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# Genetic Analysis of Vertebral Trabecular Bone Density and Cross-Sectional Area in Older Men

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## Abstract

Vertebral bone mineral density (BMD) and cross-sectional area (CSA) are important determinants of vertebral bone strength. Little is known about the specific genetic variants that influence these phenotypes in humans. We investigated the potential genetic variants associated with vertebral

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trabecular volumetric BMD (vBMD) and CSA measured by quantitative computed tomography (QCT). We initially tested for association between these phenotypes and 4608 tagging and potentially functional single nucleotide polymorphisms (SNPs) in 383 candidate genes in 862 community-dwelling Caucasian men aged 65 years in the Osteoporotic Fractures in Men Study (MrOS). The most promising SNP associations (P<0.01) were then validated by genotyping an additional 1,156 randomly sampled men from the same cohort. We identified 11 SNPs in 10 genes (TGFBR3, SOST, KL, CALCR, LEP, CSF1R, PTN, GNRH2, FGFR2, MEPE) that were consistently associated with trabecular vBMD and 5 SNPs in 5 genes (*CYP11B1, DVL2, DLX5, WNT4, PAX7*) that were consistently associated with CSA in both samples (p<0.005). None of the SNPs associated with trabecular vBMD were associated with CSA. Our findings raise the possibility that at least some of the loci for vertebral trabecular BMD and bone size may be distinct.

### Keywords

Osteoporosis; Genetics; BMD; men; QCT; Polymorphism

## Introduction

Vertebral fractures are a hallmark of osteoporosis and a contributor to back pain, height loss, disability and mortality among elderly women and men [1]. Traneclar volumetric bone mineral density (vBMD) and cross-sectional area (CSA) determines vertebral body strength and its resistance to fracture [2]. Direct measures of trabecular volumetric BMD using quantitative computed tomography (QCT) show the highest diagnostic sensitivity for vertebral fracture[3,4] and are more sensitive than DXA measures to changes in bone metabolism. In contrast, dual-energy X-ray absorptiometry (DXA) yields a measure of integral BMD (i.e., cortical and trabecular) that can be inflated erroneously by the high prevalence of extra-vertebral mineralization among older adults (e.g., osteophytes, vascular calcifications) [5-8].

Trabecular BMD and bone size are highly heritable traits [9-11]. Studies in mice suggest that separate genetic loci may influence BMD and bone size [12]. Moreover, the loci that influence vertebral trabecular BMD may be distinct from those that influence total vertebral BMD among inbred mouse strains [13]. Others have found that genetic influences on bone strength may be skeletal site specific with different genes regulating bone mass and the biomechanical properties of the femur and spine in mice [14-16]. However, there is currently a paucity of data available on the genetic determinants of vertebral trabecular BMD and vertebral bone size in humans.

The development of high-throughput genotyping technologies, identification and cataloging of millions of single nucleotide polymorphisms (SNPs) across the human genome [17] and decreased genotyping costs have made it possible to perform comprehensive association studies of bone metabolism genes. We used these advances in technology and resources to conduct a genetic association analysis of 383 bone metabolism related genes and vertebral trabecular volumetric BMD and cross-sectional area in Caucasian American men who were participants in the Osteoporotic Fractures in Men Study (MrOS).

## Methods

### Study Participants

Participants for this investigation were selected from the Osteoporotic Fractures in Men (MrOS) Study. The MrOS study is a prospective, cohort study designed to investigate

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anthropometric, lifestyle and medical factors related to bone health in older, community dwelling men. At study entry, participants were at least 65 years old, community-dwelling, ambulatory and had not had bilateral hip replacement [18]. In total, 5995 men were recruited into the MrOS Study over approximately two years using population-based mailings in 6 geographic regions in the United States including Birmingham, AL, Minneapolis, MN, Palo Alto, CA, Pittsburgh, PA, Portland, OR and San Diego ,CA [19].

All Caucasian participants with QCT scans were selected for genotyping in the current investigation if they had not reported taking bone altering medications such as androgens, anti-androgens or oral corticosteroids and had not reported being on osteoporosis treatment. Genotyping was completed in two phases using two independent random samples from the MrOS cohort: an initial discovery sample consisting of 862 Caucasian men from the Minneapolis and Pittsburgh clinic sites and a validation sample consisting of 1156 additional Caucasian men from the remainder of the clinic sites (Birmingham, Palo Alto, Portland and San Diego). All men were selected without regard to their BMD level.

## Volumetric BMD

Volumetric BMD was measured using quantitative computed tomography (QCT) of the central skeleton. Due to cost restraints, only the first 650 Caucasian participants from each study site plus all minority participants of the cohort were referred for QCT scans. There were few differences between the men who did and did not receive QCT scans except that those with QCT scans were slightly younger [20].

