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A Polymorphism in a Gene Encoding Perilipin 4 Is Associated with Height but not with Bone Measures in Individuals from the Framingham Osteoporosis Study

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Abstract

There is increasing interest in identifying new pathways and candidate genes that confer susceptibility to osteoporosis. There is evidence that adipogenesis and osteogenesis may be related, including a common bone marrow progenitor cell for both adipocytes and osteoblasts. Perilipin 1 (*PLIN1*) and Perilipin 4 (*PLIN4*) are members of the PATS family of genes and are involved in lipolysis of intracellular lipid deposits. A previous study reported gender-specific associations between one polymorphism of *PLIN1* and bone mineral density (BMD) in a Japanese population. We hypothesized that polymorphisms in *PLIN1* and *PLIN4* would be associated with bone measures in adult Caucasian participants of the Framingham Osteoporosis Study (FOS). We genotyped 1,206 male and 1,445 female participants of the FOS for four single-nucleotide polymorphisms (SNPs) in *PLIN1* and seven SNPs in *PLIN4* and tested for associations with measures of BMD, bone ultrasound, hip geometry, and height. We found several gender-specific significant associations with the measured traits. The association of *PLIN4* SNP rs8887, *G>A* with height in females trended toward significance after simulation testing (adjusted $P=0.07$) and remained significant after simulation testing in the combined-sex model (adjusted $P=0.033$). In a large study sample of men and women, we found a significant association between one SNP in *PLIN4* and height but not with bone traits, suggesting that PATS family genes are not important in the regulation of bone. Identification of genes that influence human height may lead to a better understanding of the processes involved in growth and development.

Keywords

Perilipin 1; Perilipin 4; Bone mineral density; Bone geometry; Framingham Osteoporosis Study

Osteoporosis is a skeletal disorder characterized by compromised bone strength and subsequent increase in fracture risk. In the United States alone, 10% of adults aged 50 years or more suffer from osteoporosis, and the direct care expenditures for osteoporotic fractures are estimated to be \$12–\$18 billion annually. The incidence of osteoporosis is expected to increase significantly in the next few years due to extended life expectancies and the aging of the US population [1]. Twin and family studies have implicated genetic factors in up to 75% of the intersubject variance in the regulation of bone mineral density (BMD) and other determinants of osteoporotic fracture risk, such as quantitative ultrasound properties of bone and skeletal geometry [2–5]. Height itself may also be considered a bone-related phenotype since the phenotype is largely explained by the length of the legs and vertebral segments [6–8]. Based on numerous candidate gene-association studies and recent genome-wide association studies, there is reason to believe that previously unidentified pathways and new candidate genes will be described that confer susceptibility to osteoporosis [9–13].

A common bone marrow progenitor cell for both adipocytes and osteoblasts suggests that adipogenesis and osteogenesis may be related [14–16]. Osteoporotic bone has been noted to have an expansion of adipose tissue in the bone marrow at the expense of osteogenic cells, a finding that has been reported in several species including humans and mice [17–19]. Lineage-specific transcription factors that direct differentiation of this common progenitor have been identified, including the runt-related transcription factor 2 (*Runx2*) for osteoblastogenesis and the peroxisome proliferator-activator gamma (*PPAR γ*) for adipogenesis [20, 21]. An inverse relationship appears to exist between the two lineages, with agents inducing osteoblast differentiation inhibiting adipogenesis and vice versa [21]. Polymorphisms in *PPAR γ* have been shown to affect bone mass in mice and humans [22]. If there are direct effects of adipocytes on other marrow progenitors vital to bone health, there may be associations between genes that regulate fat metabolism and skeletal integrity. Finally, an inverse relation between fat mass and bone density has been observed in both

cross-sectional and longitudinal studies in different age groups [23–25]. For these reasons, we examined two genes involved in fat metabolism to investigate their association with a variety of skeletal phenotypes.

Perilipin, whose HUGO Gene Nomenclature Committee name is now *Perilipin 1 (PLIN1)*, encodes a protein that was first identified in the late 1980s by Londos et al. [26] and named “perilipin” in reference to its physical location surrounding lipid droplets [27]. *PLIN4*, formerly known as S3-12, which has sequence similarity to *PLIN1*, was found in a screen for adipocyte-specific proteins, suggesting that it is involved in adipocyte lipid storage. As such, both *PLIN1* and *PLIN4* are members of the “PATS” family of proteins (*PLIN*, *ADFP*, *TIP-47*, *S3-12*), a group of structural proteins defined by their protein sequence similarity and association with lipid droplets [28]. These proteins are found almost exclusively in adipocytes and steroidogenic cells and play a vital role in the lipolysis of intracellular lipid deposits [29, 30]. Expression of these genes is elevated in obese animals and humans, with *PLIN1* being the most studied of the group [28–33]. Polymorphisms in *PLIN1* have been shown to have a female-specific association with obesity in various ethnic groups [31–33].

Yamada et al. [34] studied the *PLIN1* 1243C>T (rs2304796) single-nucleotide polymorphism (SNP) and its relationship to BMD in a community-dwelling Japanese population. They found a male-specific association, with the *TT* genotype having greater BMD for the total hip, lumbar spine, femoral neck, and trochanter than the combined *CC* and *CT* genotypes. Here, we studied *PLIN1* and *PLIN4* SNPs and their relation to BMD, broadband ultrasound attenuation (BUA), bone geometric traits, and height in participants of the Framingham Osteoporosis Study (FOS), a population of European ancestry. We hypothesized that SNPs in *PLIN4* and *PLIN1* would be associated with these bone properties.

