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Replication study of candidate genes/loci associated with osteoporosis based on genome-wide screening

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Conflicts of interest None.

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Abstract

Summary—Osteoporosis is a major public health problem characterized by low bone mineral density (BMD). This replication study confirmed 38 single-nucleotide polymorphisms (SNPs) out of 139 SNPs previously reported in three recent genome-wide association studies (GWASs) in an independent US white sample. Ten SNPs achieved combined $p<3.6\times10^{-4}$.

Introduction—BMD is under strong genetic control. This study aims to verify the potential associations between BMD and candidate genes/loci reported by GWAS of FHS100K, Icelandic deCODE, and UK-NL.

Methods—Eight promising (at the genome-wide significant level after Bonferroni correction) and 131 available sub-promising (at the most stringent *p* value, $p<5.5\times10^{-5}$ in the three GWASs reports) SNPs were selected. By using genotypic information from Affymetrix 500 K SNP arrays, we tested their associations with BMD in 1,000 unrelated US whites. Fisher's combined probability method was used to quantify the overall evidence of association. BMD was measured by dual energy X-ray absorptiometry.

Results—Two promising SNPs, rs3762397 and rs3736228, were replicated in the current study with $p<0.05$. Besides, 36 sub-promising SNPs were replicated at the same significant level. Ten SNPs achieved significant combined $p<3.6\times10^{-4}$ (0.05/139 SNPs, corrected for multiple testing).

Conclusions—Osteoporosis susceptibility of 38 SNPs was replicated in 1,000 unrelated US whites. This study showed promise for replication of some initial genome-wide association signals.

Keywords

Bone mineral density; Genome-wide association; Osteoporosis; Replication

Introduction

Osteoporosis is a common skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to osteoporotic fractures [1]. Osteoporosis is defined clinically through the measurement of bone mineral density (BMD, g/cm^2), which is one of the most important predictors of primary osteoporotic fractures [2,3]. Genetic factors play important roles in determining population variation of BMD [4]. More than 20 genome-wide linkage studies and hundreds of candidate gene association studies have revealed multiple genetic loci related to BMD. However, discrepant and conflicting results were reported across studies, necessitating replication studies in independent samples [5]. So far, specific genetic factors of osteoporosis are largely unknown. Identifying the genes/loci for osteoporosis is still challenging.

With the rapid advancement in high throughput single-nucleotide polymorphism (SNP) genotyping technique and determination of haplotype tagging SNPs attributed to the International HapMap Project (http://hapmap.org), genome-wide association study (GWAS) is now widely applied to dissect the genetic determination of common complex disorders, including osteoporosis. Considering conflicting results from previous association studies and probability of false positive association due to large number of tests involved in GWAS, replication studies in an independent sample are requested.

As a follow-up replication study, the present work was attempted to test in 1,000 unrelated US whites the associations between 139 candidate SNPs and BMD variation, which were originally disclosed in three recently published GWASs: FHS100K [6], Icelandic deCODE [7], and UK-NL [8].

Materials and methods

Study populations

Current GWAS replication population—The study of current 500 K GWAS was approved by the involved Institutional Review Board. Signed informed consent documents were obtained from all study participants. We studied a total of 1,000 unrelated subjects, including 501 women and 499 men. Our study subjects of US whites were identified from an established cohort containing ~6,000 subjects recruited from the Midwestern US. All subjects were normal healthy subjects defined by a comprehensive suite of exclusion criteria detailed previously [4]. Briefly, subjects with chronic diseases and conditions involving vital organs (heart, lung, liver, kidney, and brain) and severe endocrinological, metabolic, and nutritional diseases that might affect bone metabolism were excluded from this study. By following the above exclusion criteria, we expected to exclude potential confounders which may interfere with association test and increase the power of detecting modest genetic effect on BMD variation in our study population.

Previous three GWASs populations—The FHS100K GWAS in osteoporosis [6] consisted of 1,141 subjects, including 495 men and 646 women with BMD data.

The Icelandic deCODE GWAS [7] consisted of 5,861 Icelandic subjects, including 776 men and 5,085 women.

The UK-NL GWAS [8] consisted of 8,557 participants from four population-based cohorts. Among them, 2,094 women came from the TwinsUK discovery cohort, which was a population-based sample of Britons previously shown to be representative of singleton populations, and the general UK population [9]. The other three cohorts included Rotterdam cohort (4,081 subjects, 784 men, and 3,297 women), TwinsUK replication cohort (1,692 women), and Chingford cohort (690 women). All the participants were of white European ancestry. The general relevant characteristics of these four studies are summarized in Table 1.

