indices of cortical structure were calculated as previously suggested (Garn and others 1967; Karasik and others 2000a; Plato and others 1982) using the basic metacarpal measurements obtained from the *ImageTool* software, as follows:

1. **Metacarpal cortical thickness** (MCT, mm)= D50-d50
2. **Metacarpal cortical index** (MCI, %)= (D50-d50)/D50 *100
3. **Metacarpal Section Modulus** (MZ, mm³) = $\pi(\frac{1}{2} D50^4 - \frac{1}{2} d50^4) / D50$

Indices of the metacarpals 2, 3 and 4 were averaged (as suggested by Karasik and others 2000b); this also allowed avoiding the impact of the low precision of d50 at metacarpal 2. Length of the bone (L) and width in the middle of its diaphysis (D50) were studied solely for a comparison with the indices, to control for genetic influences on bone size rather than cortical structure. Therefore, together with metacarpal length and midshaft width, there were 5 phenotypes.

Other measurements—Information on age, sex, weight and height, were obtained for each individual at the time of the x-ray. 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/m². Cigarette smoking was assessed at each examination as number of cigarettes/day, and used in this study as current, former, or non-smoker at time of x-ray. For women, estrogen use and menopausal status were recorded. Each woman was assigned to one of the two “estrogenic status” groups: 1) premenopausal or postmenopausal on estrogen (estrogen-replete) or 2) postmenopausal not on estrogen (estrogen-deplete), where menopause was defined as having no menstrual period for at least one year.

Genome scan

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 (Yuan and others 1997)). 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, 94 additional markers genotyped on these 330 largest pedigrees, used to augment the original genome scan, were 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 (Levy and others 2000).

Finally, members of the 323 families with family sizes ranging from 2 to 30 genotyped individuals contributed to the linkage analyses. These pedigrees are mostly nuclear (with an average of 2.4 family members and small proportion of extended families, with 2 to 6 persons). Out of a total of 1702 genotyped Framingham participants, 1412 had metacarpal measurements. Out of these, 32 participants did not consent to genetic analyses, leaving the 1380 family members analyzed in the study. These were: men, $n=666$, women, $n=714$. The sample with genotyping and bone geometric phenotypes included the following relative pairs: 907 parent-offspring pairs, 716 sibling pairs, 453 cousin pairs, and 541 avuncular pairs.

Statistical methods

Prior to heritability and linkage analyses, multivariable regression analysis was performed in each sex (men and women) and Cohort (Original and Offspring) in order to obtain residual bone geometry phenotypes, adjusted for age, age² and estrogen status in women (Model 1) and additionally, for smoking, height, and BMI (Model 2). Combination of height and BMI simultaneously adjusts for body size and body composition (Michels and others 1998), and is widely used in epidemiological studies.

Variance component analysis (VCA): univariate—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, using adjustment Models 1 and 2 as above.

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 (Heath 1997). For chromosome X, IBD probabilities were computed using the *minx* subroutine of MERLIN (Abecasis and others 2002), which can perform multipoint linkage analysis on chromosome X. Because this program is not able to handle extended pedigrees, such pedigrees were broken down into smaller ones, by splitting families and/or deleting family members while keeping as many members with genotypes as possible. 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 (Kong and others 2002). 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 (Blangero and others 2000), 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 (Almasy and Blangero 1998; Blangero and others 2000). 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)(Goring and others 2001).

Variance component analysis: bivariate—To test the hypothesis that QTLs jointly influence variation in measures of bone geometry, we performed genome-wide bivariate linkage analyses for pairs of traits characterized by a significant additive genetic correlation (ρ_Q , $p < 0.0001$). The bivariate method that we employed is a simple extension of the univariate maximum likelihood-based VCA described above. 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 non-additive genetic factors). (Almasy and others 1997) We determined significance of the above 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 zero (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 (Almasy and others 1997; Havill and others 2006; Karasik and others 2007; Williams and others 1999).

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 geometry or related traits.

Results

Table 1 displays descriptive statistics of the radiographed study participants, by Cohort and sex. 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 compared with females. Of the total 1027 women, there were 606 estrogen-replete women (mostly from the Offspring Cohort). Thirty five percent of men and 33% of the women in the Original Cohort were currently smoking, whereas fewer Offspring Cohort members were currently smoking.

Table 2 provides estimates of heritability by the variance component analysis, which revealed a strong additive genetic component for all geometric measures, with age- and age²-adjusted h^2 values between 0.51 (MCT) and 0.82 (L). Additional adjustment for smoking, height and BMI (Model 2) apparently reduced the h^2 estimates for L and MCI, but did not meaningfully change heritability of MCT and MZ.

