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Genome-wide association study of lncRNA polymorphisms with bone mineral density

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Abstract

Recent studies suggested that long noncoding RNAs (lncRNAs) were widely transcribed in the genome, but their potential roles in the genetic complexity of human disorders required further exploration. The purpose of the present study was to explore genetic polymorphisms of lncRNAs associated with bone mineral density (BMD) and its potential value. Based on the lncRNASNP database, 55,906 lncSNPs were selected to conduct a genome-wide association study meta-analysis among 11,140 individuals of seven independent studies for BMDs at femoral neck (FN), lumbar spine, and total hip (HIP). Promising results were replicated in Genetic Factors for Osteoporosis Consortium (GEFOS Sequencing, $n = 32,965$).

We found two lncRNA loci that were significantly associated with BMD. *MEF2C* antisense RNA 1 (*MEF2C*-AS1) located at 5q14.3 was significantly associated with FN-BMD after Bonferroni correction, and the strongest association signal was detected at rs6894139 ($P = 3.03 \times 10^{-9}$). *LOC100506136* rs6465531 located at 7q21.3 showed significant association with HIP-BMD ($P = 7.43 \times 10^{-7}$). *MEF2C*-AS1 rs6894139 was replicated in GEFOS Sequencing with P -value of 1.43×10^{-23} . Our results illustrated the important role of polymorphisms in lncRNAs in determining variations of BMD and provided justification and evidence for subsequent functional studies.

Keywords

Bone mineral density; Genome-wide association study; Long noncoding RNAs; Meta-analysis

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AUTHOR CONTRIBUTIONS

TLJ, DHW, CXD, ZQ: study design; ZQ, WKH, LK, YH, ZL, SH, QT, ZLJ: data collection and analysis; ZQ, TLJ, DHW: manuscript preparation.

CONFLICTS OF INTEREST

The authors state that they have no conflict of interest.

1 | INTRODUCTION

Osteoporosis is a prevalent skeletal disorder characterized by excessive bone loss and skeletal fragility thus greatly increased risk of fractures (Liu, Zhang, Papasian, & Deng, 2014; Zheng et al., 2015). Bone mineral density (BMD) as the major predictor of osteoporotic fractures is highly heritable. Recently, large-scale meta-analysis of genome-wide association studies (GWAS) have identified a great quantity of genetic variants, both common and rare, as well as microRNA variants, associated with BMD and osteoporosis related traits (Lei, Papasian, & Deng, 2011; Liu et al., 2014; Stykarsdottir et al., 2013; Zheng et al., 2015).

Long noncoding RNAs (lncRNAs), which were previously thought to be genome “transcriptional noise,” have come to gain attention for their significant functional and structural roles and have been proposed to be key regulators of chromatin reconstitution, transcriptional regulation, posttranscriptional processing, and of protein metabolism (Mercer, Dinger, & Mattick, 2009; Ponting et al., 2009). LncRNAs, which are defined as noncoding RNA (ncRNA) transcripts longer than 200 nucleotides, can be divided into five broad categories: sense, antisense, bidirectional, intronic, and intergenic (Mattick & Rinn, 2015). They are involved in diverse biological process and pathogenesis of disorders, such as osteoblast differentiation (Zhu & Xu, 2013; Zhuang et al., 2015), osteosarcoma (Cong, Li, Jing, & Li, 2016), and cardiovascular diseases (Klattenhoff et al., 2013). Recent studies have proved that lncRNAs can physically interact with other genes to regulate their expressions. For example, the lncRNA-ANCR (anti-differentiation ncRNA) catalyzed Runx2 H3K27me3 to suppress Runx2 expression and osteoblast differentiation (Zhu & Xu, 2013); upregulation of lncRNA maternally expressed gene 3 facilitated osteogenic differentiation of mesenchymal stem cells by targeting BMP4 transcription (Zhuang et al., 2015).