BMD measurement

In current 500K GWAS, we measured BMD (g/cm^2) at the following skeletal sites with daily calibrated Hologic 4500 dual energy radiograph absorptiometry (DXA; Hologic, Bedford, MA, USA): BMD at the lumbar spine L1–L4 (SPNBMD), combined BMD of femoral neck, trochanter, and intertrochanter areas (HIPBMD), and BMD at the femoral neck (FNBMD). The coefficient of variation (CV) values of the DXA measurements were 1.98%, 1.87%, and 1.87% for SPNBMD, HIPBMD and FNBMD, respectively.

In the FHS100K GWAS, BMD was measured by Lunar DPX-L (Lunar Corp., Madison, WI, USA). The CVs for SPNBMD, trochanter BMD, and FNBMD were 0.9%, 2.5%, and 1.7%, respectively [10]. In the Icelandic deCODE GWAS, BMD was measured at the lumbar spine and hip by DXA [7]. In the UK-NL GWAS, BMD at the lumbar spine and femoral neck was measured by DXA [8].

Genotyping

Current 500K GWAS—Genomic DNA was extracted from peripheral blood sample using a commercial isolation kit (Gentra systems, Minneapolis, MN, USA). Genotyping with the Affymetrix Mapping 250K Nsp and Affymetrix Mapping 250K Sty arrays was performed by the Vanderbilt Microarray Shared Resources (http://array.mc.vanderbilt.edu/) using the standard protocol of the Affymetrix. Genotyping calls were determined from the fluorescent intensities using the dynamic model (DM) algorithm with a 0.33 *p* value setting [11] as well as the B-RLMM algorithm, an extension of the RLMM [12] developed for the Mapping 500K product. DM calls were used for quality control while the B-RLMM calls were used for all subsequent data analyses. B-RLMM clustering was performed with 94 samples per cluster.

The final average B-RLMM call rate across the entire sample was 99.14%. However, out of the initial full set of 500,568 SNPs, we discarded 32,961 SNPs with call rates <95% in the sample, an addition of 36,965 SNPs with allele frequencies deviating from Hardy–Weinberg equilibrium (HWE; $p<0.001$), and 51,323 SNPs with minor allele frequencies (MAF)<1%. Therefore, the final SNP set maintained in the subsequent analyses contained 379,319 SNPs, yielding an average SNP spacing of ~7.9 kb throughout the human genome.

The FHS100K GWAS—The Affymetrix 100K SNP GeneChip was used for genotyping. The study tested 70,987 SNPs with genotypic call rates ≥80%, HWE $p\geq0.001$, MAF≥10% for association with BMD [6].

The Icelandic deCODE GWAS—The Infinium HumanHap300 or the HumanCNV370 SNP chip from the Illumina (San Diego, CA) was used for genotyping. In total, 301,019 SNPs with genotypic call rates ≥98%, HWE $p>10^{-7}$, and MAF>5% were used for association analyses with BMD [7].

The UK-NL GWAS—The Infinium assays (Illumina, San Diego, CA), Hap300 Duo, Hap300, and Hap550, were used for genotyping TwinsUK discovery samples (2,094 women). Following the inclusion criteria of the genotypic call rates >90%, HWE $p\geq0.0001$, MAF≥1%, 314,075 SNPs were used for association analyses with BMD. These 314,075 SNPs were then assayed in the Rotterdam samples with the HumanHap 550 v3.0 assays (Illumina, San Diego, CA), applying the same quality-control criteria. In the study, significant SNPs were further replicated in the Chingford cohort and/or TwinsUK replication cohort, which were genotyped with Taqman system (Applied Biosystems, Foster City, CA, USA) [8].

Selection of SNPs for current replication study

For replication analyses in our samples, a total of 139 SNPs were selected from three previous GWASs (FHS100K, Icelandic deCODE and UK-NL). Firstly, we selected eight so-called promising SNPs (three from the FHS100K GWAS, three from the Icelandic deCODE GWAS, and two from the UK-NL GWAS), which all reached a genome-wide significant level after Bonferroni correction in the respective studies, i.e., 7.04×10^{-7} (0.05/70,987 SNPs) in the FHS100K GWAS, 1.66×10^{-7} (0.05/301,019 SNPs) in the Icelandic deCODE GWAS, and 1.59×10^{-7} (0.05/314,075 SNPs) in the UK-NL GWAS. Considering that the

Bonferroni correction is overly strict, secondly, we selected an addition of 131 available SNPs (15 from FHS100K, 82 from Icelandic deCODE, and 34 from UK-NL) with *p* value less than 5.5×10^{-5} (i.e., a most stringent cutoff *p* value for data report in the three previous GWASs) i.e., so-called sub-promising SNPs.

