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Joint study of two genome-wide association meta-analyses identified 20p12.1 and 20q13.33 for bone mineral density★

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Abstract

In the present study, aiming to identify loci associated with osteoporosis, we conducted a joint association study of 2 independent genome-wide association meta-analyses of femoral neck and lumbar spine bone mineral densities (BMDs): 1) an in-house study of 6 samples involving 7484 subjects, and 2) the GEFOS-seq study of 7 samples involving 32,965 subjects. The in-house samples were imputed by the 1000 genomes project phase 3 reference panel. SNP-based association test was applied to 7,998,108 autosomal SNPs in each meta-analysis, and for each SNP the 2 association signals were then combined for joint analysis and for mutual replication. Combining the evidence from both studies, we identified 2 novel loci associated with BMDs at the genome-wide significance level ($\alpha = 5.0 \times 10^{-8}$): 20p12.1 (*rs73100693* $p = 2.65 \times 10^{-8}$, closest gene *MACROD2*) and 20q13.33 (*rs2380128* $p = 3.44 \times 10^{-8}$, *OSBPL2*). We also replicated 7 loci

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that were reported by two recent studies on heel and total body BMD. Our findings provide useful insights that enhance our understanding of bone development, osteoporosis and fracture pathogenesis.

Keywords

Osteoporosis; Bone mineral density; Genome-wide association study; 20p12.1; 20q13.33

1. Introduction

Osteoporosis is a common bone metabolic disease among the elderly. It is characterized by low bone mass and micro-architectural deterioration of bone tissue. People with osteoporosis are predisposed to fragility fracture [1]. Fifteen per cent of white people over 50 years old suffer osteoporotic fracture in their remaining lifetime, and the projected costs expended on this disease will exceed \$25 billion in the United States alone in year 2025 [2]. Therefore, there is no doubt that osteoporosis and osteoporotic fracture will increase economic, clinical and social burden substantially.

Bone mineral density (BMD) is the gold standard for clinical assessment of osteoporosis and fracture risk. BMD is highly inherited with heritability as high as 60–80% [3]. Genome-wide association study (GWAS) and their meta-analysis are a powerful approach to identify common variants associated with complex traits including BMD. In the past decade, over one hundred BMD loci have been discovered by a number of GWAS studies [4–7], explaining 12% of BMD heritability [5]. However, the explained heritability only accounts for one-third of GWAS-attributable heritability [5]. Therefore, the majority of GWAS-attributable heritability is yet to be discovered. Future GWAS endeavors with enlarged sample size and in particular more efficient analytical strategies are warranted.

There exist a number of GWAS samples in bone research community. The UK Biobank sample and the genetic factors for osteoporosis (GEFOS) consortium represent two largest collections of existing GWAS samples. Besides them, there also exist several sporadic GWAS samples. Pooling large samples and these sporadic samples together will enlarge the sample size substantially.

In this study, aiming to identify novel loci associated with BMD, we carry out a joint study of 2 independent GWAS meta-analyses of BMDs at femoral neck (FNK) and lumbar spine (SPN). The first is an in-house study of 6 GWAS samples ($N = 7484$), and the second is the publicly available GEFOS-seq study summary results ($N = 32,965$). SNP-based meta-analysis is applied to the in-house samples, and association signals are then combined with the GEFOS-seq summary results for joint analysis and for mutual replication. This approach integrates the information from an expanded size of GWAS samples, and therefore has the potential to improve statistical power of gene mapping.

2. Materials and methods

This study incorporated samples from multiple research and/or clinical centers. All samples were approved by the respective institutional ethics review boards, and all participants provided written informed consent.

The study design of the present study is displayed in Fig. 1. Briefly, we performed a GWAS meta-analysis of spine (SPN-) and femoral neck (FNK-) BMDs in 6 samples of diverse ancestries. We also collected the publicly available GEFOS-seq European population meta-analysis summary results of the same traits. We combined the two studies for joint analysis and for mutual replication.