Selected results of linkage analyses of phenotypes adjusted for age, age², and estrogen in women (model 1) are displayed in Table 3a (two- and/or multipoint LOD ≥ 1.9). Multipoint LOD score >3.0 was obtained for midshaft width at chr. 9 (55 cM); this locus was also shared with the MZ, although with a lower LOD score (2.49). Another multipoint linkage (with LOD scores >2.0) was observed for the same two traits at chr. 11 (106 cM). On chromosome X, a long region from 143 to 164 cM provided suggestive evidence of linkage with MZ (LOD >2.77) and with the midshaft width (LODs >2.0). Modest LOD scores were observed also on chr. 6 (multipoint, at 51 cM) with width and on chr. 7 (two-point, at 149 cM) with MCI. Two-point signals in general conformed to the multipoint peaks. From this Table it is obvious that the

MCT and MCI did not share linkage loci with periosteal width and section modulus. Moreover, it is notable that metacarpal length has its own peak, not shared with the bone width and cross-sectional indices, on chr. 9 (121-144 cM), with a multipoint LOD of 1.91 in Model 1 (not shown in the Table 3a).

Adjustment for smoking, height and BMI, resulted generally in much lower LOD scores. Thus, multipoint LOD scores for midshaft width and MZ on chr. 9 (55 cM) became ~ 2.0 , decreased on chromosome X (143 to 164 cM) for MZ, and fell dramatically at chr. 11 (101-106 cM). LOD scores on chr. 7 (149 cM) with MCI and chr. X with width became very low. However, in contrast, the linkage signal for metacarpal length on chr. 9 (129 cM), increased after adjustment for smoking, height and BMI, from 1.91 to 2.84 (not shown in the Table 3b).

Since there were common linkage peaks for some of the indices, we explored the correlations among them. As shown in Table 4, there were high phenotypic correlations as well as both genetic and environmental correlations between the midshaft width and MZ ($\rho_G = 0.95$, $\rho_E = 0.88$, $p < 0.001$) and between MCT and MCI ($\rho_G = 0.91$, $\rho_E = 0.93$, $p < 0.001$). We therefore performed bivariate linkage analyses for these pairs of traits and found that the QTL on chr. 6 (51 cM) with a combination midshaft width / MZ had a LOD score 2.40 (adjusted for all covariates in Model 2; Figure 1). There was no LOD score higher than 1.5 for the combination MCT / MCI.

Discussion

In this study of metacarpal size and cross-sectional cortex adjusted for age, age², and estrogen status in women, we have shown a high heritability for all five geometrical indices studied. Further, genome-wide linkage analysis of metacarpal geometry traits identified chromosomal regions 6p21, 9p21, 11q21-q22, and Xq26-Xq27, with suggestive LOD scores (>2.0). Additional adjustment for covariates that included smoking, height, and BMI, generally reduced the LOD scores; however, periosteal bone width and midshaft section modulus (MZ) still had LOD scores >2.0 . Finally, bivariate linkage analysis confirmed that a QTL on chromosome 6 (51 cM) was shared by both width and MZ (LOD = 2.40, adjusted for all covariates). Neither metacarpal cortical thickness nor metacarpal cortical index shared linkage loci with bone width or MZ.

In this genome-wide linkage study, we focused on the genetic contribution to bone geometry, an important player in bone strength. Our choice of metacarpals was based on the historic utility of metacarpal indices as proxies in diagnosis of osteoporosis (Garn and others 1967; Haara and others 2003). Also, radiogrammetry of the metacarpals is widely accessible and inexpensive in comparison to other more recently emerged sophisticated bone assessment techniques (Barnett and Nordin 1960; Lazenby 1998). Furthermore, evidence for the role of genetics in metacarpal bone geometry has been well documented in adults (Cohen and others 2003; Karasik and others 2000b; Livshits and others 2003), and recent publications have provided evidence of significant genetic influence over bone mass in children (Duren and others 2007). Thus it is likely that there is a significant genetic influence on bone geometry throughout the lifespan. Indeed, heritability testing for all studied metacarpal geometry traits indicated a significant genetic component, ranging from 51% (MCT) and 68% (width), to as high as 82% for bone length. This latter finding is not surprising, knowing that metacarpal length is highly correlated with body stature (Kimura 1992), which in turn is highly heritable (89% in the Framingham sample (Karasik and others 2007)). As expected, adjustment for covariates including height and BMI reduced the heritability estimates for metacarpal length to 76%, but did not affect the other metacarpal indices (h^2 of MCI apparently decreased from 61 to 59%).