Statistical analyses

Statistical analysis in current replication sample—Prior to the association analyses, we adjusted the raw phenotypic values with the same covariates applied in the GWAS of FHS100K, Icelandic deCODE, and UK-NL, respectively. For those promising and sub-promising SNPs, which were missing in our 500K Affymetrix assays, we imputed the genotypes with the IMPUTE program (http://www.stats.ox.ac.uk/marchini/software/gwas/impute.html). Assuming the same genetic model in this replication study and the previous GWASs, we then used the SNPTEST program (http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html) to test the association in our sample. To quantify the overall evidence of association between SNPs and BMD, Fisher's combined probability method [13] was used to calculate a combined *p* value in both previous GWASs and the current replication study, stratified by sex and skeletal site.

Statistical analysis in three previous GWASs—In FHS100K GWAS [6], BMD values were adjusted by age, age^2, height, BMI, smoking, physical activity, and estrogen therapy. Multivariate regression analysis was performed in each sex (men and women) and cohort (original and offspring). Association analyses were performed by using both family-based association tests and additive generalized estimating equation models.

In Icelandic deCODE GWAS [7], BMD values were adjusted for age, sex, and weight. For each SNP, a linear regression analysis, with the genotype as an additive covariate and standardized BMD as the response variable, was fitted to test for association. Each SNP was tested separately for its association with HIPBMD and SPNBMD.

In UK-NL GWAS [8], BMD values were adjusted for age. Association analyses were performed using the PLINK software package (version 1.01) (http://pngu.mgh.harvard.edu/purcell/plink/), with family structure in the sample taken into account [14]. Some of the subjects are monozygotic twins, and for these sib-pairs, genotypic information for only one individual per pair was included in the analyses, since monozygotic twins share identical genetic information. Where a single dizygotic twin had missing data, or was excluded, the remaining sibling was treated as a singleton in the statistical analysis.

Quality control of the replication sample—The Structure 2.2 (http://pritch.bsd.uchicago.edu/software.html) was used to detect potential population stratification in our GWAS sample, which uses a Markov chain Monte Carlo algorithm to cluster individuals into different cryptic subpopulations on the basis of multilocus genotype data [15]. Specifically, 200 randomly selected unlinked SNPs were used for the clustering. For the reliability of our results, we performed independent analyses under three assumed numbers for population strata (k=2, 3, and 4). Existence of substructure is suggested if the subjects were clustered into two or more groups. We further tested our sample for population stratification using the genomic control method [16]. Based on genome-wide SNP information, we estimated the inflation factor (λ), a measure for population stratification. Ideally, for a homogeneous population with no stratification, the value of λ should be equal or near to 1.0.

Results

Characteristics of the current replication sample

Basic characteristics of our current replication sample are presented in Table 1. The STRUCTURE program revealed that all subjects in this US whites sample were clustered together and could not be assigned into any subgroups, indicating that there was no significant population stratification within the sample. Further, the genomic control method estimated the λ value to be 1.007, confirming the results achieved through the Structure 2.2 software, indicating that there was essentially no population stratification in this sample. The relative homogeneity of this study sample eliminates potential spurious associations due to population stratification.

Replicated SNPs

Thirty-eight SNPs among the 139 selected SNPs achieved *p* values less than 0.05 in this replication study. Sixteen out of the 38 SNPs attained *p* value less than 0.01. Specifically, (1) for the eight selected promising SNPs, two SNPs (rs3762397 and rs3736228) were replicated (see Table 2). SNP rs3762397 is located in the intron of the nuclear receptor subfamily 5, group A, member 2 (NR5A2) gene, also known as liver receptor homolog (LRH-1) gene; SNP rs3736228 is located in the coding exon 18 of the lipoprotein-receptor-related protein (LRP5) gene (see Table 2). (2) For the remaining 131 selected sub-promising SNPs, 36 SNPs were replicated (see Table 3). Among them, 17 SNPs were associated with HIPBMD, six SNPs with SPNBMD, and 13 SNPs with both HIPBMD and SPNBMD.