Materials and Methods

Study Sample

The FOS is an ancillary study of the Framingham Heart Study. The Framingham Heart Study began in 1948 with the primary goal of prospectively evaluating the multivariable risk factors for cardiovascular disease. The original cohort participants were selected from a systematic sampling of two-thirds of the households of the population of Framingham, MA, at that time. In 1971, the Framingham Offspring Cohort Study was initiated with the intent to evaluate the role of genetic factors in the etiology of coronary artery disease and was comprised of 71% of all the eligible adult offspring of couples from the original cohort and offspring’s spouses. The offspring cohort consists of 5,124 subjects, of whom 2,616 are offspring of the 1,164 original cohort spouse pairs, 34 are stepchildren, 898 are offspring of one parent in the original cohort at greater risk of cardiovascular disease, and 1,576 are spouses. After their initial evaluation, these individuals underwent repeat examinations approximately every 4 years and participated in the FOS at either examination cycle 6 or 7 from 1996 to 2001. The design of this study has been described previously [35–38].

Our genetic analysis included an unselected subset of 2,651 (1,206 men and 1,445 women) offspring subjects (age range 29–94 years) who had both genotyping and at least one of the measured phenotypes available. Among these 2,651 participants with *PLIN1* or *PLIN4* genotyping, 2,448 (55.08% female, age 62.06 ± 10.59 , range 29–94) had valid BMD measures of the total hip, 2,495 (55.37% female, age 62.14 ± 10.30 , range 29–90) had BMD measures of the spine, 2,504 (55.3% female, age 62.1 ± 10.3 , range 29–90) had BMD measures of the trochanter, 2,505 (55.4% female, age 62.1 ± 10.38 , range 29–90) had BMD measures of the femoral neck, 2,435 (56.07% female, age 62.51 ± 10.86 , range 29–94) had BUA, 2,468 (55.4% female, age 62.0 ± 10.2 , range 31–90) had femoral neck length

measurements, and 1,400 (53.4% female, age 54.5 ± 8.9 , range 29–81) had metacarpal length measurements. All participants had height measurements obtained at or several years before the time of the bone exam. Neither the Framingham original nor offspring cohort was selected on the basis of cardiovascular diseases or osteoporosis. This study was approved by the Institutional Review Board for Human Subjects Research of Boston University, Tufts University, and the Hebrew Rehabilitation Center.

Measurement of BMD and Quantitative Ultrasound

Participants underwent bone densitometry by DXA with a Lunar (Madison, WI) DPX-L from 1996 to 2001. The coefficients of variation (CV) in normal subjects for the DPX-L have been previously noted to be 0.9% for the lumbar spine, 1.7% for the femoral neck, and 2.5% for the trochanter [37]. Calcaneal quantitative bone ultrasonography was performed to obtain right calcaneal BUA with a portable device, the Sahara[®] bone sonometer (Hologic, Waltham, MA), in members of the offspring cohort between 1996 and 2001. Based on duplicate, same-day measurements on 29 subjects, the CV for BUA was 5.3% [38].

Measurement of Bone Geometry

Hip Geometry—DXA scans obtained in 1992–1993 or 1996–1997 (for 31 original cohort subjects who missed the earlier measured DXAs) were taken with an interactive computer program [39]. The program derived a number of proximal femoral structural variables, including femoral neck length (FNL), defined as the distance from the center of the femoral head to the intersection of the neck and shaft axes; the outer diameters at both the femoral neck and the femoral shaft; as well as the neck-shaft angle (see [40] for details). CVs for the different geometric indices were previously reported, ranging from 3.3% (femoral neck outer diameter) to 9.1% (FNL) [12].

Metacarpal Measurements—As part of a study investigating the heritability of osteoarthritis, participants of the FOS who had at least one parent (member of the original cohort) with hand radiographs were radiographed using the same techniques in 1993–1995. Hand radiographs were digitized at 450-dpi resolution using a COBRAScan 612 SL Digitizer (Radiographic Digital Imaging, Compton, CA) and Windows Imaging software (Microsoft, Redmond, WA) to acquire an image with a pixel size of 0.12 mm and 1,024 gray levels. A custom-written script developed for ImageTool software (UTHSCSA, San Antonio, TX) was used to semiautomatically obtain measurements of the second, third, and fourth metacarpals, by outlining the contour of the digitized metacarpal bones of interest on a flat-screen monitor. The algorithm implemented in the software then determined the coordinates of outlined bone, generated a mass profile, and converted the data into the cortical macrostructure. For our analyses we focused on lengths of the second through fourth metacarpals and averaged them as previously described [41]. Finally, considering height to be a bone-related phenotype, it was measured to the nearest one-fourth inch using a stadiometer [35, 37].