GWAS found that a greater than anticipated part of the identified variants were not located in the coding region, rather mapped to noncoding regions (Hindorff et al., 2009; Jin et al., 2011). Recently, accumulating evidence has suggested an influential role of genetic polymorphisms of lncRNAs with diseases' occurrence and progress, mostly in cancer and carcinogenesis (Jin et al., 2011). Jin et al. (2011) performed a meta-analysis using the 1000 Genomes data and found that prostate cancer susceptibility loci were abundant in lncRNAs. A recent study detected the association of lncRNA polymorphisms with lung cancer and found that genetic variants in lncRNAs HOTTIP, HOTAIR, CCAT2, ANRIL, and MALAT1 were significantly associated with lung cancer susceptibility or chemotherapy response (Gong et al., 2016). A case-control study of breast cancer in a Chinese population identified three polymorphisms in the lncRNA HOTAIR associated with breast cancer (Yan et al., 2015). However, to date, there is no specific research about the association between lncRNA's polymorphisms and osteoporosis risk.

To explore osteoporosis susceptibility loci at lncRNAs, we performed a meta-analysis of multiple GWASs with diverse ancestries for polymorphisms of lncRNAs and BMD. Furthermore, we analyzed the potential effects on lncRNA structure and function of interested single nucleotide polymorphisms (SNPs).

2 | MATERIALS AND METHODS

2.1 | Study samples

Discovery sample: Our study consisted of 11,140 individuals from seven GWASs, three of which were “in-house” studies: (1) Omaha Osteoporosis Study (OOS; $N = 987$, Caucasian ancestry), (2) Kansas-City Osteoporosis Study (KCOS; $N = 2250$, Caucasian ancestry), and (3) China Osteoporosis Study (COS; $N = 1547$, Han Chinese ancestry); the other four were “external” studies, downloaded from Genotypes and Phenotypes (dbGaP) (<https://www.ncbi.nlm.nih.gov/gap/>): (1) Framingham Heart Study (FHS; $N = 3747$, Caucasian ancestry), (2) Indiana Fragility Study (IFS; $N = 1488$, Caucasian ancestry), (3) Women's Health Initiative (WHI) Observational Study African-American Sub-study (WHI-AA; $N = 712$, African ancestry), and (4) WHI Observational Study Hispanic Sub-study (WHI-HIS; $N = 409$, Hispanic ancestry).

BMD (g/cm^2) at lumbar spine (L1–L4) and hip, including femoral neck, trochanter, and intertrochanter, were measured using dual-energy x-ray absorptiometry scanners (either Lunar Corp., Madison, WI, or Hologic Inc., Bedford, MA), and details of bone densitometer of each sample are given in Table 1. The total hip BMD was a combined value at the three measured regions (Tan et al., 2015). The densitometer was calibrated daily, and long-term precision was monitored with the control vertebral phantom (Tan et al., 2015). The coefficient of variation of measured BMD values was 1.01% and 1.34% at the spine total hip (Tan et al., 2015). Hologic BMD was converted to Lunar BMD using published equations derived from women for lumbar spine and femoral neck (Genant et al., 1995; Lu, Fuerst, Hui, & Genant, 2001; Shepherd et al., 2002). Conversion equations in men for Hologic to Lunar BMD were derived using Deming regression analysis (Ganda, Nguyen, & Pocock, 2014). BMD was calculated separately using Hologic and Lunar BMD at the lumbar spine, femoral neck, and total hip.

All samples were approved by the Institutional Review Board or Research Administration of the institutions involved. Signed informed consent documents were obtained from all study participants before entering the study. Detailed samples information was described previously (Zhang et al., 2014).

Replication sample: The replication dataset was the meta-analysis from the Genetic Factors for Osteoporosis Consortium (GEFOS Sequencing), the largest meta-analysis to date in the bone field, including 32,965 individuals of Caucasians descent with the study detailed in a recent publication (Zheng et al., 2015). Each SNP and its association to femoral neck and lumbar spine BMD in the meta-analysis were downloaded from <http://www.gefos.org/>. FHS, IFS and WHI samples in the GEFOS Sequencing dataset were overlapped with the samples in the discovery dataset. Since the GEFOS dataset only provides the summary statistic data of all samples, we cannot exclude the four dbGAP samples from the replication sample.