QCT measurement of the lumbar spine was obtained using an anatomical region 5mm above the L1 superior endplate to 5mm below the L2 inferior endplate. Lumbar spine images were acquired using a setting of 120 kVp, 150 mA, 1-mm slice thickness and 512×512 matrix in spiral reconstruction mode. For this analysis a scan of the entire second lumbar vertebra excluding the transverse processes was measured. Different scanners were used at different clinic sites. Specifically, the images were acquired on a GE Prospeed at the Birmingham clinic, a GE Hispeed Advantage at the Minneapolis clinic, a Phillips MX-8000 at the Palo Alto clinic, a Seimans Somatom +4 at the Pittsburgh clinic, either a Phillips CT-Twin or Toshiba Acquilion at the Portland site, and a Picker PQ-5000 in San Diego.

QCT images were processed at the University of California at San Francisco using a standardized protocol. Each participant scan included a calibration standard of three hydroxyapatite concentrations (150, 75 and 0 mg/cm3) and these were used to convert between Hounsfield units and vBMD. Differences between the clinic sites exist and are statistically adjusted for in all analyses. To derive trabecular volumetric BMD and vertebral cross-sectional area, previously described analytical techniques [21] were employed to orient the vertebrae so that the vertebral cross-sections were obtained in a plane parallel to the two endplates and to segment the vertebral body from the scans. Vertebral trabecular BMD was determined in a region containing most of the trabecular bone in the vertebral body. Vertebral CSA was determined by reconstructing a 10-mm thick cross-section through the mid-vertebral plane. The CSA was taken as the periosteal area of the vertebra excluding the posterior elements and spinous processes.

## Covariates

Participant characteristics including age, medical history and prescription medication use in the past 30 days were obtained by a self-administered questionnaire at the baseline visit. Height was measured by Harpenden stadiometer and weight was measured by either a balance beam scale or a digital scale.

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### Candidate Gene and Single Nucleotide Polymorphism (SNP) Selection

Candidate genes of potential physiological importance were identified from publicly available resources. Specifically, literature searches were conducted using Pubmed, evidence of gene expression in normal human trabecular bone cells was obtained from the Skeletal Gene Database (sgd.nia.nih.gov, no longer available) and the NCBI UniGene database (www.ncbi.nlm.nih.gov/sites/entrez?db=unigene), genes with functions of interest (e.g., "regulation of bone mineralization" or "skeletal development") were obtained from Entrez Gene (www.ncbi.nlm.nih.gov/sites/entrez?db=gene) and Amigo (amigo.geneontology.org/cgi-bin/amigo/go.cgi), genes with a skeletal phenotype in mice were identified from the Jackson Laboratory Mouse Genome Informatics database (http://www.informatics.jax.org/) and the Online Mendelian Inheritance in Man database (www.ncbi.nlm.nih.gov/omim/) was queried for evidence of genes implicated in skeletal conditions in humans. In total, 383 candidate genes were identified for genotyping (Table 1).

Publicly available databases were then interrogated for SNP variation in the region surrounding the candidate gene. For the first phase of genotyping (the discovery sample) two SNP selection strategies were utilized. In the first strategy, genetic variation in the region spanning 30kb upstream and 10kb downstream of each candidate gene was captured by creating a reference SNP panel of variants with a minor allele frequency (MAF) of at least 5% in Phase I of the International HapMap Project (www.hapmap.org) [22]. Tag SNPs were then selected using a pair-wise correlation method ($r^2$ 0.80) [23]. Candidate genes that were clustered near each other on the chromosome were tagged as a unit spanning all loci of interest. For example, IGFBP2 and IGFBP5 are located only 7.6kb from each other on chromosome 2. Since the region of interest for these two candidates overlapped they were tagged as a unit. In the second strategy, potentially functional SNPs that were either non-synonymous coding variants, predicted to alter a putative transcription factor binding site in the promoter region, or a putative exon splice enhancer with MAF 1% were selected for genotyping using the PupaSNP (pupasuite.bioinfo.cipf.es/) and Promolign (polly.wustl.edu/promolign/main.html) databases [23,24].

Follow-up genotyping was conducted in a validation sample for promising SNP associations identified from the discovery sample. Specifically, SNPs with a p-value 0.015 for either vertebral trabecular vBMD or CSA in the discovery sample were genotyped in the validation sample. Additionally, SNPs with a p-value 0.05 in a gene that also had a SNP with p-value 0.015 in the discovery sample were included in the second phase of genotyping.