Covariables

Information including age, sex, weight, and, for women, estrogen use and menopausal status was obtained at the time of bone density/ultrasound and geometry measurements. Weight was measured using a standardized balance beam scale. Body mass index (BMI, kg/m^2) was calculated using height measured as above. Each woman was assigned to one of the two estrogen status groups: (1) premenopausal or postmenopausal women taking estrogen (estrogen-replete) or (2) postmenopausal not on estrogen (estrogen-deplete), where menopause was defined as having no menstrual period for at least 1 year [35, 37].

SNP Selection and Genotyping

The variants chosen for *PLINI*—rs2289487, *T>C* (intron 2), named *PLINI* SNP; rs894160, *G>A* (intron 6), named *PLIN4* SNP; rs2304795, *A>G* (exon 8), named *PLIN5* SNP; and rs1052700, *A>T* (exon 9), named *PLIN6* SNP—were studied based in part on our results from previous publications [32, 42]. To assist selection of SNPs in the *PLINI* locus, we performed a search of the CEU population HapMap PHASE II data for polymorphic alleles with a minor allele frequency (MAF) $\geq 5\%$ at the *PLINI* locus, defined as 5 kb upstream of the predicted start codon and 2 kb downstream of the termination codon. Eleven SNPs were identified within this region in the CEU population of HapMap, but only five had MAFs $\geq 5\%$. The rs894160 SNP was in linkage disequilibrium (LD) with rs8179043, having $r^2 = 1$, so rs894160 was used as a tag SNP. These four SNPs provide coverage of all common variation within the *PLINI* locus, as defined above.

To identify common SNPs in the human *PLIN4* locus, we performed a search of the HapMap database for polymorphic alleles with an MAF $\geq 5\%$. The *PLIN4* locus was defined as 5 kb upstream of the predicted start codon and 2 kb downstream of the termination codon, a region spanning approximately 25.1 kb. Thirteen such SNPs were identified in this region. We chose eight of these SNPs for genotyping: two promoter (rs884164 and rs1609717), one exonic missense (rs7250947), one 3'-UTR (rs8887), and four intronic (rs8102428, rs892158, rs4807598, and rs11673616). Analysis in the CEU population of the Hap-Map database with the Haploview program determined rs892158 (rs7260518 and rs10406797), rs7250947 (rs8102428 and rs884164), rs11673616 (rs4991027), and rs1609717 (rs4807598) to be tag SNPs capturing variants (in parentheses) in LD with $r^2 > 0.8$ over 11 of 13 SNPs in the region.

DNA was isolated from blood samples using DNA blood Midi kits (Qiagen, Hilden, Germany) according to the vendor's recommended protocol. Ready-made 5' nucleic allelic discrimination assays were available from Applied Biosystems (Foster City, CA) for the *PLIN4* SNPs rs8887, rs11673616, rs892158, rs8102428, and rs884164. We used the Applied Biosystems Custom Assay design Web tool to generate functional assays for SNPs rs1609717 and rs7250947 (appliedbiosystems.com). We performed genotyping of *PLINI* and *PLIN4* SNPs, using TaqMan assays on the ABIPrism 7900HT Sequence Detection System (Applied Biosystems). Standard laboratory practices were used to ensure the accuracy of the data.

Statistical Analysis

MAF estimation and Hardy-Weinberg equilibrium (HWE) testing were performed using unrelated individuals. We used an exact Chi-squared test statistic (Genetic Package) to compare observed genotype frequencies to those expected under HWE. We excluded one SNP (*PLIN4*, rs4807598) from analysis because of significant deviation from HWE ($P = 0.001$).

To test for association between SNPs and height, BMD, geometry, and metacarpal measures, we used sex-specific regression models with height and bone measures as dependent variables and SNPs and covariates as independent variables. To account for within-familial correlation, linear mixed effects (LME) regression was used (*Imekin*, R-Kinship Package). We used a codominant model (comparing three genotype groups, 2 df test) for five SNPs with $\geq 20\%$ MAF and a dominant model (combining the heterozygotes and minor allele homozygotes) for the remaining six SNPs with $<20\%$ MAF. Association analyses between SNPs and height were adjusted for age and estrogen status in women, and those for bone measures were adjusted for age, BMI, height, and estrogen status in women. We also performed sex-combined analyses, additionally adjusting for sex.

We used simulation methods to adjust for the number of statistical tests while accounting for the dependency of tests due to LD among the SNPs and correlation among phenotypes. First, independently of phenotypes and covariates, we simulated SNP genotypes with allele frequencies and LDs similar to the observed genotype data (*simqtl*, SOLAR, <http://www.sfbr.org> [38]). Second, we merged the set of phenotypes and covariates for each individual with the simulated genotype, while maintaining the allele frequency, LD information, Mendelian consistency, and correlation among phenotypes and covariates. Then, using the simulated data, we performed LME regression analysis to obtain sex- and gene-specific minimum *P* values. This procedure was repeated 1,000 times, and for each test the adjusted *P* value was calculated as the proportion of simulated data sets in which the minimum *P* value was less than the observed *P* value. “Adjusted *P* values” are significant when they are below $P < 0.05$ as these have been adjusted for multiple comparisons using simulation testing.

We calculated the proportion of variance explained by SNP effects for those traits with significant *P* values. Among significant *P* values in men, h^2 ranged from 0.35% to 0.58%. Among significant *P* values in women, h^2 ranged from 0.26% to 0.87%. We then used QUANTO (<http://hydra.usc.edu/gxe/>) to compute the power for males ($n = 1,206$) and females ($n = 1,446$) using a significance level of 0.05. With h^2 of 0.5%, the power is 70% for males and 77% for females.