2.2 | Genotyping and imputation

Each GWAS sample was genotyped by high-throughput SNP genotyping array. Genotyping quality control criteria were used as follows: individual missing data < 5%, SNP call rate > 95%, and Hardy–Weinberg equilibrium P -value > 1.00×10^{-5} . Genotypes of each individual study sample were imputed by the relevant reference panel in the 1000 Genome Project. SNPs (at the very least one sample) and minor allele frequency (MAF) > 0.05 (at the very least one sample) were included in the association analysis. For detailed genotyping method, please refer to previous study (Zhang et al., 2014).

2.3 | LncRNA SNP selection

LncRNA data were downloaded from the LncRNASNP-human database (<http://bioinfo.life.hust.edu.cn/lncRNASNP>), obtained from dbSNP of NCBI and LNCipedia database, which contains 495,729 SNPs of 32,108 human lncRNA transcripts from 17,436 lncRNA genes (human GRCh37) (Gong, Liu, Zhang, Miao, & Guo, 2015). The database also provided synthetic and integrated information about lncRNA SNPs and their potential functions and interrelation with other loci (Gong et al., 2015). When comparing the genotyped SNPs in our GWAS samples with SNPs from LncRNASNP-human database, 55,906 lncRNA SNPs were shared and selected in the subsequent analysis.

2.4 | GWAS meta-analysis

For each GWAS study, BMD measurements were adjusted by significant covariates: sex, age, weight, height, scan side (in FHS), and scanner ID (in WHI-AA and WHI-HIS) by a stepwise linear regression model, and principal component analysis was computed to correct for potential population stratification (Zhang et al., 2014). For each unrelated subjects, association was computed by the linear regression statistical model using MACH2QTL (Li, Willer, Ding, Scheet, & Abecasis, 2010), and allele dosage was used as a phenotype prognosticator. In total, 5,842,825 SNPs were qualified in the meta-analyses, and overall genomic control inflation factors for three BMD traits ranged from 0.99 to 1.04 (Zhang et al., 2014).

METAL (<https://www.sph.umich.edu/csg/abecasis/metal/>) were used to implement weighted fixed-effect meta-analysis, and Cochrane's Q statistic and I^2 were calculated as measures of between-study heterogeneity (Tan et al., 2014; Willer, Li, & Abecasis, 2010). Random-effect meta-analyses were performed for SNPs with a Q statistic P -value < 0.05 or I^2 > 50% (Tan et al., 2014). Forest plots were performed to present the effect size of identified candidate SNPs in meta-analysis, and odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated using Review Manager (<https://ims.cochrane.org/revman>) (Tan et al., 2014).

To constrain the probability of false positives, Bonferroni correction was applied for multiple tests. $P = 8.94 \times 10^{-7}$ (i.e., $0.05/55906$) was applied to as a genome-wide significant criterion, while $P = 1.00 \times 10^{-4}$ was regarded as suggestively significant level.

2.5 | LncRNA secondary structures prediction

The structural features of lncRNAs play important roles, by acting as a scaffold, and a portion of lncRNAs can exert their functions by scaffolding protein complexes (Gong et al.,

2015; Zhu, Fu, Wu, & Zheng, 2013). SNPs located in lncRNA may affect the lncRNA secondary structure, stability, expression, and functions (Gong et al., 2015; Nagano & Fraser, 2011). To access the influence of polymorphism alleles on lncRNAs' secondary structure, the RNAfold online tool (<https://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) was applied to illustrate the change of secondary structure and calculate the Minimum Free Energy (MFE) (Gruber, Lorenz, Bernhart, Neubock, & Hofacker, 2008).

2.6 | miRNA-lncRNA interactions prediction

lncRNAs SNPs in the miRNA target sites may create or break miRNA binding sites, and polymorphisms would lead to change of miRNA-lncRNA interactions (Gong et al., 2015). SNPs impact of miRNA-lncRNA interactions was predicted from lncRNASNP database (Gong et al., 2015). The lncR-NASNP database applied miRanda and TargetScan to predict potential miRNA target sites around lncRNA SNPs (± 25 bp around SNP) (Gong et al., 2015).