### Genotyping

Genomic DNA from frozen whole blood specimens was extracted using the Flexigene protocol (Qiagen; Valencia, CA). Genotyping was completed using the Illumina Golden Gate custom assay. For the discovery sample, 37 participant samples were genotyped in duplicate and 4 internal controls were included per plate to ensure reproducibility. We observed 100% reproducibility among the internal controls and 99.9% reproducibility among replicate participant samples. In the validation sample, 26 participant samples and 4 internal controls per plate were included for quality control. We observed 99.9% reproducibility among internal controls and among duplicate participant samples. To ensure maximum genotyping completeness in the validation sample, loci of interest that could not be genotyped using the Illumina Golden Gate assay were genotyped using one of two platforms: the TaqMan allelic discrimination assay system (Applied Biosystems, Foster City, CA) on a 7900HT Real-time PCR instrument with probes and reagents purchased from Applied Biosystems or Sequenom MassARRAY iPLEX Gold technology (Sequenom, Inc; San Diego, CA) with PCR primers purchased from Invitrogen (Carlsbad, CA). We observed

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an average reproducibility of 99.8% and 99.9% for duplicate samples included in the TaqMan (n=26) and Sequenom (n=44) platforms, respectively.

Loci with a minor allele frequency less than 1% in the genotyping sample (N=129), that did not conform to the expectations of Hardy-Weinberg equilibrium (P<0.0005; N=123) or that had a low call rate (<85%; N=241) were excluded from statistical analysis. Individual samples with a low call rate (<85%) (N=14) or that were highly correlated with another sample (indicating relatedness; n=13) were excluded from the analysis.

### Statistical analysis

Although only Caucasian individuals were investigated in this study, population stratification is a potential concern in large-scale genetic association analyses [24]. Stratification was initially assessed using the program Structure [25]. We found little evidence of population stratification in the discovery, validation or pooled samples. Nevertheless, we accounted for potential fine scale population sub-structure by employing a principal components method of analysis using uncorrelated SNPs (r<0.2) to calculate the principal components [26]. The first principal component explained very little variation in trabecular BMD or CSA (<0.5%) in the discovery, validation or pooled samples. None of the principal components explained a substantial proportion of the phenotypic variation in the discovery or validation samples. The first principal component was significantly associated with trabecular vBMD in the pooled sample (p=0.02) but not with CSA in the pooled sample. Therefore, the first principal component was included as a covariate in all subsequent analyses to account for any fine scale substructure.

The distribution of vertebral trabecular BMD and vertebral CSA were assessed for normality and outliers (+/−4 std. dev from the mean) were trimmed for subsequent analysis. Analyses of genotype and the skeletal traits assumed an additive and recessive model of inheritance. Linear regression was used to test for an additive association between the number of copies of the minor allele and the skeletal traits. For the recessive model, regression methods were implemented to determine if individuals having two copies of the minor allele differed from those with the other two genotypes. SNPs with  10 individuals having the rare genotype were not tested for the recessive model to minimize spurious findings based on small sample size. All analyses adjusted for participant age and clinic site in addition to the first principal component of the population sub-structure analysis.

Single nucleotide polymorphisms that were consistently and significantly associated with vertebral phenotypes in the discovery and validation sample were examined further in the pooled sample. In addition to the analyses described above, further adjustments for height and weight were made in the pooled sample to determine if body size attenuated the relationship between genotype and the skeletal traits. Linear regression analysis was used to determine the amount of phenotypic variation explained by all of the significant replicated SNPs. Since SNPs in the same gene region are often correlated, the collinearity of individual SNPs in the model was assessed. One pair of SNPs in the trabecular vBMD analysis (rs6604050 and rs1805113) was highly correlated and the SNP with the most missing genotypes (rs6604050) was dropped from the regression modeling.

## Results

The average age of men in the pooled analysis was 74 years (range, 65 to 100 years). The men in the discovery and validation samples were similar in age. Men in the validation sample had slightly lower body weight, taller stature, and slightly lower BMI, trabecular vBMD and CSA (Table 2).

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In the discovery sample, 4108 of the 4608 attempted SNPs passed quality control criteria and were analyzed for their association with trabecular vBMD and CSA at the lumbar spine. Tag SNPs were selected from Phase I of the International HapMap Project. These SNPs captured 64% (range, 1%-100%) of the common SNPs (MAF >5%) in Phase II of the International HapMap project (www.hapmap.org).

In the discovery sample, 192 SNPs in 66 genes were associated with vertebral trabecular vBMD (p  0.015) and were genotyped in the validation sample. 11 SNPs in 10 different genes (TGFBR3, SOST, KL, CALCR, LEP, CSF1R, PTN, GNRH2, FGFR2, MEPE) were significantly associated with vertebral trabecular vBMD in both the discovery and validation samples and the direction of association was the same in both samples (Table 3). The most significant association in the pooled sample was for rs1877632 in SOST where the less common A/A genotype was associated with 10.2% higher vBMD than the more common G/G genotype (p=0.00005). Each of the 11 replicated SNPs explained between 0.35% and 0.83% of the variation in vBMD in the pooled sample. Regression modeling including all 11 replicated SNPs explained 4.7% of the variation in vBMD after accounting for age, clinic, population substructure, height and weight (Table 3).