Results

Subject Characteristics

Table 1 contains the descriptive characteristics of FOS participants included in this analysis by gender with bone density/ultrasound measures and bone geometric traits. Both men and women were of similar age (mean 62 years) and similar BMI measurements (mean 28 kg/m²). Among the 1,445 women, 42% were classified as estrogen status–positive, defined as premenopausal women or postmenopausal women taking estrogen supplementation. In general, compared to women, men had higher BMD/BUA measurements by 0.11–0.18 g/cm² for the femoral neck, trochanter, spine, and total hip and by 12.5 db/mHz for BUA. In addition, men had greater values for the bone geometric traits compared to women, with larger values of FNL and metacarpal length by 0.88 and 0.58 cm, respectively.

Single-Nucleotide Polymorphisms

Table 2 contains detailed information regarding the seven polymorphisms in *PLIN4* and the four polymorphisms in *PLIN1* evaluated in this analysis. One of the *PLIN4* SNPs (rs4807598) was not in HWE and was therefore not included in this analysis. Figure 1a shows the LD for each pair of *PLIN4* SNP markers using D' and r^2 calculated from estimated haplotype frequencies. Figure 1b shows the *PLIN1* LD for each pair of markers. MAFs of the SNPs ranged from 4.7% to 44.7%, with no significant differences in the genotype frequencies by sex.

Individual SNP Associations with BMD/BUA and Geometric Traits

Figure 2a contains the multivariate adjusted, sex-specific (age, BMI, height, and estrogen status in women) *P* values for the associations between the seven SNPs in the *PLIN4* gene and the four SNPs in the *PLIN1* gene with BMD for the total hip, femoral neck, trochanter, and spine and BUA. Figure 2b contains the multivariate adjusted (age, BMI, height, and estrogen status in women) *P* values for the associations between SNPs in the *PLIN4* and *PLIN1* genes and height, FNL, and metacarpal length. Table 3 displays only the significant ($P < 0.05$) gender-specific associations and the magnitude of these associations in men and women between the *PLIN4* and *PLIN1* SNPs and BMD/BUA and geometric traits, including

the data for nonsignificant findings in the other gender. Our analysis revealed that eight of the 11 SNPs were associated at a nominal significance level with one or more of the measurements of bone density/ultrasound or geometry after adjustment for covariates.

The combined-gender results showed significant associations at a nominal level between *PLIN4* SNP 1 (rs8887, *G>A*) with height and metacarpal length; *PLIN4* SNP 2 (rs11673616, *A>G*) with height and BMD of the spine, femoral neck, and total hip; *PLIN4* SNP 3 (rs892158, *G>A*) with height and BMD of the femoral neck, trochanter, and total hip; *PLIN4* SNP 4 (rs7250947, *G>A*) with height; and *PLIN4* SNP 5 (rs8102428, *A>G*) with metacarpal length and BMD of the trochanter. *PLIN1* SNPs were not associated with any of the phenotypes examined in the combined-gender analysis.

Among men, we found significant associations at a nominal level between *PLIN4* SNP 2 (rs11673616, *A>G*) with height and BMD of the spine; *PLIN4* SNP 3 (rs892158, *G>A*) with BMD of the femoral neck, trochanter, and total hip; *PLIN4* SNP 6 (rs1609717, *A>G*) with BMD of the spine; *PLIN4* SNP 7 (rs884164, *A>G*) with BMD of the trochanter; and *PLIN1* SNP 9 (rs2304795, *A>G*) with BMD of the total hip. In women, we noted significant associations between *PLIN4* SNP 1 (rs8887, *G>A*) with metacarpal length and height and SNP 6 (rs1609717, *A>G*) with height. None of the studied *PLIN4* SNPs was significantly associated with the measured BMD/BUA traits in women.

Accounting for the multiple testing, the association of SNP 1 with height remained significant in the combined-sex results with adjusted $P = 0.033$. None of the gender-specific associations remained significant for either *PLIN1* or *PLIN4*, although the association of *PLIN4* SNP 1 (rs8887, *G>A*) with height in females was borderline significant with adjusted $P = 0.07$.

Combined SNP Associations across *PLIN1* and *PLIN4* with BMD/BUA and Geometric Traits

SNPs that were shown to have significant individual association(s) with the studied BMD, BUA, and bone geometric traits as shown in Table 3 were selected for additional analyses to evaluate the combined effect of these SNPs (SNP 1, SNP 2, SNP 3, SNP 6, SNP 7, and SNP 9) on the selected traits. Since SNP 2 and SNP 3 have high LD ($r^2 = 0.77$), we also evaluated a model excluding SNP 3 (model 2) and a model excluding SNP 2 (model 3). In these models, we adjusted for age, BMI, height, and estrogen status in women. To evaluate the combined effect on height, we adjusted for age and estrogen status (in women only).