2.1. Part 1: in-house meta-analysis

We previously conducted a GWAS meta-analysis of FNK- and SPN-BMDs in 7 samples of diverse ancestries [8]. The use of diverse ancestral samples was solely for maximizing sample size and statistical power of association test, with the hypothesis that diverse populations share common genetic effects to complex traits. Of these 7 samples, the Framingham heart study (FHS) overlaps with the GEFOS-seq study. To keep the in-house analysis independent of the GEFOS-seq study, the FHS sample was excluded. Sample collection procedure, quality control (QC) and statistical analyses were described elsewhere [8]. Briefly, the first sample comprises 1000 unrelated subjects from the Omaha osteoporosis study (OOS). The second sample comprises 2286 unrelated subjects from the Kansas city osteoporosis study (KCOS). The third sample comprises 1627 unrelated subjects of Chinese Han ancestry from the China osteoporosis study (COS). The forth sample is the Indiana fragility study (IFS) that was accessed through the dbGAP. The IFS is a cross-sectional cohort comprising 1493 premenopausal sister pairs of European ancestry [9]. Both the fifth and sixth samples are from the Women's health initiative (WHI) observational study that was accessed through the dbGAP. The WHI is a partial factorial randomized and longitudinal cohort with >12,000 genotyped women aged 50–79 years, of African-American or Hispanic ancestry [10]. Participants in both populations were chosen based on their availability of BMD phenotypes. The fifth sample comprises 845 subjects of African-American ancestry (WHI-AA), and the sixth sample comprises 446 subjects of Hispanic ancestry (WHI-HIS).

BMD was measured with DXA bone densitometer (Lunar Corp., Madison, WI, USA; or Hologic Inc., Bedford, MA, USA), following the manufacturers' protocols. Covariates (including gender, age, age squared, height and the top 10 principal components derived from genome-wide genotype data) were screened for significance with the step-wise linear regression model. Raw BMD value was adjusted by significant covariates, and the residuals were normalized by inverse quantiles of standard normal distribution.

All individual samples were genotyped by high-throughput SNP genotyping arrays (Affymetrix Inc., Santa Clara, CA, USA; or Illumina Inc., San Diego, CA, USA), following the manufacturers' protocols. QC within each sample was implemented at both individual level and SNP level. At the individual level, sex compatibility was checked by imputing sex from X-chromosome genotype data with PLINK [11]. Individuals of ambiguous imputed sex

or of imputed sex inconsistent with reported sex were removed. At the SNP level, SNPs violating the Hardy-Weinberg equilibrium (HWE) rule ($p\text{-value} < 1.0 \times 10^{-5}$) were removed. Population outliers were monitored by principal components derived from genome-wide genotype data, and were removed if present.

Each GWAS sample was imputed by the 1000 genomes project phase 3 sequence variants (as of May 2013) [12]. Genotype imputation reference panels of 503 individuals of European ancestry, of 504 individuals of East Asian ancestry, of 661 of African ancestry, and of 347 of admixed American ancestry, were downloaded from the project website. Each GWAS sample was imputed by respective reference panel with the closest ancestry. Haplotypes of bi-allelic variants, including SNPs and bi-allelic insertions/deletions, were extracted to form reference panels for imputation. As a QC procedure, variants with zero or one copy of minor alleles were removed. Imputation was implemented with FISH [13], a fast and accurate diploid genotype imputation algorithm that we previously developed.

Each GWAS sample was tested for association between normalized phenotype residuals and genotyped and imputed genotypes under an additive mode of inheritance. For the unrelated samples, association was examined by the linear regression model with MACH2QTL [14], in which allele dosage was taken as the predictor for the phenotype. For the familial sample IFS, a mixed linear model was used to account for genetic relatedness within each pedigree [15].

Association summary statistics from individual GWAS samples were combined to perform inverse variance weighted fixed-effects meta-analysis with METAL [16]. Between-study heterogeneity effect was measured by I^2 and Q p-value [17]. Genomic control inflation factor was estimated in both individual samples and in meta-analysis [18].