In the discovery sample, 98 SNPs in 53 genes were associated with vertebral CSA in the discovery sample (p  0.015) and were selected for genotyping in the validation sample. 5 SNPs in 5 genes (CYP11B1, DVL2, DLX5, WNT4, PAX7) were significantly associated with vertebral CSA in the discovery and validation samples and the direction of association was the same in both samples (Table 4). The most significant association in the pooled sample was for rs6410 in CYP11B1. Men with the less common A/A genotype had 2.6% lower CSA than men with the more common G/G genotype (p=0.0002). The largest difference between genotypes was for rs1215 in DVL2. Men with the less common G/G genotype had 7.4% higher CSA than those with the more common A/A genotype (p=0.002).

Each of the 5 replicated SNPs explained between 0.2% and 0.5% of the variation in vertebral CSA in the pooled sample. Regression modeling including all 5 replicated SNPs explained 1.4% of the variation in vertebral CSA after accounting for age, clinic, population substructure, height and weight (Table 4).

## Discussion

Bone mineral density and cross-sectional area are highly heritable traits that contribute to bone strength. We conducted a systematic pathway-based analysis of candidate genes and vertebral trabecular volumetric BMD (vBMD) and cross-sectional area (CSA) measured by QCT in a well-characterized cohort of older Caucasian men. We identified 11 SNPs in 10 genes that showed consistent association with trabecular vBMD and 5 SNPs in 5 genes that showed consistent association with CSA. We found no overlap between the SNP associations with trabecular vBMD and CSA. Our findings, together with those from mice [9-13] suggest that vertebral trabecular BMD and bone size may be at least to some extent under distinct genetic regulation. A better understanding of the genes and variants involved should advance our understanding of bone strength and individual susceptibility to osteoporosis.

The strongest single SNP association from our analysis was with rs1877632 in the gene encoding sclerostin (*SOST*). This SNP accounted for 0.83% of the total phenotypic variance in vertebral trabecular vBMD with a difference in means of 10% between homozygous genotypes. Sclerostin appears to be primarily expressed in osteocytes, antagonizes canonical Wnt and bone morphogenetic protein signaling, and is a negative regulator of bone formation [27, 28]. SOST knockout mice have increased bone formation rates and markedly

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increased BMD, trabecular number, and trabecular thickness leading to increased bone strength [29]. In contrast, overexpression of normal human *SOST* alleles causes osteopenia in mice [30]. Loss of function mutations or deletions in the human *SOST* gene cause the rare high bone mass disorders, sclerosteosis and van Buchem disease [28]. The associated SNP in our study is located ~31 kb 3′ from the *SOST* gene, but is close to the van Buchem disease deletion which disrupts a distal enhancer element for *SOST* [30]. Several SNPs in this region have been associated with DXA measures areal BMD in older Caucasian women and men [31-33]. Interestingly, one recent study found that 11 SNPs in the *SOST* region were associated with DXA measures of lumbar spine, but not femoral neck, areal BMD [33]. The associated SNP in our study is in high linkage disequilibrium with the 11 SNPs in this study (D'>0.7; $r^2$>0.7).

An intronic SNP within the gene encoding the calcitonin receptor (*CALCR*) was associated with trabecular vBMD. Several studies found associations between a Pro447Leu (rs1801197) polymorphism and osteoporosis related phenotypes including lumbar spine areal BMD [34-40]. However, this non-synonymous substitution does not appear to affect calcitonin binding or signaling of the receptor[41] and was not associated with vertebral measures in our analysis.

A SNP (rs1888057) in the first intron of the gene encoding Klotho (KL) was associated with trabecular vBMD. Klotho deficient mice experience bone loss due to a decreased number of osteoblast progenitors and impaired osteoclast differentiation leading to low-turnover osteopenia [42]. Although we are unaware of previous studies of rs1888057 and osteoporosis phenotypes, associations between polymorphisms in *KL* and DXA measures of areal BMD have been reported in European and Asian women and European men [43-46] including a Phe352Val (rs9536314) substitution and a G→A substitution at position −395. We genotyped rs9536314 in the current study but were unable to document an association with vertebral measures.