Model 1 SNP1 + SNP2 + SNP3 + SNP6 + SNP7 + SNP9 + age + BMI + height + estrogen status

Model 2 SNP1 + SNP2 + SNP6 + SNP7 + SNP9 + age + BMI + height + estrogen status

Model 3 SNP1 + SNP3 + SNP6 + SNP7 + SNP9 + age + BMI + height + estrogen status

Among men, we found a significant combined SNP effect on spine BMD in model 2 ($P = 0.0375$) and model 3 ($P = 0.0485$), where SNP 2 and SNP 6 have significant individual P values. Trochanter BMD had a significant combined SNP effect in model 1 ($P = 0.0363$) and model 3 ($P = 0.0397$), where SNP 3 and SNP 7 have significant individual P values. Total hip BMD had a significant combined SNP effect in model 1 ($P = 0.0420$) and model 3 ($P = 0.0312$), where SNP 3 and SNP 9 have significant individual P values.

Among women, we found a significant combined SNP effect on height in all three models, model 1 ($P = 0.028$), model 2 ($P = 0.0155$), and model 3 ($P = 0.0147$), where SNP 1 and SNP 6 have significant individual P values.

Discussion

We evaluated several polymorphisms in the *PLIN1* and *PLIN4* genes for associations with BMD/BUA, geometric traits, and height in this large subset from the FOS.

In this cohort of Caucasians mostly from European ancestry, we detected several significant ($P < 0.05$) gender-specific and combined-sex associations in the measured traits, including metacarpal length and height. There were several significant associations with BMD found in men only. Using simulation to adjust for multiple testing, the association of SNP 1 (rs8887, $G > A$) with height in females trended toward significance with adjusted $P = 0.07$, and in the combined-sex model this association remained significant ($P = 0.03$).

To date, there are only two references in the literature that investigated *PLIN1* and bone density. Yamada et al. [34] studied a cohort of 1,122 men and 1,112 women, ages 40–79, as part of the Japanese National Institute for Longevity Sciences-Longitudinal Study of Aging. These investigators studied only a single polymorphism in *PLIN1*, 1243C>T (rs2304796), and their analysis revealed a relationship in men only between the *TT* genotype and greater BMD for the total hip, lumbar spine, femoral neck, and trochanter compared to the combined *CC* and *CT* genotypes. They found no relationship between this polymorphism and BMD in Japanese women, regardless of estrogen status. The *PLIN1* SNP studied by Yamada et al. does not appear to be present in the Caucasian population, and it is therefore not possible to establish if this SNP is in LD with any of the SNPs evaluated in this analysis. A recent genome-wide analysis of the FOS population [12] found one SNP in the *PLIN1* gene, rs8179043 in intron 6 (MAF = 0.30), to be associated with lumbar spine BMD in men only, with a P value of 0.01. This SNP is in strong LD with rs894160 (SNP 10 in this analysis) with $r^2 \sim 0.97$; however, no significant associations were found in our study with this SNP. Differences in the size and characteristics of the population evaluated in our analysis and this genome-wide analysis may account for the null result seen in our study.

Our study has several strengths, including having a large sample size. In addition, this is the first analysis to study a potential relationship between *PLIN1* and *PLIN4* with bone phenotypes in a Caucasian population. SNP selection covered the *PLIN1* gene adequately to enable us to detect associations, and we included *PLIN4*, a related gene that has not been previously studied. We found several gender-specific associations between various SNPs and the measured traits, consistent with the previous findings by Yamada et al. [34] as well as previous reports of gender-specific linkage of osteoporosis-related phenotypes [3, 39, 40]. After adjustment for multiple testing, we found a trend toward significance in the association between one of the studied *PLIN4* polymorphisms and height in females and a significant result in the sex-combined sample. However, we were unable to detect a significant association with this SNP and metacarpal length, which might have been expected if height were reflecting bone growth. Previously, height was shown to be a bone-related phenotype since the phenotype is largely explained by the length of the extremities and vertebral segment, which are a function of bone length [6–8]. Therefore, unsurprisingly, genome-wide association studies of adult height identified several bone- and cartilage-related genes including *GDF5* (growth differentiation factor 5), a cartilage-derived morphogenetic protein [7, 8]. Sanna et al. [7] stated that “identifying genetic variants that influence human height will advance our understanding of skeletal growth and development.”

Limitations of this study include the fact that the phenotypes used in our analyses are proxies representing the end product of complex biological processes, rather than the endophenotypes, most proximal to the effects of genes. Other factors, including unknown and unmeasured environmental as well as stochastic factors, may contribute to both the bone and anthropometric traits studied, especially in a sample that is beyond the age of peak bone

density and long bone growth. Also, areal BMD combines the trabecular and cortical compartments, making it impossible to distinguish between potentially more important trabecular effects of genes related to marrow fat handling. Future studies should repeat our analyses using volumetric trabecular bone density phenotypes.

There is growing evidence of a close relationship between lipid metabolism and bone remodeling. Osteoblasts and adipocytes share a common progenitor cell in the bone marrow, and an inverse relationship exists between the two lineages, with agents inducing adipogenesis inhibiting osteoblast differentiation and therefore promoting bone loss [23–25]. Thiazolidinediones, inducers of PPAR γ and subsequent induction of the mesenchymal stem cell into adipocytes, have been associated with bone loss and osteoporosis [43]. Osteocalcin is a protein produced by osteoblasts that acts as a regulator of bone formation, and osteocalcin-null mice have been found to be obese [44]. There may be profound associations between genes that regulate fat metabolism and skeletal integrity, and future research is necessary to investigate this relationship further, perhaps focusing on other genes involved in stem cell differentiation, rather than fat handling.