2.2. Part 2: GEFOS-seq meta-analysis summary results

The GEFOS-seq consortium recently conducted a sequencing- and imputation-based GWAS meta-analysis of FNK- and SPN-BMDs in over 50,000 (discovery + replication) subjects of European ancestry. Details of the study samples, QC and statistical analyses were described in the consortium publication [4]. In brief, the GEFOS-seq study performed whole-genome sequencing in 2882 subjects from 2 cohorts in the UK10K project, whole-exome sequencing in 3549 subjects from 5 cohorts, and genome-wide genotype imputation in 26,534 subjects. All samples were meta-analyzed, and the summary results of the discovery stage (up to 32,965 subjects) were released to be publicly available. We downloaded the summary results from the consortium website (<http://www.gefos.org/?q=content/data-release-2015>).

2.3. SNP inclusion criteria

In the part 1 in-house meta-analysis, we adopted the following QC criteria to include SNPs into gene based analysis: genetic heterogeneity measure $I^2 < 50\%$.

In the part 2 GEFOS-seq summary results, we adopted the following QC criteria: 1) genetic heterogeneity measure $I^2 < 50\%$; 2) minor allele frequency (MAF) $> 1\%$.

The in-house samples are of diverse populations, and different populations may have different MAFs. Therefore we did not apply a unified MAF criteria in the in-house samples. In contrast, all the GEFOS samples are of the European population. Therefore we applied a MAF cutoff 1% to focus on common to less common SNPs in the European population.

SNPs present in both parts were retained for subsequent joint analysis, so that the included SNPs in both studies were identical.

2.4. Joint analysis

We again applied an inverse variance weighted meta-analysis for joint analysis of the two studies. Given the two regression coefficients β_1 and β_2 and their standard errors se_1 and se_2 , the joint analysis statistics were defined as

$$\beta = \frac{\frac{\beta_1}{se_1^2} + \frac{\beta_2}{se_2^2}}{\frac{1}{se_1^2} + \frac{1}{se_2^2}}, \quad se = \sqrt{\frac{1}{\frac{1}{se_1^2} + \frac{1}{se_2^2}}}.$$

Under the null hypothesis of no association, the statistic $z = \beta/se$ approximately follows a standard normal distribution. GWS level was set at 5.0×10^{-8} .

2.5. In silico replication analysis

Kemp et al. [5] recently reported a largest genome-wide association study (GWAS) of BMD as estimated by quantitative ultrasound of the heel in 142,487 individuals from the UK Biobank. The summary results are publicly available in the GEFOS website. We downloaded the summary statistics and performed *in silico* replication analysis in the UK Biobank study. Replication success criteria was defined as nominally significant p-value 0.05 at the same effect direction.

2.6. cis-eQTL analysis

We performed cis-eQTL analysis by checking the association between SNP polymorphisms and nearby gene expressions. The datasets we used include the following three empirical studies. The first one is the GTEx project dataset [19]. The GTEx project collected and RNA-sequenced multiple human tissues (up to 11,614) from donors who were also densely genotyped, and analyzed associations between SNPs and global RNA expression within individual tissues. The second one is the Westra et al.'s study [20]. This study performed an eQTL meta-analysis in 5311 peripheral blood subjects from 7 studies, and performed replication analysis in another 2775 subjects. Significant cis-eQTL results were downloaded from the study website (<http://www.genenetwork.nl/bloodeqtlbrowser/>). The third one is the Kabachiev et al.'s study [21]. This study performed cis- and trans-eQTL analyses in ileal biopsy specimens from 173 subjects. Our eQTL analyses were mainly performed via the Haploreg web portal [22].

2.7. Regulation pattern exploration

To further explore potential regulation patterns at the identified regions, we used the UCSC web genome browser to annotate them by CHIP-seq experiments of the ENCODE project [23]. We checked H3K27AC histone mark activity, DNase hypersensitivity site (DHS) prediction and transcription factor binding site activity. H3K27AC is the acetylation of lysine 27 of the H3 histone protein. It is often found near active regulatory regions and is thought to enhance transcription. We used the following 7 cell lines rendered at the UCSC genome browser by default: GM12878 lymphoblastoid cell, H1-hESC human embryonic stem cell, HSMM human skeletal muscle myoblasts, HUVEC human umbilical vein endothelial cells, K562 human erythroleukemic cell line, NHEK normal human epidermal keratinocytes (NHEK), and NHLF normal human lung fibroblasts.