Genome-wide association analysis has identified associations between 5 SNPs (rs17131547, rs12403389, rs4658112, rs17131544, rs2087299) within a ~ 7 kb intronic region in *TGFBR3* and DXA measures of lumbar spine areal BMD, which were replicated across diverse ethnic groups [47]. The synonymous coding SNP that was associated with trabecular vBMD in our study (rs1805113) was ~26 to ~33 kb away from this intronic region and only low linkage disequilibrium was observed with these other SNPs ($r^2$<0.16). Recent genome-wide association analyses have not detected associations between SNPs within or flanking *TGFBR3* with areal BMD at a genome-wide significance level [32, 33,48-50].

An SNP (rs727420) located about 3 kb 5′ of *MEPE* was associated with trabecular vBMD in our cohort. *MEPE* encodes matrix extracellular phosphoglycoprotein which is highly expressed in osteocytes as well as osteoblasts [51-54] and this expression is linked to mineralization and bone formation [52,54]. Deletion of *MEPE* in mice causes increased trabecular bone volume, number and thickness and resistance to aging-associated trabecular bone loss [55]. Associations between SNPs located 3′ of *MEPE* and DXA measures of lumbar spine areal BMD were also recently identified by genome-wide association analysis in older Caucasian women and men [50]. The top associated SNP (rs1471403) identified in the genome-wide analysis is in moderate linkage disequilibrium with the associated SNP in our study ($r^2$=0.45).

We also identified several new associations between vertebral trabecular vBMD and SNPs in PTN, FGFR2, LEP, CSF1R, GNRH2. Two SNPs (rs322236, rs3959914) in distinct linkage disequilibrium blocks within the first intron of the gene encoding pleiotrophin (PTN) were associated with vBMD. Pleiotrophin is released by osteoblasts and stimulates

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osteoprogenitor cells [56,57]. A SNP in fibroblast growth factor receptor 2 which mediates FGF signaling was associated with vBMD. Mutations in FGFR2 are associated with familial craniosynostosis and altered bone mineralization [58,59]. Leptin (LEP) is most commonly known for its role in regulating food intake and energy expenditure, but leptin also regulates bone metabolism and bone mass [60]. Colony-stimulating factor 1 deficiency leads to osteopetrosis due to an absence of osteoclasts [61]. Gonadotropin-releasing hormone (GNRH) is a central neuroendocrine regulator of the hypothalamic-pituitary-gonadal axis and may also have regulatory functions in extra-pituitary compartments [62].

We also identified several novel SNP associations with vertebral body CSA which were independent of height and body weight. CYP11B1 encodes 11-beta-hydroxylase, an enzyme involved in adrenal steroidogenesis. Mutations in CYP11B1 that result in 11β-hydroxlase deficiency have been associated with altered bone growth [63]. The genes encoding WNT4 and dishevelled 2 (DVL2) are members of the WNT signaling pathway, which is involved in skeletal development [64]. Targeted inactivation of the distal-less homeobox gene (Dlx5) in mice results in severe retardation in chondrocyte and osteoblast differentiation and axial skeletal abnormalities including stunted bone growth and kinked tail vertebrae [65]. The paired-box transcription factor gene PAX7 regulates the entry of myogenic progenitor cells into a program of skeletal muscle differentiation [66] but a direct influence on bone has not been demonstrated to our knowledge.

Twin and family studies indicate that as much as 60-70% of the normal variation in BMD and bone size may be attributable to inherited variation, although the specific genes and allelic variants involved are not well defined [6-9]. Our analysis identified SNPs in several genes that may explain ~4% of the variance in vertebral trabecular vBMD and ~2% of the variance in vertebral CSA after controlling for age, clinic, population substructure, height and weight. Although the individual and cumulative effect of these SNPs is relatively weak, the effect size is very consistent with emerging data from genome-wide association studies for quantitative traits such as BMD and height [48-50,67]. It is possible that less common SNPs, rare variants and other types of variants such as structural variants will explain additional phenotypic variance in vertebral trabecular vBMD and CSA. In addition, gene-gene and gene-environment interactions may play an equally if not more important role in the regulation of skeletal traits. Indeed, studies in inbred strains of mice indicate that the genetic component to bone strength related traits results from multiple loci each with small to moderate individual effects, and from additive or non-additive interactions of unlinked loci [68,69]. Such analyses will be important to investigate in the future to fully understand the genetic architecture of trabecular vBMD and bone size.

Our analysis has several potential limitations including its focus on only white men and those aged  65 years. Thus, the present results may have limited generalizability, particular among men of other ethnicity, women or younger individuals. Indeed, there is some evidence to suggest that genetic effects on bone related traits may be gender specific [70]. Additional studies will be needed to determine if the genetic associations that we detected are restricted to older white men or are more globally represented across other population groups. Another potential limitation of the analysis is the effect of variations in vertebral marrow fat content on the trabecular BMD measurement [71].