3 | RESULTS

The main characteristics of samples were summarized in Table 1. Chinese and Hispanic BMD were relatively lower than that of Caucasians and African Americans.

We identified two significant loci in our GWAS meta-analysis. *MEF2C* antisense RNA 1 (*MEF2C*-AS1) located at 5q14.3 was significantly associated with femoral neck BMD (FN-BMD), of which the strongest association was rs6894139 ($P = 3.03 \times 10^{-9}$) and allele-specific OR (95% CI) ranged from 0.89 to 0.93. rs6894139 was previously reported to be associated with BMD in our earlier GWAS, but it was annotated in the *MEF2C* gene (Zhang et al., 2014). Our previous study did not highlight the important role of rs6894139 in osteoporosis. *LOC100506136* located at 7q21.3, of which the strongest association was rs6465531 ($P = 7.43 \times 10^{-7}$ for total hip BMD (HIP-BMD)). Allele-specific OR (95% CI) ranged from 0.88 to 0.98 for HIP-BMD. Figure 1 showed the forest plot of the two identified SNPs, *MEF2C*-AS1 rs6894139 (T/G) for FN-BMD and *LOC100506136* rs6465531 (G/A) for HIP-BMD. Additionally, 24 loci showed nominally significant associations ($P < 10^{-4}$). We listed results of GWAS meta-analysis associations of SNPs with BMD (with $P < 10^{-4}$) in Tables 2–4.

To validate these findings, these 26 loci of potential interest (2 significant and 24 nominally significant SNPs) were replicated in GEFOS Sequencing dataset encompassing 32,965 individuals with BMD at femoral neck and lumbar spine. *MEF2C*-AS1 rs6894139 were significantly associated with FN-BMD ($P = 1.43 \times 10^{-23}$), which was not reported in earlier GEFOS Sequencing (Estrada et al., 2012; Rivadeneira et al., 2009). Of the nominally significant SNPs, *LOC100506136* rs10242216 ($P = 9.80 \times 10^{-5}$) was significantly associated with FN-BMD in the GEFOS Sequencing dataset. *LOC105369209* rs1572095 ($P = 2.87 \times 10^{-5}$) and rs1572094 ($P = 3.55 \times 10^{-5}$), rs3761226 ($P = 8.98 \times 10^{-5}$) and *BDNF*-AS rs1808124 ($P = 5.11 \times 10^{-4}$) was significantly associated with lumbar spine BMD (SPN-BMD) in the GEFOS Sequencing dataset.

RNAfold predicted that rs6894139 might change the secondary structure and MFE of *MEF2C*-AS1. Figure 2 shows that the secondary structure of *MEF2C*-AS1 transcript (NR_109941.1) carried rs6894139-T allele or rs6894139-G allele, respectively. The optimal secondary structures for these two forms had different MFEs (−191.20 kcal/mol for rs6894139-T allele and −193.90 kcal/mol for rs6894139-G allele), and the latter structure becomes more stable. They also have different MFE structure, encoding base-pair probabilities and folding patterns in the middle of the secondary structures, as it was pointed out by the arrow in the diagram. Since the conformation of the rs6894139-T allele with a higher MFE (MFE = 2.7 kcal/mol) differs from that of the rs6894139-G allele, the *MEF2C*-AS1 rs6894139 could potentially affect lncRNA function.

We also predicted miRNA target sites around the lnc-SNPs region. We found that a G > T change at rs6894139 may destroy the binding sites of hsa-miR-369-3p and hsa-miR-8084 on lncRNA *MEF2C*-AS1, which may cause loss of function of miRNA-lncRNA interactions. A G > A change at rs6465531 may give rise to gain function and develop the binding sites of hsa-miR-4302 and hsa-miR-199a-5p on lncRNA LOC100506136. The miRNA may regulate the transcription of lncRNA; therefore, the destruction of miRNA-lncRNA interaction may cause allelic-specific lncRNA expression.