In this study, we have analyzed linear geometric indices of bone size (length and width) as well as cross-sectional cortical geometry indices representative of the area in which a long bone is likely to fracture, namely the midshaft (mid-diaphysis) region; our measure of midshaft width is equivalent to the periosteal diameter. We used MCT in this area that is a direct measure of cortical envelope thickness, as well as MCI, which measures cortical bone as a percentage of total bone in a one-dimensional space. Section modulus, MZ, takes into account both the cross-sectional moment of inertia and diameter of bone, and as such is an indicator of resistance to bending. This latter index is especially important when considering tubular structures (such as long bone filled with a marrow cavity as opposed to a solid bone), which can resist bending due to the optimal spatial distribution of their material. An increase in periosteal diameter with a concurrent decrease in cortical thickness will increase the bending strength even further (Duan and others 2003). Notable, these indices were calculated using the “circular ring model” assumption, from the midshaft endosteal and periosteal diameters.

Results of our analyses point out several chromosomal regions with some indication of linkage. Midshaft periosteal width shared a locus with section modulus (MZ) at chr. 9 (55 cM), with multipoint LOD scores 3.42 and 2.49, respectively. The degree of excess allele sharing at marker D9S922 indicates that a genetic variant governing both bone width and MZ may reside around this locus. Another linkage signal (with multipoint LOD scores >2.0) was observed for the same two traits at chr. 11 (around 106 cM). There was a long QTL at Xq26.1-Xq27.3, where MZ (less strongly, bone width) was linked, with multipoint LOD scores up to 2.97. Modest linkage signals were on chr. 6 (51 cM), with midshaft width, and on chr. 7 (149 cM), with MCI. It is notable that although all four traits are derived from endosteal and periosteal midshaft width, the MCT and MCI did not share linkage loci with either bone width or section modulus, suggesting that bending strength is governed by a set of unique genes. Similarly, metacarpal length has its individual peak, not shared with the bone width and cross-sectional indices, on chr. 9 (121-144 cM). Of note, in our linkage analysis of femoral geometry in the same sample (Demissie and others 2007), we found linkage of femoral narrow neck cross-sectional area near the same locus (LOD = 2.49 at 128 cM). When adjusted for covariates including height and BMI, the linkage signal for metacarpal length here increased from LOD=1.91 to 2.84, suggesting that this locus may govern length of the metacarpal bone independent of body size. This is especially intriguing since we recently found linkage to BMI at this locus (Karasik and others 2007); this may indicate that gene(s) for both bone length and body size reside in the region 9q31-q32.

Linkage of the remaining traits, however, may be in some part due to the effects on body size; thus, LOD scores for midshaft width and MZ at chr. 11 (106 cM) fell dramatically after the adjustment for covariates including height and BMI; the same was true for the signals on chromosome X for MZ and bone width. Modest multipoint LOD scores on chr. 7 (149 cM) with MCI decreased even further. This finding points out a possibility of a shared genetic regulation between metacarpal width and cortical bending strength with body size. For example, 11q22-q24 showed evidence of linkage with body size traits (BMI and percentage body fat) in several studies, including the Framingham Heart Study sample, Pima Indians, and Nigerians (for details, see (Baier and Hanson 2004)). It has been previously reported that metacarpal length, the measure of bone size, is highly correlated with body size, especially height (Kimura 1992). We therefore performed an additional analysis of the cross-sectional cortical indices, by using metacarpal length as a covariate (in addition to the Model 2 covariates). However, this adjustment for bone size, generally, did not change the results for these indices: heritability estimates stayed the same, as well as the majority of LOD scores in Table 3b. This finding confirms the results of the recent-most QTL mapping for the lengths of the bones in a mouse intercross (Norgard 2008). In particular, the region homologous to 11q22-24 was identified in mice, which harbors approximately 134 positional candidate genes (Norgard 2008), therefore, there is a need of an extensive fine-mapping here.

Despite generally low significance of the observed linkage, it should be noted that this study is the first genome-wide linkage analysis of metacarpal size and cross-sectional indices. The one previous linkage study of the same phenotypes was restricted to a chromosome 11q region (Cohen and others 2003). There have also been recent studies in animals (sheep (Beraldi and others 2007) and deer (Maqbool and others 2007)), although limited to metacarpal length only. Therefore, the genome regions identified in this study deserve some attention. Indeed, the QTL shared by bone width and section modulus on chromosome 6p21.3 has been shown to be associated with susceptibility for Paget's disease of bone. The region at chr. 9p21 around marker D9S1121 (49 cM), which is linked with midshaft periosteal width and section modulus, was implicated in the autosomal dominant progressive limb-girdle myopathy with bone fragility. In a recent study, this disease was linked to the above marker and the ensuing haplotype analysis mapped the putative disease gene within a 15 Mb interval at chr. 9p21 (Watts and others 2005). This region also localizes diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH). An involvement of these disease genes in the normal variation in bone geometry is not obvious, but tantalizing and requires further study.