Fisher's combined probability test

Among the 38 replicated SNPs, 10 SNPs achieved a significant combined *p* value, i.e., less than 3.6×10^{-4} (0.05/139 SNPs, with multiple-testing of SNPs taken into account). The combined *p* values of the 10 SNPs are presented in Table 4.

Notably, two SNPs located within the LRP5 gene, rs3736228 and rs2306862, achieved combined *p* values of 5.3×10^{-12} and 6.0×10^{-6} for SPNBMD, respectively, in a combined test for UK-NL sample and this replication sample. These two SNPs were also found to be associated with SPNBMD in the entire population of Icelandic deCODE study with *p* values of 6.5×10^{-4} and 4.9×10^{-4}, respectively. The other two SNPs, rs3020331 in the estrogen receptor 1 (ESR1) gene and rs4870044, ~45 kb upstream of the ESR1 gene and in the intron of the chromosome 6 open reading frame 97 (C6orf97) gene, were associated with SPNBMD, with combined *p* values of 1.2×10^{-6} and 4.9×10^{-8} in the whole populations of Icelandic deCODE GWAS and this replication sample. Besides the above four SNPs in the well-known osteoporosis candidate genes, the other six SNPs were also found to play important roles in the BMD variation at different skeletal site. Three SNPs, rs11898505 in SPTBN1 gene, rs4276378 in ADCY2 gene, and rs11239762 in BMS1L gene, were found to be associated with SPNBMD. Two SNPs, rs12437971 in ADAMTS17 gene and rs6696981 in an anonymous gene, were associated with HIPBMD. The SNP, rs1823926 in LOC51334 gene, was associated with FNBMD.

Discussion

Following up three recently published GWASs on osteoporosis conducted in different cohorts (FHS100K, Icelandic deCODE, and UK-NL) [6–8], the present study explored the association of the top significant SNPs with BMD variation in an independent sample of 1,000 unrelated US whites. We selected a total of 139 SNPs for replication test, including eight promising SNPs and 131 sub-promising SNPs available from the three previous GWASs reports. Among them, two promising and 36 sub-promising SNPs were replicated

when the significant threshold of *p* value was set at 0.05. The average replication rate was 25% for the promising SNPs, compared to 27% for the sub-promising SNPs. After combining outcomes from previous GWAS and the current replication study, 10 SNPs attained significant level even after stringent Bonferroni correction. In general, our data showed that significant and suggestive GWAS findings are likely to be replicated by independent study. Suggestive SNPs, though not attaining genome-wide significance threshold in initial GWAS sample, should also be valued for evaluation in independent samples. Therefore, besides the 20–50 top significant SNPs generally listed in GWAS reports, we suggest more comprehensive data be released for further exploration and replication in genetic research community.

One of the significant SNP replicated in this study, rs3762397, is located in a novel candidate gene NR5A2 at 1q32. This gene is expressed in all major steroidogenic tissues and tissues such as skeletal muscle, bone marrow [17,18]. NR5A2 is the major NR5A subfamily member expressed in the preovulatory follicle and the corpus luteum. It may play a key role in the regulation of gonadal steroidogenic gene expression [17], accordingly affecting bone metabolism via hormonal regulation.

Another significant SNP replicated in the current study, rs3736228, is located in LRP5 gene, which encodes a transmembrane protein from the low density lipoprotein receptor family, the low-density LRP5. LRP5 is expressed in osteoblasts [19]. As a well-known Wnt coreceptor, the protein transduces Wnt signaling and affects bone mass [20,21]. Relationship between LRP5 polymorphisms and BMD has been widely investigated [22,23]. Some polymorphisms in the LRP5 gene (e.g., rs4988321, rs312009, rs2508836, rs729635, rs643892) were associated with reduced bone mass and/or increased fracture risk [24,25], while some other LRP5 mutations (e.g. $LRP5_{171V}$) were associated with high bone mass [26,27]. The UK-NL GWAS [8] disclosed the essential effect of rs3736228 on decreased BMD. In a recent meta-analysis comprised of 16,705 individuals, a significant association between rs3736228 and decreased SPNBMD was revealed in both Asian and Caucasian population, although the effect of this LRP5 gene polymorphism on BMD variation was modest [28]. Our current replication study found consistent association of rs3736228 with SPNBMD in the whole population (p=0.028) and in female subgroup (p=0.019). These findings were strengthened by the following combined analyses, in which rs3736228 ranked as the top significant SNP, indicating a strong association with SPNBMD in the overall sample of 9,557 subjects (8,557 from UK-NL and 1,000 individuals from current study). And the SNP was significantly associated with SPNBMD in 2,595 women (2,094 from Twins UK discovery and 501 from our current replication study). The other SNP in LRP5 gene, rs2306862, was also significantly associated with SPNBMD in this 2,595 female subgroup. Interestingly, the two replicated SNPs in LRP5 gene, rs2306862 and rs3736228, were found interacted with physical activity on SPNBMD in a recent study [29].