3. Results

3.1. Joint meta-analysis

Basic characteristics of the in-house analysis samples are listed in Table 1. The samples were genotyped by high-throughput SNP genotyping arrays. Each GWAS sample was imputed by the 1000 genomes project phase 3 sequence variants (as of May 2013) [12]. Imputation was performed with FISH [13], a fast and accurate diploid genotype imputation algorithm that we previously developed.

We used 10 principal components (PCs) to correct for population stratification in each individual study. Overall genomic control inflation factors are small in both individual samples ($\lambda = 0.87\text{--}0.99$) and in meta-analyses of SPN- and FNK-BMDs ($\lambda = 1.03$ and 1.02), implying the limited effects of potential population stratification. As a quality control (QC) step, we required meta-analysis genetic heterogeneity measure $I^2 < 50\%$.

The GEFOS-seq summary results include a total of 10,586,901 SNPs. We applied the following 2 inclusion criteria: genetic heterogeneity measure $I^2 < 50\%$ and minor allele frequency (MAF) $> 1\%$. After QC, 9,315,050 SNPs are left. A total of 7,998,108 SNPs are present in both studies, and were used for subsequent analyses.

Joint analysis of both studies identified 45 loci that are associated with the two BMD traits (Supplementary Table 1). Many of these loci are significant at the GWS level in the GEFOS-seq sample alone, and are further replicated by the in-house analysis. Nine loci are significant in the combined analyses, but not in either the GEFOS study or the in-house study, at the GWS level. Of them, 4 (10q26.13, 11q14.2, 12q21.33 and 15q22.33) were reported by the UK Biobank study. During the revision of this manuscript, another 3 loci (2p13.3, 7q22.1, 15q14) were reported by Medina-Gomez et al. [7]. The remaining 2 loci locate outside 1 MB from either direction of previously reported lead SNPs: 20p12.1 and 20q13.33. Manhattan plot is displayed in Fig. 2, and main results of the 2 lead SNPs are listed in Table 2. Regional plot drawn by LocusZoom [24] is displayed in Fig. 3.

3.2. In silico replication analysis

The 2 lead SNPs at the identified novel loci were subjected to *in silico* replication analysis in the UK Biobank study. Both SNPs are consistent in effect direction between the UK Biobank study and the present study. The p-value of *rs73100693* is close to significant ($p = 0.09$). Considering the one-side nature of replication effort, it is indeed significant (one sided $p = 0.04$). *rs2380128* is not significant (two-sided $p = 0.71$).

3.3. Newly identified loci

3.3.1. 20p12.1 (MACROD2)—Two SNPs *rs73100693* and *rs73100690* are associated with FNK-BMD, with the lead SNP being *rs73100693* ($p_1 = 0.02$, $p_2 = 9.80 \times 10^{-7}$, $p_{12} = 2.65 \times 10^{-8}$). It is located in the intron region of the *MACROD2* (MACRO domain containing 2) gene. Previous study of femoral microCT screen of knockout mice *versus* normal controls identified *MACROD2* as a novel candidate gene regulating bone [25].

3.3.2. 20q13.33 (OSBPL2)—A total of 4 SNPs are associated with SPN-BMD, with the lead SNP being *rs12481249* ($p = 2.90 \times 10^{-8}$). However, 3 SNPs including *rs12481249* present modest heterogeneity effects ($I^2 = 28.7$ – 31.4%), though none is significant. The last SNP *rs2380128* ($p_1 = 6.43 \times 10^{-4}$, $p_2 = 1.46 \times 10^{-5}$, $p_{12} = 3.44 \times 10^{-8}$, $I^2 = 0.0\%$) is located in the intron region of the *OSBPL2* (Oxysterol Binding Protein Like 2) gene.