The findings of this study should be regarded as hypothesis-generating and indicate that assessment in other populations is needed. Future investigation is necessary to differentiate the role that proteins important in adipocyte metabolism, including PLIN and PLIN4 of the PAT family of proteins, have with regard to bone properties and subsequently fracture risk.

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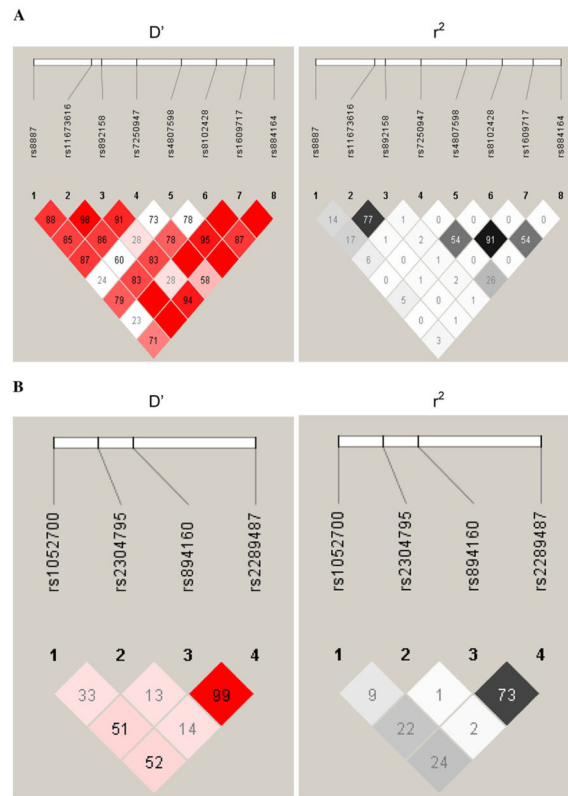
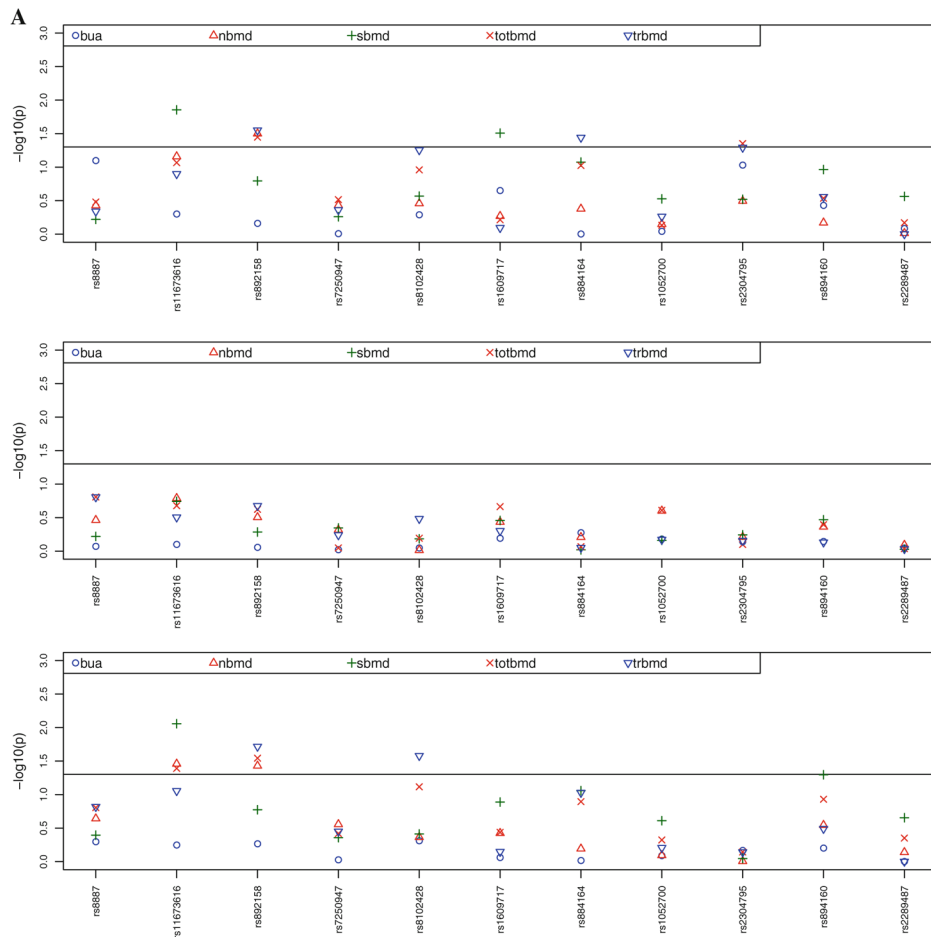
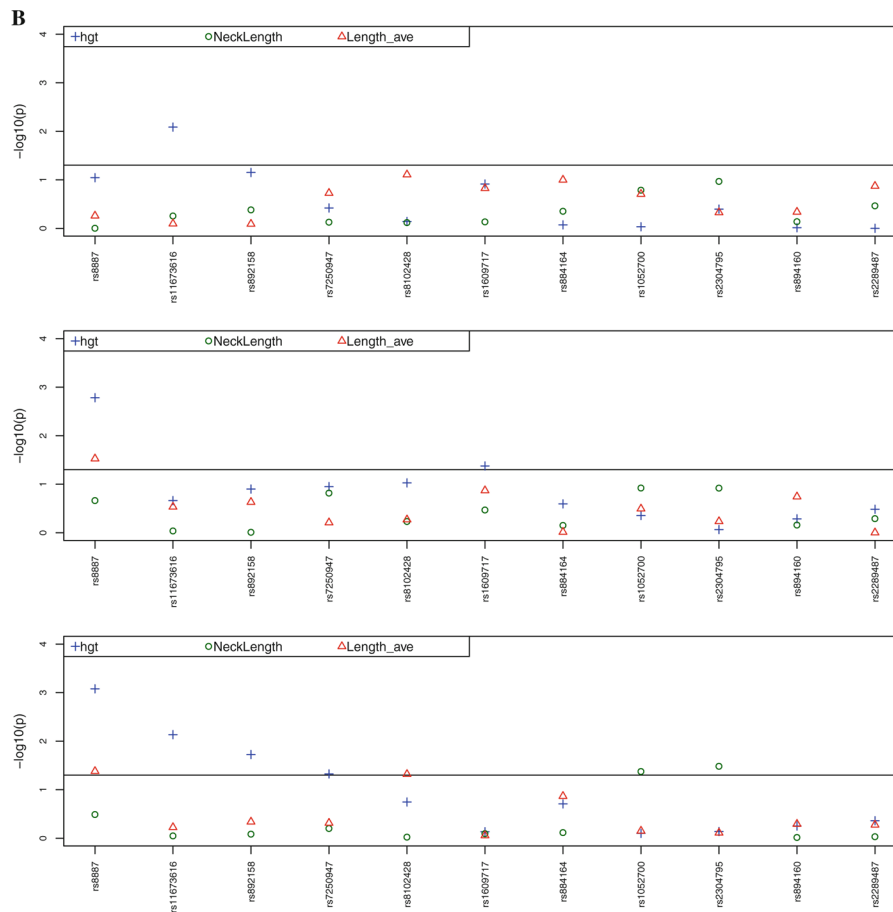