4 | DISCUSSION

In the present study, we explored the important roles of lncRNAs polymorphism in determining variations of BMD for the first time. We found that *MEF2C*-AS1 rs6894139 was associated with FN-BMD and *LOC100506136* rs6465531 was associated with HIP-BMD.

The intergenic polymorphism rs6894139 can influence the *MEF2C*-AS1 transcript secondary structure by changing the folding pattern and MFEs and thus may potentially affect the function of *MEF2C*-AS1, such as signal, decoy, guide, or scaffold proteins and transcriptional regulation of gene expression. SNP rs6894139 in the miRNA target sites may destroy hsa-miR-369-3p and hsa-miR-8084 binding sites on *MEF2C*-AS1 with a G > T alteration and may cause loss of function of miRNA-lncRNA interactions. Therefore, SNP rs6894139 may potentially influence the expression of *MEF2C*-AS1.

A previous study showed that a mouse BMD quantitative trait locus was mapped in *MEF2C*-AS1 region (Ackert-Bicknell et al., 2010). Mo et al. (2015) showed that *MEF2C*-AS1 was associated with FN-BMD ($P = 3.72 \times 10^{-8}$) using gene-based analysis with GEFOS-2 data. Hence, *MEF2C*-AS1 is an important locus in determining BMD variation.

Antisense RNA transcription is initiated in the opposite strand either within the gene body or at the end of its sense transcript (Katayama et al., 2005). Relative to sense genes, antisense genes are transcribed head-to-head, tail-to-tail, or fully overlapping (Katayama et al., 2005). Antisense genes can interact with sense genes. For example, antisense gene *AS-RBM15* enhanced its sense gene, and RBM15 protein translation in a head-to-head overlapping in the 5'UTR (Tran et al., 2016). Antisense gene *FGFR3*-AS1 through pairing with *FGFR3* in tail-to-tail overlapping in the 3'UTR, increased *FGFR3* mRNA stability and upregulated *FGFR3*

expression (Sun et al., 2016). In our study, the antisense MEF2C-AS1 transcript overlapped with the 5' UTR of MEF2C, and we speculate that lncRNA MEF2C-AS1 may play a role in MEF2C expression and/or translation (Chen, Sun, Hurst, Carmichael, & Rowley, 2005; Tran et al., 2016). A functional study showed that expression of a dominant-negative *MEF2C* mutant or genetic deletion of *MEF2C* impaired cartilage angiogenesis, ossification, and longitudinal bone growth, on the contrary, superactivated *MEF2C* caused precocious chondrocyte hypertrophy, ossification of growth plates, and dwarfism (Arnold et al., 2007). We assume that lncRNA MEF2C-AS1 through affecting MEF2C expression and/or translation may indirectly influence the endochondral bone development. However, the hypotheses need further functional studies.

SNP rs6465531 is located in the intron region of NR_038948.1 (uncharacterized *LOC100506136*). A G > A alteration of SNP rs6465531 may create the binding sites of hsa-miR-4302 and hsa-miR-199a-5p on lncRNA. Previous studies revealed that hsa-miR-199a-5p regulated osteogenic differentiation of human MSCs at early and late stages via the HIF1a pathway, and miR-199a-5p overexpression could improve the *in vivo* regeneration of bone (Chen et al., 2015). These findings imply the potential role of *LOC100506136* rs6465531 in bone metabolism.

There are some limitations in the present study. First, four samples overlapped between our discovery study and GEFOS Sequencing sample. Because GEFOS Sequencing data did not provide detailed results of single sample subset, we cannot exclude the four dbGAP samples from the replication sample. Besides, small sample size and imbalanced sex ratio may lead to limited statistical power. However, our findings can provide new insights on the lncSNP role in determining BMD variation.

In summary, in our present study, we have identified two lncRNAs polymorphisms associated with BMD. Although we have no specifically experimental proof to figure out the biologic mechanisms underlying the GWAS association, our results enhance the information and awareness of the associations between lncRNAs polymorphisms and BMD, and they supply a clue for succeeding functional studies of these relevant lncSNPs in osteoporosis pathophysiology.