3.4. Previously reported loci

The joint analysis also identified additional 7 loci that are not significant in either individual analysis at the GWS level. These loci were independently reported by the UK Biobank study and/or the study of Medina-Gomez et al. [7], during the preparation and revision of the present manuscript. These loci include 2p13.3 (*rs10048745*, $p_1 = 0.02$, $p_2 = 1.03 \times 10^{-6}$, $p_{12} = 3.57 \times 10^{-8}$, closest gene *ARHGAP25*), 7q22.1 (*rs34670419*, $p_1 = 0.09$, $p_2 = 9.28 \times 10^{-8}$, $p_{12} = 1.01 \times 10^{-8}$, *ZKSCAN5*), 10q26.13 (*rs2292626*, $p_1 = 0.04$, $p_2 = 3.07 \times 10^{-7}$, $p_{12} = 1.95 \times 10^{-8}$, *PLEKHA1*), 11q14.2 (*rs664398*, $p_1 = 5.35 \times 10^{-3}$, $p_2 = 1.83 \times 10^{-6}$, $p_{12} = 1.83 \times 10^{-8}$, *TMEM135*), 12q21.33 (*rs4573745*, $p_1 = 3.22 \times 10^{-4}$, $p_2 = 1.45 \times 10^{-5}$, $p_{12} = 2.05 \times 10^{-8}$, *LOC338758*), 15q14 (*rs7176106*, $p_1 = 4.40 \times 10^{-3}$, $p_2 = 2.27 \times 10^{-6}$, $p_{12} = 2.16 \times 10^{-8}$, *TMC5A*) and 15q22.33 (*rs11631380*, $p_1 = 5.60 \times 10^{-3}$, $p_2 = 1.65 \times 10^{-6}$, $p_{12} = 2.11 \times 10^{-8}$, *SMAD3*).

3.5. eQTL analysis

We performed eQTL analysis of the identified SNPs in 3 empirical studies. Of the 2 newly identified SNPs, *rs2380128* is associated with the expression of an unknown function gene *RP11-157P1.5* in two heart tissues (atrial appendage and left ventricle, $p = 2.10 \times 10^{-11}$ and 8.60×10^{-11}) and in artery aorta ($p = 8.30 \times 10^{-6}$) of the GTEx project datasets. The association is also observed for two neighboring genes *LAMA5* ($p = 6.30 \times 10^{-6}$) and *OSBPL2* ($p = 1.70 \times 10^{-5}$) in esophagus mucosa. For *rs73100693*, one of its neighboring SNPs *rs12625875* (LD $r^2 = 0.79$, 29.7 kb) is associated with *FLRT3* expression ($p = 2.10 \times 10^{-6}$) in peripheral blood monocyte cells [21].

Of the previously reported loci, *rs10048745* is significantly associated with *ARHGAP25* expression in three brain tissues (anterior cingulate cortex, cortex and frontal cortex) of the

GTEX datasets. The most significant association is observed in anterior cingulate cortex region ($p = 3.70 \times 10^{-9}$). *rs34670419* is associated with the expressions of up to 4 genes in 4 tissues (*CYP3A7* $p = 3.60 \times 10^{-6}$ in adrenal gland, *OR2AE1* $p = 1.00 \times 10^{-5}$ in lower leg skin, *ZKSCAN5* $p = 2.50 \times 10^{-5}$ in suprapubic skin, and *GSI-259H13.2* $p = 5.50 \times 10^{-5}$ in esophagus mucosa). *rs2292626* is associated with *PLEKHA1* expression in whole blood ($p = 2.41 \times 10^{-40}$). At last, *rs7176106* is associated with *RP11-1008C21.1* expression ($p = 3.40 \times 10^{-3}$) in whole blood.

3.6. Regulation pattern exploration

We explored potential regulation patterns at the identified regions by checking the CHIP-seq experiments of the ENCODE project for H3K27AC histone mark activity, DNase hypersensitivity site (DHS) prediction and transcription factor binding site activity. Both regions (200 kb each) encompassing the 2 lead novel SNPs are predicted to bind to transcription factors. In addition, *rs2380128* is predicted to have DHS activity.

4. Discussion

In the present study, we have performed a joint study of 2 genome-wide association meta-analyses. By combining the GEFOS-seq results and the in-house analysis, we have identified 2 novel loci that are associated with FNK- and/or SPN-BMDs. Extensive functional annotations have supported the regulatory roles of the identified variants in bone metabolism.