Our study also has several notable strengths. Most candidate gene association studies to date have tested few polymorphisms – oftentimes focusing only on a single polymorphism in a single candidate gene. The current study was more extensive than most previous candidate gene studies, genotyping over 4,000 tagging and potentially functional SNPs within or flanking 383 bone metabolism related genes. Past candidate gene association studies of BMD were also often limited by small sample size, did not attempt to replicate findings, and

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did not assess and control for potential population structure. Further, most genetic studies in humans have focused on trying to identify the genetic variants associated with DXA measures of lumbar spine BMD, which are influenced erroneously by spinal degenerative disease and aortic calcifications and do not provide a separate measure of trabecular BMD. We addressed all of these important limitations in the current study.

In conclusion, the current study identified SNPs in 10 genes that were associated with vertebral trabecular volumetric BMD and SNPs in 5 genes that were associated with vertebral cross-sectional area independent of weight and height. Several of these SNP associations were novel. There was no overlap between SNPs or genes associated with vertebral BMD and cross-sectional area. Our results suggest that there may be distinct loci for vertebral trabecular volumetric BMD and bone size that are independent of body weight and body size.

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## Table 1

Candidate Genes Screened for Association in the Discovery Sample.

| | |
|---|---|
| Chr1 | DVL1, TNFRSF1B, PAX7, ALPL, WNT4, ID3, CSF3R, LEPR, TGFBR3, ERBP, CSF1, HSD3B2, HSD3B1, NOTCH2, GNRHR2, CTSK, IL6R, ZFP67, BGLAP1, MEF2D, NTRK1, RXRG, ADIPOR1, MYOG, HSD11B1, TGFB2, WNT9A, WNT3A |
| Chr2 | ID2, NCOA1, POMC, LTBP1, CYP1B1, LHCGR, PPP3R1, IL1R2, IL1R1, IL1A, IL1B, IL1RN, EN1, GLI2, LCT, NR4A2, TANK, DLX1, DLX2, ATF2, HOXD13, HOXD12, HOXD11, HOXD10, HOXD9, HOXD8, HOXD4, HOXD3, HOXD1, FRZB, GDF8, STAT1, CASP8, FZD7, BMPR2, FZD5, IGFBP2, IGFBP5, WNT10A, IHH, PAX3, IRS1, TWIST2 |