Interestingly, the two replicated genes, LRP5 and NR5A2 (LRH-1), are involved in lipid and lipoprotein metabolism [30–34]. LRP5 deficiency mice had increased plasma cholesterol levels [31]. LRH-1 directly regulates apolipoprotein M (APOM) transcription by binding to an LRH-1 response element located at the APOM promoter region [32]. In addition, studies reported associations between serum lipid and BMD [35,36], suggesting a close relationship between lipids and bone metabolism.

GWAS with a larger sample size has greater power to detect genetic variants that confer modest disease risks without relying on prior knowledge of any specific genes/genomic regions [37,38]. Population stratification is an important source of spurious association in genetic association studies [39,40]. The study cohort, used for replication test, came from an apparently homogenous US midwest white population, living in Omaha, Nebraska and its

surrounding areas. This population is largely dominant of Caucasians as the major ethnic group in this area for many generations. Consistently, no significant population stratification was detected, which strongly warranted the reliability of the replication results.

We noted that a majority of the promising SNPs previously found associated with BMD were not replicated in this study. Potential reasons might be: (1) study methods differ among GWAS studies. For example, phenotype data is not completely the same across the GWASs: SPNBMD was a combination of L2–L4 in FHS100K and Icelandic deCODE but a combination of L1–L4 in UK-NL and the present replication study. Different genotyping platforms (i.e., Affymetrix in current GWAS, Illumina in UK-NL) and different SNP genotype quality control criteria was used among studies. (2) The result probably also partially reflect the complexity of osteoporosis pathogenesis, which is determined not only by genetic factors but also by environmental factors and their interactions. Populations from the same ethnic origin but different geographic regions or with different cultures have different exposures to environmental factors. Factors such as smoking, alcohol drinking, nutritional status, and exercises may have significant influence on BMD determination in humans. These factors, however, are sometimes difficult to assess, quantify, and controlled accurately, and their influence on BMD variation could not be judiciously accounted for thus brings interfering noise for statistical analyses.

In summary, following up three previous GWASs on osteoporosis, this study tested association with BMD for 139 significant or suggestive SNPs in an independent US whites sample, with 38 SNPs replicated. This study showed promise for replication of initial genome-wide association signals in an independent study.

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Table 1

Basic characteristics of the replication sample and initial GWAS samples

	Current Study	FHS 100K	Icelandic deCODE	UK-NL			
				TwinsUK Discovery	Rotterdam	TwinsUK replication	Chingford
Number of Subjects	1,000	1,141	5,861	2,094	4,081	1,692	690
Men	499	495	776	0	784	0	0
Women	501	646	5,085	2,094	3,297	1,692	690
Age (years)	Total: 50.3 (18.3)	Total: 62.5	Total: 60.3 (14.1)	49.7 (13.1)	Total 68.9 (8.8)	49.7 (14.1)	62.1 (6.0)
	Men: 50.1 (17.7)		Men: 66.2 (14.3)				
	Women: 50.5 (18.9)		Women: 59.4 (14.1)				
Weight (kg)	Total: 80.1 (11.7)	–	Total: 72.2 (13.4)	–	–	–	–
	Men: 89.0 (14.9)		Men:83.4 (14.6)				
	Women: 71.2 (15.9)		Women: 70.5 (13.2)				
SPNBMD (g/cm^2)	Total:1.03 (0.16)	–	Total: 0.95 (0.17)	Women: 1.00 (0.14)	Total: 1.06 (0.19)	Women: 0.97 (0.14)	Women: 0.95 (0.15)
	Men: 1.07 (0.17)		Men: 1.02 (0.18)				
	Women: 0.99 (0.15)		Women: 0.94 (0.17)				
Observation number	996		5,858	2,094	4,081	1,692	690
HIPBMD (g/cm^2)	Total: 0.97 (0.16)		Total: 0.83 (0.16)	–	–	–	–
	Men: 1.04 (0.15)		Men: 0.93 (0.16)				
	Women: 0.91 (0.14)		Women: 0.82 (0.16)				
Observation number	994		5,715				
FNBMD (g/cm^2)	Total: 0.81 (0.14)	–	–	0.80 (0.13)	0.85 (0.14)	0.80 (0.13)	0.75 (0.12)
	Men: 0.85 (0.15)						
	Women: 0.77 (0.13)						
Observation number	983			2,094	4,081	1,692	690
Bone scanner	Hologic QDR4500A	Lunar DPX-L	Hologic QDR4500A	Hologic QDR 2000 W	Hologic QDR 2000 W	Hologic QDR 2000 W	Hologic QDR 2000 W