It is clear that the lead SNPs at the identified novel loci are associated with the expression level of nearby genes, as shown by eQTL analysis. The regulation mechanism may involve binding to transcription factors. At gene level, it would be informative to evaluate the role of relevant genes and pathways in bone biology taking all the identified loci into account. After prioritizing seemingly causal genes at all the identified loci by DEPICT [26], and then constructing a protein-protein interaction network with STRING [27], none of the genes identified at the novel loci interacts with other bone regulating genes (Supplementary Fig. 1). This implies the newly identified genes participate into pathways of unknown function to bone biology.

The newly identified genes may imply new bone modulating mechanisms. At 20p12.1, the gene *MACROD2* is of particular interest. It is not a member of classical bone regulating pathways. By surveying the international mouse phenotyping consortium (IMPC) website (<http://www.mousephenotype.org>) [28], we found that homozygous knockout mice of *MACROD2* have increased BMD ($p = 2.66 \times 10^{-6}$), decreased body length ($p = 3.48 \times 10^{-8}$) and lean mass (5.08×10^{-10}) compared to wild-type controls. These observations are consistent with the study of Adams et al. [25], who used an un-biased high-throughput phenotypic screen of transgenic mice and observed that knockout mice of *MACROD2* presented shorter femur length compared to controls [25]. Together, these animal models imply that *MACROD2* may participate into bone regulation pathway in an unknown way.

At 20q13.33, a cluster of related SNPs are associated with SPN-BMD. Though *rs6143006* is the lead SNP, a modest level of heterogeneity is observed on it. We reported *rs2380128* as

tag SNP. In a previous study by Niu et al. [29], two SNPs *rs6089342* and *rs6121978* annotated as microRNA target sites at this locus are associated with SPN-BMD, though neither p-value is significant at the GWS level. Both SNPs are in strong LD level with *rs2380128* (*rs6089342* $r^2 = 0.88$, *rs6121978* $r^2 = 0.88$). In the present study, the p-values of both SNPs are close to significant at the GWS level ($p = 8.60 \times 10^{-8}$ and 9.16×10^{-8}). Their associations imply that the regulation pattern at this locus might involve binding to microRNA.

Our joint analysis replicated 4 loci reported by the UK Biobank study of heel BMD. These loci were not reported by previous GEFOS studies. Among them, 15q22.33 contains a gene *SMAD3* that is a member of several pathways participating bone metabolism, such as the WNT signaling regulation pathway and the osteoblast differentiation regulation pathway. In addition, it is also a component of the *TGF*-beta signaling pathway, acting as transducers of signals from *TGF*-beta receptors [30]. In the canonical signaling pathway, receptor-activated *SMADs* are phosphorylated on C-terminal serines by the type I *TGF*-beta receptor, after which they partner with *SMAD4*. The resulting complex translocates to the nucleus where it regulates the activities of target genes [30]. In the absence of *SMAD3*, *TGF*-beta can no longer inhibit the differentiation of osteoblast [31]. Mice with targeted deletion of *SMAD3* are osteopenic with less cortical and cancellous bone compared with wild-type littermates [32]. Another region 11q14.2 was previously reported to be associated with bone related traits. Moayyeri et al. reported that *rs597319* was associated with broadband ultrasound attenuation (BUA) and velocity of sound (VOS), another two alternative bone properties [33]. The association was confirmed in a recent large-scale GWAS meta-analysis [34] and in an East-Asian population [35].

Our analysis also replicated 3 loci reported by the latest study of Medina-Gomez et al. [7], for total body BMD. At 2p13.3, the lead SNP *rs10048745* is located at the 5'-UTR region of *ARHGAP25* gene, and is associated with its expression. However, the role of *ARHGAP25* played in bone biology is largely unknown. Noticeably, a neighboring gene *BMP10* at this locus is a well-known bone candidate gene. At 7q22.1, the lead SNP *rs34670419* is located at the 3'-UTR region of the *ZKSCAN5* gene. In a previous study, Zhai et al. [36] reported the association of another SNP *rs11761528* at the same locus with dehydroepiandrosterone sulphate (DHEAS) level. *rs34670419* and *rs11761528* are in modest level of LD ($r^2 = 0.49$), therefore the two association signals might emerge from same source. The functional relevance of *ZKSCAN5* to bone development remains unclear. We notice that a cluster of cytochrome P450 family 3 subfamily A (CYP3A) members, such as *CYP3A4*, *CYP3A5*, and *CYP3A7*, are also localized at this locus. In our eQTL analysis, *rs34670419* is associated with the *CYP3A7* expression. The associations of polymorphisms in *CYP3A7* with both BMD and DHEAS have also been extensively studied [37,38].