Fig. 1. **a** LD for each pair of *PLIN4* SNP markers using D' and r^2 calculated from estimated haplotype frequencies. **b** LD for each pair of *PLIN1* SNP markers using D' and r^2 calculated from estimated haplotype frequencies



**Fig. 2.**

a Adjusted P values for the associations between SNPs in the *PLIN4* and *PLIN1* genes and BMD for the total hip (*totbmd*), femoral neck (*nbmd*), trochanter (*trbmd*), spine (*sbmd*), and right calcaneal broadband ultrasound attenuation (*bu*) (adjusted for age, BMI, height, and estrogen status in women). SNPs rs8887, rs11673616, rs892158, rs7250947, rs8102428, rs1609717, and rs884164 are from *PLIN4* and rs1052700, rs2304795, rs894160, and rs2289487 are from *PLIN1*. *First panel* represents the results for males, *second panel* for females, and *third panel* the combined-gender results. **b** Adjusted P values for the associations between SNPs in the *PLIN4* and *PLIN1* genes and height (*hgt*, adjusted for age and estrogen status in women), femoral neck length (*NeckLength*), and metacarpal length (*Length_ave*) (adjusted for age, BMI, height, and estrogen status in women). *First panel* represents the results for males, *second panel* for females, and *third panel* the combined-gender results

Table 1

Subject characteristics^a

Characteristics	Males (n = 1,206)		Females (n = 1,446)		P
	n	Mean ± SD	n	Mean ± SD	
Age (years)	1,078	62.4 ± 10.0	1,317	61.8 ± 10.3	0.1124
BMI (kg/m ²)	1,074	28.7 ± 4.5	1,313	27.5 ± 5.6	<0.0001
Height (cm)	1,074	174.5 ± 7.1	1,313	160.5 ± 6.6	<0.0001
Estrogen status positive ^b (%)	N/A		562/1,316 (42.71%) ^c		N/A
Bone density/ultrasound measurements					
Spine BMD (g/cm ²)	1,063	1.33 ± 0.20	1,302	1.15 ± 0.20	<0.0001
Femoral neck BMD (g/cm ²)	1,068	0.97 ± 0.14	1,306	0.86 ± 0.15	<0.0001
Trochanter BMD (g/cm ²)	1,068	0.89 ± 0.14	1,305	0.71 ± 0.14	<0.0001
Total hip BMD (g/cm ²)	1,050	1.04 ± 0.14	1,275	0.91 ± 0.15	<0.0001
BUA (db/mHz)	1,022	82.7 ± 18.9	1,290	70.2 ± 19.2	<0.0001
Bone geometry measurements					
Femoral neck length (cm)	1,053	5.97 ± 5.97	1,289	5.09 ± 0.67	<0.0001
Metacarpal length (cm)	613	6.71 ± 0.38	701	6.13 ± 0.34	<0.0001
Age (years)	613	54.9 ± 8.9	701	54.3 ± 9.0	0.2493
BMI (kg/m ²)	610	28.4 ± 4.0	697	27.0 ± 5.7	<0.0001
Height (cm)	610	175.0 ± 6.9	697	161.3 ± 5.8	<0.0001
Estrogen status positive ^b (%)	N/A		339/698 (48.57%) ^c		N/A