Presented are the mean (SD)

SPNBMD combined value at the L2–L4 in FHS100K and Icelandic deCODE, and the combined value at the L1–L4 in UK-NL (Discovery) and current study; *HIPBMD* combined BMD at the femoral neck, trochanter and intertrochanter region; *FNBMD* BMD at the femoral neck;"–" data is not available from the initial report

Table 2

Replication outcome of the promising SNPs selected from initial GWASs

Initial GWAS	dbSNP	Position	Role	Chro.	Alleles[a]	Gene	Initial *p* value	Replication *p* value
FHS100K	rs3762397	198356842	Intron	1q32.1	C/*T*	NR5A2	2.8×10^{-7} (TRBMD)	2.8×10^{-2} (HIPBMD[c])
	rs9317284	62532351	Unknown	13q21.31	G/*T*		2.4×10^{-7} (FNBMD[b])	Not replicated
	rs4087296	80935282	Unknown	16q23.3	C/*T*		3.1×10^{-7} (TRBMD[c])	Not replicated
Icelandic deCODE	rs2504063	152132400	Unknown	6q25.1	G/*A*	ESR1	5.7×10^{-8} (SPNBMD)	Not replicated
	rs851982	152066678	Unknown	6q25.1	T/*C*	ESR1	1.6×10^{-7} (SPNBMD)	Not replicated
	rs9594759	41930593	Unknown	13q14.11	T/*C*	RANKL, AKAP11	1.2×10^{-8} (SPNBMD)	Not replicated
UK-NL	rs4355801	119993054	Unknown	8q24.12	A/*G*	TNFRSF11B	7.6×10^{-10} (SPNBMD) 3.3×10^{-8} (FNBMD)	Not replicated
	rs3736228	67957871	Coding exon	11q13.2	C/*T*	LRP5	6.3×10^{-12} (SPNBMD)	2.8×10^{-2} (SPNBMD) 1.9×10^{-2} (SPNBMD[c])

SPNBMD combined value at the L2–L4 in FHS100K and Icelandic deCODE and the combined value at the L1–L4 in UK-NL and current study; *HIPBMD* combined BMD at the femoral neck, trochanter, and intertrochanter region; *FNBMD* BMD at the femoral neck; *TRBMD* BMD at the trochanter

[a]The second allele is the minor allele in current study

[b]Male sample

[c]Female sample

Table 3

Replication outcome of the sub-promising SNPs ($p<5.5\times10^{-5}$) selected from initial GWASs