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FIGURE 1. Forest plot of the association of two identified SNPs, MEF2C-AS1 rs6894139 (allele T) for FN-BMD (upper) and LOC100506136 rs6465531 (allele G) for HIP-BMD phenotype (lower)

FIGURE 2.

Predicted secondary structure of MEF2C-AS1 transcript carried rs6894139-T allele (left) or rs6894139-G allele (right) by RNAfold. MFE: minimum free energy [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Basic characteristics of the samples

Sample	Sample size	Population	Female (%)	Age (yr)	Height (m)	Weight (kg)	SPN (g/cm ²)	HIP (g/cm ²)	FN (g/cm ²)	Bone densitometer
OOS	987	Caucasian	49.6	50.3(18.3)	1.71(0.10)	80.10(17.72)	1.03(0.16)	0.97(0.16)	0.81(0.14)	Hologic QDR 4500W
KCOS	2250	Caucasian	75.9	51.4(13.8)	1.66(0.08)	75.16(17.47)	1.02(0.16)	0.97(0.17)	0.80(0.15)	Hologic QDR 4500W
COS	1547	Han Chinese	50.7	34.8(13.4)	1.64(0.08)	60.27(10.54)	0.95(0.13)	0.92(0.13)	0.81(0.13)	Hologic QDR 4500W
FHS	3747	Caucasian	57.3	60.3(10.7)	1.66(0.10)	77.00(16.99)	1.22(0.23)	0.95(0.17)	0.89(0.16)	Lunar DPX-L
IFS	1488	Caucasian	100.0	32.7(7.2)	1.65(0.06)	71.66(16.90)	–	–	–	Lunar DPX-L
WHI-AA	712	African American	100.0	60.9(6.9)	1.62(0.06)	83.15(17.72)	1.05(0.17)	0.95(0.15)	0.83(0.14)	Hologic QDR-2000
WHI-HIS	409	Hispanic	100.0	60.7(7.2)	1.57(0.06)	73.87(15.62)	0.97(0.16)	0.86(0.13)	0.73(0.12)	Hologic QDR-2000

Data were presented as mean (SD).

Legend: OOS: Omaha osteoporosis study; KCOS: Kansas-city osteoporosis study; COS: China osteoporosis study; FHS: Framingham heart study; IFS: Indiana fragility study; WHI-AA: Women's health initiative African American sample; WHI-HIS: Women's health initiative Hispanic sample. SPN: spine; HIP: total hip; FN: femoral neck; g/cm²: the unit of BMD measurement; –: raw phenotypes/information was not available at the dbGAP for the IFS dataset.

TABLE 2

Results of GWAS meta-analysis associations of lncSNPs with FN-BMD (with P -value less than 10^{-4})