Our linkages on chromosome X deserve special attention. Our linkage peak at Xq26-Xq27 with MZ (less with width), spanning from 129,387,868 to 142,768,992 bp, corresponded to linkage findings of femoral neck cortical thickness reported by others (Xiong and others 2006)). (Also, the same group, Xiao and others (2006) achieved a linkage for wrist BMD at Xq27). There are some potential candidate genes of interest in the identified chromosomal region, including fibroblast growth factor 13 (Lovec and others 1997), and two rare skeletal dysplasia syndromes have been mapped to Xq27 (Dlouhy and others 1987; Zhang and others 2000) but other unknown genes may also reside here. There also is a promising overlap between regions identified in this study and findings in animal models, including femoral cross-sectional area in a large genetically heterogeneous population of mice. (Klein and others 2002) In particular, Klein et al. (2002) found linkages on mouse chromosome X (syntenic with Xq26-Xq27), where MZ and the width, respectively, were linked in our sample. Similarly, Masinde et al. (Masinde and others 2003) found linkage of murine periosteal circumference with DXMit208, which again corresponds to Xq26 in humans. This long chromosomal region thus deserves more attention for follow-up (fine-mapping) studies. However, given that our analyses did not take the possible X inactivation into a consideration, we interpret the results on chromosome X with caution.

A plethora of factors regulate bone's ability to withstand the loads and to redistribute the mass in accordance with new demands, including age, applied stress, sex hormones, and behavioral factors, such as exercise and smoking. We did not have measures of physical activity that involved hand bones available for the Framingham Original Cohort measured in the late 1960s; however, we consistently adjusted our analyses for smoking and estrogen history in women. It is well known that increased bone loss after menopause is correlated with increased periosteal apposition, thus the use of hormone therapy can alter both processes, in turn affecting bone width and MZ. Although environmental factors are important, the genetic component for age-related bone change is substantial and thus is important for understanding the underlying processes of endosteal bone loss and periosteal apposition.

In this study, we did not perform correction for the overall false positive rate (Feingold and others 1993) and multiple testing (Camp and Farnham 2001). Our linkage peaks are relatively low using the criteria discussed in detail in Lander and Kruglyak (Lander and Kruglyak 1995) for the autosomes; additional adjustment for multiple testing would have further decreased the significance of the linkage signal. There are also indications that threshold for significance for the X-chromosome should be higher (Nyholt 2000). We therefore consider the linkage peaks reported in this study as "suggestive" and hypothesis-generating rather than providing decisive indication of the QTLs in the chromosomal regions listed above.

In summary, several chromosomal regions are suggested to host the genes regulating metacarpal bone size and cross-sectional geometry, including 6p21, 9p21, 11q21-q22, and Xq26-Xq27. Genes in some of these regions (most obviously 11q21-q22) seem to regulate bone geometry via effects on body size. This study contributes to the ongoing research that focuses on discovery of genes involved in the determination of bone geometry, an important component of bone strength. The identification of genes involved in the determination of bone geometry may lead to a better understanding of the genetics of modeling and remodeling of the skeleton at various stages of life. Ultimately, elucidation of candidate genes for these processes can provide insight to the complexities of long bone fracture and pave the way for future research of therapeutic targets in osteoporosis fracture prevention.

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Figure 1. Univariate and Bivariate linkage results (Multipoint LOD scores), Chromosome 6

Horizontal line: "suggestive" linkage threshold (LOD = 1.9)

MZ_ave – metacarpal section modulus (average of 3 metacarpal bones)

D_outer_ave – metacarpal midshaft width (average of 3 metacarpal bones)

MZ_ave & D_outer_ave – a combination of the above.

Table 1

Characteristics of the studied sample, by Cohort and gender*.