Initial GWAS	dbSNP	Position	Role	Chro.	Alleles[a]	Gene	Initial *p* value	Replication *p* value
FHS100K	rs1538173	166908686	Unknown	1q24.2	C/*T*		2.8×10^{-6} (FNBMD)	2.3×10^{-2} (SPNBMD[b]) 1.7×10^{-3} (FNBMD[b])
	rs914951	241018701	Unknown	1q43	G/*T*		8.8×10^{-6} (TRBMD)	1.3×10^{-2} (FNBMD)
	rs10510628	29828407	Intron	3p24.1	G/*A*	RBMS3	2.8×10^{-6} (TRBMD[b])	2.4×10^{-2} (FNBMD[b]) 2.1×10^{-3} (HIPBMD)
	rs922028	181024748	Unknown	4q34.3	A/*G*		1.5×10^{-5} (FNBMD)	1.0×10^{-2} (HIPBMD[b])
	rs1823926	119882620	Intron	5q23.1	C/*A*	PRR16	1.3×10^{-5} (FNBMD)	3.3×10^{-2} (FNBMD) 5.8×10^{-3} (HIPBMD) 9.5×10^{-3} (SPNBMD)
	rs10514345	90460035	Intron	5q14.3	C/*T*	GPR98	2.2×10^{-6} (SPNBMD[c])	2.3×10^{-2} (HIPBMD[c]) 5.8×10^{-3} (HIPBMD) 9.5×10^{-3} (SPNBMD)
	rs10506701	72872477	Unknown	12q21.1	T/*G*		1.4×10^{-6} (TRBMD)	3.5×10^{-2} (FNBMD) 6.1×10^{-3} (HIPBMD) 9.5×10^{-3} (SPNBMD)
	rs10508076	101270253	Intron	13q33.1	T/*C*	FGF14	4.4×10^{-6} (FNBMD[b])	3.1×10^{-2} (FNBMD)
Icelandic deCODE	rs7543680	22603856	Unknown	1p36.12	G/*A*	ZBTB40	4.8×10^{-5} (HIPBMD)	2.9×10^{-2} (FNBMD[c]) 5.1×10^{-3} (HIPBMD[c])
	rs7524102	22571034	Unknown	1p36.12	A/*G*	ZBTB40, WNT4	2.6×10^{-6} (HIPBMD)	3.7×10^{-2} (SPNBMD[c]) 3.7×10^{-2} (SPNBMD)
	rs6696981	22575445	Unknown	1p36.12	G/*T*		1.2×10^{-6} (HIPBMD)	3.5×10^{-2} (HIPBMD) 3.9×10^{-2} (FNBMD)
	rs7524281	151049879	Promoter	1q21.3	C/*A*	LCE1B	4.1×10^{-5} (HIPBMD)	2.7×10^{-2} (FNBMD[c])
	rs1332498	150957072	Downstream	1q21.3	T/*C*	LCE4A	3.3×10^{-5} (HIPBMD)	3.5×10^{-2} (FNBMD)
	rs11898505	54538061	Unknown	2p16.2	G/*A*	SPTBN1	1.7×10^{-6} (SPNBMD)	2.5×10^{-3} (FNBMD[c]) 2.5×10^{-3} (SPNBMD[c]) 5.1×10^{-3} (SPNBMD) 3.6×10^{-4} (HIPBMD[c]) 1.3×10^{-2} (HIPBMD)
	rs3020331	152050473	Unknown	6q25.1	C/*T*	ESR1	8.2×10^{-6} (HIPBMD) 3.4×10^{-6} (SPNBMD)	2.0×10^{-2} (SPNBMD[c]) 1.7×10^{-2} (SPNBMD[b]) 2.0×10^{-2} (SPNBMD)