SNP	lncRNA region	Refseq gene	Alleles	MAF	Weight	Z-score	P-value	Direction	I^2	Q_P-value
rs6894139	5q14.3	<i>MEF2C-AS1</i>	T/G	0.49	11,125	-5.93	3.03E-09	---	0	0.859
rs6465531	7q21.3	<i>LOC100506136</i>	G/A	0.25	11,125	-4.708	2.50E-06	---	23.4	0.250
rs2585	11-2150351-2151194	<i>IGF2</i>	T/C	0.32	11,125	4.684	2.81E-06	+++++	70.5	0.002
rs2060103	8:64378407-64388040	<i>LOC102724612</i>	C/T	0.16	11,125	-4.661	3.15E-06	---	0	0.561
rs247859	16q24.1	<i>COTL1</i>	A/G	0.14	11,125	4.445	8.77E-06	+++++	0	0.850
rs247862	16q24.1	<i>COTL1</i>	A/G	0.14	11,125	4.37	1.25E-05	+++++	0	0.796
rs17605231	15:86848282-86860404	<i>AGBL1-AS1</i>	C/G	0.28	11,125	-4.36	1.30E-05	+-	35.7	0.156
rs7458	16q24.1	<i>COTL1</i>	A/G	0.14	11,125	4.351	1.36E-05	+++++	0	0.802
rs247861	16q24.1	<i>COTL1</i>	T/C	0.14	11,125	4.333	1.47E-05	+++++	0	0.801
rs4920861	5:116256539-116260291	<i>LOC105379137</i>	T/A	0.15	11,125	4.235	2.29E-05	+++++	0	0.732
rs1166231	12q24.32	<i>LOC440117</i>	C/G	0.49	11,125	-4.213	2.52E-05	---	30.4	0.196
rs16991920	22:33562373-33617737	<i>LOC105373005</i>	A/T	0.01	2331	4.119	3.81E-05	??+??+?	0	0.717
rs17613339	1p12	<i>LOC100996263</i>	T/C	0.12	11,125	4.074	4.62E-05	+++++	46.6	0.081
rs7707920	5:116256539-116260291	<i>LOC105379137</i>	G/A	0.24	11,125	4.045	5.24E-05	+++++	0	0.660
rs34754277	1:118139492-118147980	<i>LOC100996263</i>	C/T	0.16	11,125	4.037	5.42E-05	+++++	37.5	0.143
rs10242216	7q21.3	<i>LOC100506136</i>	G/A	0.17	11,125	-4.014	5.97E-05	---	31.9	0.185
rs11862851	16:84376070-84377013		A/G	0.09	11,125	3.904	9.45E-05	+++++	43	0.104

Legend: SNP: single nucleotide polymorphisms; FN: femoral neck BMD; MAF: minor allele frequency; weight: the sum of the individual study weights for this marker; Z-score: the combined z-statistic for this marker, is the ratio of effect size estimate and standard error, for each allele are combined across of studies in an inverse variance; P-value: meta-analysis P-value; Direction: summary of effect direction for each study, with one "+" or "-" per study; I^2 : heterogeneity measure. Bold SNPs was P -value $< 10^{-4}$ in GEPOS Sequencing dataset.

TABLE 3

Results of GWAS meta-analysis associations of IncSNPs with HIP-BMD (with P -value $< 10^{-4}$)

SNP	IncRNA region	Refseq gene	Alleles	MAF	Weight	Z-score	P-value	Direction	I ²	Q P-value
rs6465531	7q21.3	<i>LOC100506136</i>	G/A	0.25	10,444	-4.95	7.43E-07	—	0	0.607
rs10242216	7q21.3	<i>LOC100506136</i>	G/A	0.17	10,444	-4.367	1.26E-05	—	19.3	0.282
rs34569456	20:33864148-33865830	<i>MMP24-AS1</i>	A/G	0.02	8113	4.349	1.37E-05	++?+—?	59.4	0.043
rs9907987	17:46656992-46659621	<i>HOXB3</i>	T/C	0.11	10,444	4.179	2.93E-05	++++++	0	0.975
rs12526269	6p24.3	<i>LINC00518</i>	A/T	0.21	10,444	-4.116	3.86E-05	—	0	0.575
rs61734458	9:109040673-109367076		C/T	0.0001	710	4.074	4.62E-05	?????—?	0	1
rs4688011	3q13.33	<i>TIMMDC1</i>	A/G	0.18	10,444	4.052	5.08E-05	++++++	0	0.825
rs4688013	3q13.33	<i>TIMMDC1</i>	A/G	0.18	10,444	4.011	6.04E-05	++++++	0	0.827
rs11714738	3:119226932-119230323	<i>TIMMDC1</i>	G/C	0.25	10,444	4	6.32E-05	++++++	19.8	0.278
rs34754277	1:118139492-118147980	<i>LOC10096263</i>	C/T	0.15	10,444	3.986	6.71E-05	++++++	40.7	0.120
rs709400	14q32.33	<i>KLC1</i>	G/A	0.32	10,444	3.976	7.01E-05	++++++	0	0.698
rs17605231	15:86848282-86860404	<i>AGBL1-AS1</i>	C/G	0.28	10,444	-3.97	7.19E-05	+—	53.7	0.044
rs1961132	3:119226932-119230323	<i>TIMMDC1</i>	T/C	0.25	10,444	3.968	7.26E-05	++++++	22.8	0.255
rs8024631	15:38360991-38365024		T/C	0.22	10,444	-3.948	7.88E-05	—	0	0.820
rs247859	16q24.1	<i>COTL1</i>	A/G	0.14	10,444	3.918	8.91E-05	++++++	0	0.695
rs3915733	14q32.33	<i>KLC1</i>	A/G	0.32	10,444	3.907	9.34E-05	++++++	0	0.701