Original cohort

	Male (N=290)	Female (N=326)
Age (yrs)	58.6±7.5	57.8±7.2
Weight (lb)	173.7±27.4	143.7±21.9
Height (inch)	67.2±2.7	62.0±2.4
BMI (kg/m ²)	27.0±3.6	26.3±4.1
current smoking	35.2%	33.1%
estrogen-replete**	-	21.8%
Length (mm)	66.2±3.5	60.4±3.7
Width (mm)	9.0±0.6	7.6±0.5
MCI (%)	41.2±6.7	40.7±8.8
MCT (mm)	3.7±0.6	3.0±0.6
MZ (mm ³)	1029.7±200.3	609.0±121.7

Offspring cohort

	Male (N=642)	Female (N=701)
Age (yrs)	54.8±9.3	54.8±9.7
Weight (lb)	192.9±29.6	156.3±35.9
Height (inch)	69.0±2.6	63.6±2.3
BMI (kg/m ²)	28.5±4.0	27.1±6.0
current smoking	14.8%	14.4%
estrogen-replete	-	49.9%
Length (mm)	67.2±3.8	61.4±3.4
Width (mm)	8.9±0.6	7.6±0.5
MCI (%)	46.5±7.5	46.4±9.9
MCT (mm)	4.1±0.6	3.5±0.7
MZ (mm ³)	1050.3±199.3	637.5±127.7

* mean ± S.D. (unless specified) for all available participants with radiographic measurements.

** Estrogenic status groups: 1) premenopausal or postmenopausal on estrogen (estrogen-replete) or 2) postmenopausal not on estrogen (estrogen-deplete).

Table 2
Heritability testing using 2 models of adjustment for covariates

Index	h^2 Adjusted for age, age ² (estrogen in women)	h^2 Adjusted for age, age ² , smoking, height, BMI (estrogen in women)
Metacarpal length	0.82 ± 0.04	0.76 ± 0.04
Metacarpal width	0.68 ± 0.05	0.72 ± 0.05
MCI	0.61 ± 0.05	0.59 ± 0.05
MCT	0.51 ± 0.05	0.51 ± 0.05
MZ	0.56 ± 0.05	0.58 ± 0.05

All significant at $p < 0.001$

Table 3
Linkage analysis using 2 models of adjustment for covariates
Table 3a. Adjusted for age, age² (estrogen in women)

Chrom.	marker (position, cM)	length	width	MCT	MCI	MZ
LOD scores: two-point (multipoint)						
6	D6S1051 (51)	-	0 (2.17)	1.31 (1.40)	2.26 (1.89)	0 (1.12)
7	D7S3061 (149)	-	0 (0) *	-	-	-
9	D9S319 (55)	-	1.48 (3.42)	-	-	1.03 (2.49)
11	D11S2000 (101)	<1.0 (<1.0)	2.01 (1.32)	-	-	2.97 (1.61)
	D11S1986 (106)	-	2.28 (1.94)	-	-	2.11 (2.29)
X	DXS1047 (143)	-	1.60 (2.03)	-	-	2.37(2.97)
	DXS9908 (164)	-	2.13 (2.14)	-	-	2.36 (2.77)

Table 3b. Adjusted for age, age², smoking, height, and BMI (estrogen in women)

Chrom.	marker (position, cM)	length	width	MCT	MCI	MZ
LOD scores: two-point (multipoint)						
6	D6S1051 (51)	-	0 (2.19)	1.51 (0.96)	2.15 (1.37)	0 (<1.0)
7	D7S3061 (149)	-	0 (0)	-	-	-
9	D9S319 (55)	-	<1.0 (2.35)	-	-	<1.0 (1.91)
11	D11S2000 (101)	<1.0 (<1.0)	<1.0 (<1.0)	0 (<1.0)	-	<1.0 (<1.0)
	D11S1986 (106)	-	1.53 (<1.0)	-	-	1.14 (<1.0)
X	DXS1047 (143)	-	<1.0 (<1.0)	-	-	2.16 (2.53)
	DXS9908 (164)	-	<1.0 (<1.0)	-	-	1.67 (2.23)

* two-point (multipoint)

Table 4

The phenotypic, genetic, and environmental correlations between bone geometric parameters*.

	width	MCT	MCI	MZ
		$\rho_P; \rho_G; \rho_E^{**}$		
length	0.653‡, 0.223‡, 0.274*	0.320‡, 0.072, -0.096	-0.028, -0.031, -0.174	0.659‡, 0.267‡, 0.168
width	--	0.314‡, -0.146, 0.248‡	-0.224‡, -0.534‡, -0.094	0.964‡, 0.948‡, 0.876‡
MCT	--	--	0.848‡, 0.908‡, 0.925‡	0.508‡, 0.135, 0.537‡
MCI	--	--	--	-0.01, -0.275‡, 0.215‡
MZ	--	--	--	--

* Adjusted for age, age², smoking, height, and BMI (estrogen in women)** ρ_P - phenotypic correlations, ρ_G - genetic correlations, and ρ_E - environmental correlationsSignificance levels for correlation coefficients: ‡: $p < 0.0001$; †: $0.0001 < p < 0.05$; * $p = 0.05$; the rest, non-significant ($p > 0.05$)