| Chr3 | IRAK2, GHRL, PPARG, WNT7A, THRB, TGFBR2, MYD88, ACVR2B, CTNNB1, PTHR1, WNT5A, POU1F1, GSK3B, CASR, GATA2, TRH, SHOX2, GHSR, TNFSF10, CHRD, AHSG, ACDC, OSTN, HES1 |
| Chr4 | FGFR3, MSX1, BAPX1, PPARGC1A, KDR, GNRHR, GC, BMP2K, BMP3, DMP1, IBSP, MEPE, SPP1, BMPR1B, NFKB1, DKK2, LEF1, EGF, FGF2, SMAD1, SFRP2, CASP3 |
| Chr5 | LIFR, PTGER4, GHR, FST, IL6ST, MAP3K1, CRHBP, MEF2C, APC, HSD17B4, CSF2, PDLIM4, TCF7, HDAC3, FGF1, NR3C1, CSF1R, SPARC, FGF18, MSX2, PROP1 |
| Chr6 | RIPK1, SOX4, TNFA, CYP21A2, RXRB, PPARD, MAPK14, CDKN1A, VEGF, RUNX2, OSTM1, WISP3, ENPP1, CTGF, TNFAIP3, ESR1, IGF2R |
| Chr7 | TWIST, IL6, GPNMB, HOXA1, HOXA2, HOXA3, HOXA4, HOXA5, HOXA6, HOXA7, HOXA9, HOXA10, HOXA11, HOXA13, CRHR2, GHRHR, SFRP4, GLI3, IGFBP1, IGFBP3, EGFR, FZD1, CDK6, CALCR, DLX6, DLX5, CYP3A4, LEP, SMO, NRF1, PTN, TRPV5, CASP2, SHH |
| Chr8 | EGR3, TNFRSF10A, GNRH1, STAR, FGFR1, SFRP1, IKBKB, DKK4, CRH, NCOA2, HEY1, TIEG, FZD6, EXT1, TNFRSF11B, FBXO32, WISP1, CYP11B1 |
| Chr9 | CER1, IFNB1, CNTFR, OSTF1, NTRK2, ROR2, OGN, PTCH, HSD17B3, TGFBR1, TLR4, TRAF1, WDR5, RXRA, NOTCH1, TRAF2 |
| Chr10 | DKK1, EGR2, BMPR1A, CHUK, CYP17A1, FGFR2 |
| Chr11 | IGF2, CDKN1C, DKK3, PTH, CALCA, SOX6, MYOD1, BDNF, TRAF6, EXT2, CNTF, ESRRA, LTBP3, FOSL1, TCIRG1, LRP5, CCND1, FGF3, FADD, CHRDL2, ARRB1, WNT11, FZD4 |
| Chr12 | WNT5B, ADIPOR2, FGF23, NTF3, TNFRSF1A, LRP6, MGP, SOX5, PTHLH, VDR, WNT10B, WNT1, IGFBP6, SP7, CYP27B1, WIF1, IRAK3, MYF6, MYF5, DCN, IGF1, TBX3, TCF1, P2RX7 |
| Chr13 | FLT1, KL, POSTN, TNFSF11 |
| Chr14 | NFKBIA, PAX9, BMP4, ESR2, LTBP2, FOS, TGFB3, TSHR, DLK1, AKT1 |
| Chr15 | CKTSF1B1, CYP19A1, MAP2K1, SMAD3, CYP11A1, CYP1A1, CYP1A2, NTRK3, IGF1R, MEF2A |
| Chr16 | AXIN1, CLCN7, IGFALS, MAPK3, RBL2, CBFB, TRADD, HSD17B2, FOXC2 |
| Chr17 | ALOX15, ARRB2, DVL2, SHBG, PI3K, G-CSF, THRA, IGFBP4, HSD17B1, SOST, MAP3K14, CRHR1, PHOSPHO1, DLX3, COL1A1, TOB1, NOG, TBX2, TBX4, GH1, SOX9 |
| Chr18 | MC2R, SMAD2, SMAD4, TCF4, TNFRSF11A, BCL2, NFATC1 |
| Chr19 | GPR54, MAP2K2, MEF2B, CEBPA, NFKBIB, DLL3, AKT2, TGFB1, FOSB, BAX, LHB, OSCAR |
| Chr20 | GNRH2, BMP2, JAG1, PAX1, ID1, E2F1, GDF5, RBL1, GHRH, SRC, WISP2, MMP9, NCOA3, CEBPB, CYP24A1, BMP7 |
| Chr21 | IFNAR2, IFNAR1, RUNX1, ETS2 |
| Chr22 | COMT, KREMEN1, LIF, CSF2RB, ATF4, GPR24, PPARA |
| X Chr | STS, RPS6KA3, GATA1, AR, BGN, IRAK1, IKBKG |