Legend: SNP: single nucleotide polymorphisms; HIP: total hip BMD; MAF: minor allele frequency; Weight: the sum of the individual study weights for this marker; Z-score: the combined z-statistic for this marker, it is the ratio of effect size estimate and standard error, for each allele are combined across of studies in an inverse variance; P-value: meta-analysis P-value; Direction: summary of effect direction for each study, with one “+” or “—” per study; I²: heterogeneity measure.

TABLE 4
Results of GWAS meta-analysis associations of lncSNPs with SPN-BMD (with P -value $< 10^{-4}$)

SNP	lncRNA region	Refseq gene	Alleles	MAF	Weight	Z-score	P-value	Direction	I^2	Q_P-value
rs6089695	20:60807016-60811355	<i>LOC105369209</i>	T/C	0.46	11.030	4.721	2.35E-06	+++++--	0	0.663
rs1572095	20:60809846-60812575	<i>LOC105369209</i>	T/C	0.46	11.030	4.628	3.70E-06	+++++--	0	0.659
rs1572094	20:60809846-60812575	<i>LOC105369209</i>	A/G	0.46	11.030	4.627	3.70E-06	+++++--	0	0.659
rs2606181	8q24.21	<i>PCAT1</i>	A/C	0.06	11.030	-4.322	1.55E-05	---+-	0	0.803
rs6804138	3q26.31	<i>LOC105374219</i>	A/C	0.02	11.030	-4.274	1.92E-05	--- -	20.3	0.275
rs11914753	3q26.31	<i>LOC105374219</i>	T/C	0.02	9409	-4.214	2.50E-05	-?_--	28.5	0.222
rs57582432	3q26.31	<i>LOC105374219</i>	A/G	0.97	9409	-4.172	3.02E-05	-?_--	31.5	0.199
rs59731247	3q26.31	<i>LOC105374219</i>	A/G	0.02	11.030	-4.156	3.24E-05	--- -	18.8	0.286
rs57580622	3q26.31	<i>LOC105374219</i>	C/G	0.02	9409	-4.147	3.37E-05	-?_--	29.8	0.212
rs3761226	20:60807016-60811355		A/C	0.50	11.030	4.135	3.55E-05	+++++--	0	0.795
rs2060103	8:64378407-64388040	<i>LOC102724612</i>	T/C	0.84	11.030	-4.126	3.70E-05	--- -	0	0.482
rs1808124	11:27686807-27699354	<i>BDNF-AS</i>	T/C	0.28	11.030	-4.049	5.15E-05	--- +	0	0.789
rs7309459	12q15	<i>LOC101927901</i>	T/C	0.41	11.030	-3.999	6.37E-05	--- -	0	0.547
rs3088238	20:60880488-60881452	<i>ADRM1</i>	A/G	0.23	11.030	3.977	6.97E-05	+++++--	44.6	0.094

Legend: SNP: single nucleotide polymorphisms; SPN: spine BMD; MAF: minor allele frequency; Weight: the sum of the individual study weights for this marker; Z-score: the combined z-statistic for this marker, it is the ratio of effect size estimate and standard error, for each allele are combined across of studies in an inverse variance; P-value: meta-analysis P-value; Direction: summary of effect direction for each study, with one '+', '-' or '.' per study; I^2 : heterogeneity measure. Bold SNPs was P -value $< 10^{-4}$ in GEFOS Sequencing dataset.