Certain limitation exists in the present study. The in-house analysis combined samples of diverse ancestral populations. This may complicate the interpretation of the results in the presence of population specificity for associated loci. The purpose of including trans-ethnic samples is to maximize sample size and statistical power of association testing, under the hypothesis that phenotypic traits in different ethnic populations may have a common genetic basis.

In conclusion, by performing a joint study of 2 genome-wide association meta-analyses, we have identified 2 novel loci associated with BMD. Our results provide useful insights that enhance our understanding of bone development, osteoporosis and fracture pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Workflow of the present study. Two independent GWAS meta-analyses of femoral neck and lumbar spine BMDs were collected. One was from the in-house analysis ($N = 7484$) and the other was downloaded from the GEFOS-seq website ($N = 32,965$). SNPs present in both studies after QC were selected for joint association analysis.

Fig. 2. Manhattan plot. Known loci were retrieved from the EBI GWAS catalog website. Top SNPs and their flanking 1 MB regions to either direction were marked in grey.

Fig. 3.
Regional plot.

Table 1

Basic characteristics of the in-house analysis samples.

Sample	Source	Anc.	N	Female (%)	Age	Height (m)	FNK-BMD (g/cm ²)	SPN-BMD (g/cm ²)	DXA machine
OOS	In-house	EUR	990	49.9	50.2 (18.3)	1.71 (0.10)	0.81 (0.15)	1.03 (0.16)	Hologic
KCOS	In-house	EUR	2219	76.7	51.5 (13.7)	1.66 (0.08)	0.79 (0.15)	1.02 (0.16)	Hologic
IFS	dbGAP	EUR	1483	100.0	32.7 (7.2)	1.65 (0.06)	-	-	Lunar
COS	In-house	EAS	1539	50.6	34.7 (13.4)	1.64 (0.08)	0.81 (0.13)	0.95 (0.13)	Hologic
WHI-AA	dbGAP	AFR	849	100.0	61.2 (7.3)	1.63 (0.06)	0.82 (0.14)	1.05 (0.17)	Hologic
WHI-HIS	dbGAP	AMR	446	100.0	60.1 (7.5)	1.58 (0.06)	0.73 (0.11)	0.97 (0.16)	Hologic

Notes: OOS, Omaha osteoporosis study; KCOS, Kansas-city osteoporosis study; IFS, Indiana fragility study; COS, Chinese osteoporosis study; WHI-AA, Women's health initiative study African- American sub-sample; WHI-HIS, Women's health initiative study Hispanic sub-sample. EUR, European population; EAS, East Asian population; AFR, African population; AMR, Admixed American population. Sample size after quality control was reported.

Table 2

Main results of the 2 identified novel loci.

Site	Locus	Tag SNP	Alleles	EAF	Gene	In-house analysis			GEFOS			Combined		
						(N = 7484)			(N = 32,965)			(N = 40,449)		
						BETA	SE	P	BETA	SE	P	BETA	SE	P
FNK	20p12.1	rs73100693	A/T	0.22	MACROD2	0.05	0.02	0.02	0.05	0.01	9.80×10^{-7}	0.05	0.01	2.65×10^{-8}
SPN	20q13.33	rs2380128	T/C	0.54	OSBPL2	0.06	0.02	6.44×10^{-4}	0.04	0.01	1.46×10^{-5}	0.04	0.01	3.44×10^{-8}
														Q_p

Notes: FNK, femoral neck; SPN, lumbar spine. Alleles were presented as effect allele/other allele. EAF: effect allele frequency in the European population. I2: heterogeneity I2 measure; Q_p: heterogeneity Q p-value. p-Values significant at the genome-wide significance level ($\alpha = 5.0 \times 10^{-8}$) were marked in bold.