Gene symbols are presented by chromosome in order across the given chromosome.

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**Table 2**

Participant Characteristics (Mean and Standard Deviation)

| | Discovery Sample N=862 | Validation Sample N=1156 | Pooled Sample (Discovery + Validation) N=2018 |
|---|---|---|---|
| Age | 74 (5.8) | 74 (6.0) | 74 (5.9) |
| Weight (kg) | 85.3 (14.1) | 82.9 (12.5) * | 83.9 (13.3) |
| Height (cm) | 173.6 (6.7) | 174.9 (6.7) * | 174.3 (6.7) |
| BMI | 28.3 (4.1) | 27.1 (3.6) * | 27.6 (3.9) |
| Vertebral Trabecular vBMD (g/cm3) | 0.118 (0.039) | 0.108 (0.038) * | 0.112 (0.039) |
| Vertebral CSA (cm2) | 13.04 (2.01) | 12.67 (1.86) * | 12.83 (1.94) |

* Significant difference between discovery and validation sample ($p<0.001$)

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## Table 3

Single Nucleotide Polymorphisms and Genes Associated with Vertebral Trabecular Volumetric BMD in Older Men.

| | | | | Discovery Sample | | Validation Sample | | Pooled Sample | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Adjustment 1[b] | | Adjustment 1[b] | | Adjustment 1[b] | | Adjustment 2[c] | | |
| Gene | SNP | Alleles | Frequency[a] | β | p-value | β | p-value | β | p-value | β | p-value | $r^2$ |
| TGFBR3 | rs1805113 | A-->G | 0.43 | 0.0069 | 0.0344 [R] | 0.0071 | 0.0104 [R] | 0.0073 | 0.0005 [R] | 0.0070 | 0.0008 [R] | 0.0058 |
| SOST | rs1877632 | G-->A | 0.31 | 0.0134 | 0.0020 [R] | 0.0096 | 0.0077 [R] | 0.0113 | 0.0001 [R] | 0.0109 | 0.0001 [R] | 0.0083 |
| KL | rs1888057 | G-->A | 0.21 | −0.0048 | 0.0430 [A] | −0.0038 | 0.0419 [A] | −0.0042 | 0.0048 [A] | −0.0038 | 0.0082 [A] | 0.0035 |
| CALCR | rs2051748 | A-->G | 0.44 | −0.0048 | 0.0078 [A] | −0.0056 | 0.0340 [R] | −0.0034 | 0.0034 [A] | −0.0031 | 0.0065 [A] | 0.0037 |
| LEP | rs2060715 | G-->A | 0.47 | −0.0085 | 0.0089 [R] | −0.0050 | 0.0414 [R] | −0.0061 | 0.0022 [A] | −0.0052 | 0.0076 [R] | 0.0035 |
| CSF1R | rs2276983 | A-->G | 0.20 | 0.0051 | 0.0236 [A] | 0.0039 | 0.0331 [A] | 0.0044 | 0.0022 [A] | 0.0044 | 0.0018 [A] | 0.0047 |
| PTN | rs322236 | A-->G | 0.23 | 0.0061 | 0.0035 [A] | 0.0037 | 0.0366 [A] | 0.0047 | 0.0005 [R] | 0.0046 | 0.0006 [A] | 0.0056 |
| PTN | rs3959914 | A-->G | 0.33 | −0.0080 | 0.0558 [R] | −0.0101 | 0.0027 [R] | −0.0091 | 0.0006 [A] | −0.0087 | 0.0007 [R] | 0.0057 |
| GNRH2 | rs6138982 | T-->A | 0.23 | 0.0056 | 0.0104 [A] | 0.0101 | 0.0338 [R] | 0.0039 | 0.0050 [A] | 0.0038 | 0.0048 [A] | 0.0041 |
| FGFR2 | rs7090018 | A-->C | 0.27 | 0.0051 | 0.0139 [A] | 0.0037 | 0.0274 [A] | 0.0044 | 0.0008 [A] | 0.0042 | 0.0012 [A] | 0.0053 |
| MEPE | rs727420 | T-->A | 0.27 | 0.0064 | 0.0038[A] | 0.0035 | 0.0343 [A] | 0.0045 | 0.0006 [A] | 0.0044 | 0.0007 [A] | 0.0061 |

Additive and recessive models were tested for each SNP and the regression parameter and p-value from the most significant genetic model (additive ore recessive) is shown.

[A]: p-value from the additive model;

[R]: p-value from recessive model

[a)]Minor allele frequency in the pooled sample

[b)]Adjustment 1: Age, clinic site, population sub-structure

[c)]Adjustment 2: Age, clinic site, population sub-structure, height, body weight

[d)]Amount of variation explained after adjusting for age, site, population sub-structure, height, body weight

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## Table 4

Single Nucleotide Polymorphisms and Genes Associated with vertebral Cross-Sectional Area Among Older Men.

| | | | | Discovery Sample | | Validation Sample | | Pooled Sample | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Adjustment 1 | | Adjustment 1 | | Adjustment 1 | | Adjustment 2 | | |
| Gene | SNP | Alleles | Frequency | β | p-value | β | p-value | β | p-value | β | p-value | $r^2$ |
| CYP11B1 | rs6410 | G-->A | 0.46 | −0.4771 | 0.0036 $^R$ | −0.2755 | 0.0311 $^R$ | −0.3686 | 0.00025 $^R$ | −0.2896 | 0.0018 $^R$ | 0.0048 |
| DVL2 | rs1215 | A-->G | 0.15 | 1.1240 | 0.0059 $^R$ | 1.0219 | 0.0192 $^R$ | 0.9254 | 0.0019 $^R$ | 0.1684 | 0.0279 $^A$ | 0.0020 |
| DLX5 | rs6960249 | A-->C | 0.45 | −0.4086 | 0.0134 $^R$ | −0.2577 | 0.0546 $^R$ | −0.3035 | 0.0035 $^R$ | −0.2031 | 0.0336 $^R$ | 0.0021 |
| WNT4 | rs7517829 | A-->G | 0.42 | 0.2154 | 0.0231 $^A$ | 0.2712 | 0.0494 $^R$ | 0.3034 | 0.0051 $^R$ | 0.2741 | 0.0059 $^R$ | 0.0046 |
| PAX7 | rs2236817 | C-->A | 0.12 | 0.4623 | 0.0009 $^A$ | 0.9597 | 0.0203 $^R$ | 0.2527 | 0.0040 $^A$ | 0.2196 | 0.0066 $^A$ | 0.0050 |

Additive and recessive models were tested for each SNP and the regression parameter and p-value from the most significant genetic model (additive ore recessive) is shown.

$^A$: p-value from the additive model;

$^R$: p-value from recessive model

b) Minor allele frequency in the pooled sample

b) Adjustment 1: Age, clinic site, population sub-structure

c) Adjustment 2: Age, clinic site, population sub-structure, height, body weight

d) Amount of variation explained after adjusting for age, site, population sub-structure, height, body weight