^aValues taken for subjects with *PLIN1* genotyping; not statistically different from the subgroup of subjects with *PLIN4* genotyping^bEstrogen status positive includes premenopausal women and postmenopausal women taking estrogen^cPrevalence count/total count (percentage)

Table 2

PLIN1 and *PLIN4* SNPs genotyped in this study

	Gene	SNP rs number	Position ^a	Location	Alleles	MAF (%)	HWE <i>P</i>
1	<i>PLIN4</i>	rs8887	4453201	Downstream	G>A	44.7	0.92
2	<i>PLIN4</i>	rs11673616	4457915	Intron 4	A>G	13.2	0.68
3	<i>PLIN4</i>	rs892158	4458716	Intron 4	G>A	16.2	0.38
4	<i>PLIN4</i>	rs7250947	4461530	Exon 3	G>A	9.5	0.40
<i>b</i>	<i>PLIN4</i>	rs4807598	4465135	Intron 2	T>C	4.7	0.001
5	<i>PLIN4</i>	rs8102428	4467982	Intron 1	A>G	10.5	0.80
6	<i>PLIN4</i>	rs1609717	4470450	Promoter	A>G	4.7	0.05
7	<i>PLIN4</i>	rs884164	4472625	Downstream	A>G	7.6	0.61
8	<i>PLIN1</i>	rs1052700	88009314	3'-UTR	T>A	34.2	0.64
9	<i>PLIN1</i>	rs2304795	88011267	Pro371Pro	T>C	38	0.25
10	<i>PLIN1</i>	rs894160	88012827	Intron 6	C>T	29.8	0.69
11	<i>PLIN1</i>	rs2289487	88018100	Intron 2	T>C	36.8	0.76

^aPosition relative to NCBI coordinates (viewed 08/20/08)

^bThis SNP was not in Hardy-Weinberg equilibrium and is not included in this analysis

Table 3
Gender-specific associations of selected *PLIN1* and *PLIN4* SNPs and bone density/ultrasound and geometric traits

Men	Genotype	Adjusted mean	P	Women	Genotype	Adjusted mean	P
Geometry							
SNP 1: Metacarpal length	<i>GG</i>	6.70	0.550	SNP 1: Metacarpal length	<i>GG</i>	6.10	0.030
	<i>GA</i>	6.72			<i>GA</i>	6.12	
	<i>AA</i>	6.72			<i>AA</i>	6.18	
SNP 1: Height	<i>GG</i>	68.97	0.090	SNP 1: Height	<i>GG</i>	63.28	0.002
	<i>GA</i>	68.79			<i>GA</i>	63.47	
	<i>AA</i>	68.46			<i>AA</i>	62.87	
SNP 2: Height	<i>AA</i>	68.90	0.008	SNP 2: Height	<i>AA</i>	63.34	0.218
	<i>AG and GG</i>	68.40			<i>AG and GG</i>	63.15	
SNP 6: Height	<i>AA</i>	68.74	0.122	SNP 6: Height	<i>AA</i>	63.34	0.042
	<i>AG and</i>	69.18			<i>AG and GG</i>	62.89	
Bone density/ultrasound							
SNP 2: Spine BMD	<i>AA</i>	1.34	0.014	SNP 2: Spine BMD	<i>AA</i>	1.16	0.181
	<i>AG and GG</i>	1.30			<i>AG and GG</i>	1.14	
SNP 3: Neck BMD	<i>GG</i>	0.98	0.032	SNP 3: Neck BMD	<i>GG</i>	0.86	0.312
	<i>GA and AA</i>	0.96			<i>GA and AA</i>	0.86	
SNP 3: Trochanter BMD	<i>GG</i>	0.89	0.028	SNP 3: Trochanter BMD	<i>GG</i>	0.72	0.211
	<i>GA and AA</i>	0.87			<i>GA and AA</i>	0.71	
SNP 3: Total hip BMD	<i>GG</i>	1.05	0.036	SNP 3: Total hip BMD	<i>GG</i>	0.91	0.238
	<i>GA and AA</i>	1.03			<i>GA and AA</i>	0.90	
SNP 6: Spine BMD	<i>AA</i>	1.32	0.031	SNP 6: Spine BMD	<i>AA</i>	1.15	0.348
	<i>AG and GG</i>	1.37			<i>AG and GG</i>	1.17	
SNP 7: Trochanter BMD	<i>AA</i>	0.88	0.036	SNP 7: Trochanter BMD	<i>AA</i>	0.71	0.889
	<i>AG and GG</i>	0.91			<i>AG and GG</i>	0.71	
SNP 9: Total hip BMD	<i>TT</i>	1.05	0.044	SNP 9: Total hip BMD	<i>TT</i>	0.91	0.794
	<i>TC</i>	1.04			<i>TC</i>	0.91	
	<i>CC</i>	1.02			<i>CC</i>	0.91	

Results multivariate adjusted, only results with $P < 0.05$ or the corresponding data for the other gender when one gender is significant
P values in *bold* are significant