Initial GWAS	dbSNP	Position	Role	Chro.	Alleles[a]	Gene	Initial *p* value	Replication *p* value
	rs4870044	151943102	Intron	6q25.1	C/*T*	ESR1, C6orf97	4.1×10^{-6} (SPNBMD)	1.8×10^{-2} (FNBMD[c]) 2.9×10^{-2} (FNBMD) 1.3×10^{-2} (SPNBMD[c]) 5.7×10^{-4} (SPNBMD) 6.6×10^{-4} (HIPBMD[c]) 8.5×10^{-3} (HIPBMD)
	rs7753676	151918508	Intron	6q25.1	A/*G*	C6orf97	3.6×10^{-5} (SPNBMD)	3.7×10^{-2} (HIPBMD[c])
	rs10125592	122201556	Intron	9q33.2	A/*G*	CDK5RAP2	8.1×10^{-6} (SPNBMD)	2.6×10^{-2} (FNBMD[b]) 5.7×10^{-2} (SPNBMD[b])
	rs3780674	122206740	Intron	9q33.2	C/*T*	CDK5RAP2	7.2×10^{-6} (SPNBMD)	8.9×10^{-3} (HIPBMD[b]) 4.3×10^{-2} (SPNBMD[b]) 2.0×10^{-2} (FNBMD[b])
	rs1007738	46805936	Intron	11p11.2	A/*G*	CKAP5	7.1×10^{-6} (HIPBMD)	4.1×10^{-2} (FNBMD[c])
	rs3783833	90505343	Intron	14q32.12	T/*C*	RPS6KA5	2.0×10^{-5} (SPNBMD)	3.6×10^{-3} (FNBMD[b]) 3.0×10^{-3} (FNBMD) 7.5×10^{-3} (HIPBMD[b]) 1.6×10^{-3} (HIPBMD)
	rs12437971	98662088	Intron	15q26.3	A/*G*	ADAMTS17	2.1×10^{-5} (HIPBMD)	4.5×10^{-2} (HIPBMD[c]) 4.9×10^{-2} (HIPBMD)
	rs8100029	7354832	Unknown	19p13.2	A/*G*	ARHGEF18	1.3×10^{-5} (HIPBMD)	4.4×10^{-2} (HIPBMD[b])
	rs1006899	14766923	Unknown	21q11.2	A/*G*	SAMSN1	3.9×10^{-5} (SPNBMD)	1.9×10^{-2} (FNBMD[c]) 1.6×10^{-2} (SPNBMD[c])
UK–NL	rs4276378	7297491	Unknown	5p15.31	A/*G*	ADCY2	1.2×10^{-5} (SPNBMD)	2.6×10^{-2} (FNBMD[c]) 4.2×10^{-2} (HIPBMD[c]) 4.5×10^{-2} (SPNBMD[c])
	rs286810	107486235	Intron	5q21.3	T/*C*	FBXL17	3.3×10^{-5} (FNBMD)	3.9×10^{-3} (HIPBMD[c]) 1.2×10^{-2} (HIPBMD) 2.9×10^{-2} (SPNBMD[c]) 2.4×10^{-2} (SPNBMD)
	rs2445803	174706595	Unknown	5q35.2	G/*A*	DRD1	4.3×10^{-5} (FNBMD)	1.8×10^{-2} (HIPBMD) 2.2×10^{-2} (FNBMD) 2.4×10^{-2} (FNBMD[b]) 2.2×10^{-2} (SPNBMD[b]) 2.3×10^{-3} (SPNBMD)
	rs851993	152047704	Unknown	6q25.1	A/*G*	C6orf97	4.8×10^{-5} (FNBMD)	2.0×10^{-3} (SPNBMD[b])

Initial GWAS	dbSNP	Position	Role	Chro.	Alleles[a]	Gene	Initial *p* value	Replication *p* value
	rs2892937	19624914	Unknown	7p15.3	T/*C*		4.8×10^{-5} (FNBMD)	2.5×10^{-2} (FNBMD)
	rs11135929	241018701	Unknown	8p21.2	T/*C*	PPP2R2A	3.4×10^{-5} (SPNMD)	1.7×10^{-2} (FNBMD[c]) 3.1×10^{-2} (FNBMD) 1.6×10^{-2} (HIPBMD)
	rs1397966	115097126	Unknown	8q23.3	A/*G*	CSMD3	6.0×10^{-6} (FNBMD)	4.9×10^{-2} (HIPBMD) 2.3×10^{-2} (SPNBMD[b])
	rs12675001	115188615	Unknown	8q23.3	G/*T*		3.5×10^{-5} (FNBMD)	4.3×10^{-2} (FNBMD[b])
	rs11239762	42582267	Unknown	10q11.21	G/*A*	BMS1L	2.0×10^{-6} (SPNBMD)	8.9×10^{-3} (SPNBMD[c])
	rs2306862	67934086	Coding exon	11q13.2	C/*T*	LRP5	4.8×10^{-5} (SPNBMD)	7.9×10^{-3} (SPNBMD[c])
	rs869878	74180896	Unknown	13q22.1	T/*C*	LOC400145	1.2×10^{-5} (SPNBMD)	8.7×10^{-3} (SPNBMD[b]) 2.6×10^{-2} (SPNBMD)
	rs1561389	74405696	Unknown	18q23	T/*C*	SALL3	3.1×10^{-5} (SPNBMD)	3.9×10^{-2} (FNBMD[b])

[a]Second allele is the minor allele in current study

[b]Male sample

[c]Female sample

SPNBMD combined value at the L2–L4 in FHS100K and Icelandic deCODE, and the combined value at the L1–L4 in UK-NL and current study; *HIPBMD* combined BMD at the femoral neck, trochanter, and intertrochanter region; *FNBMD* BMD at the femoral neck; *TRBMD* BMD at the trochanter

Table 4

Replicated SNPs with significant *p* values of Fisher's combined probability test

Phenotype	dbSNP	Position	Role	Chro.	Gene	*p* value				
						FHS100k	Icelandic deCODE	UK-NL	Current	Combined
SPNBMD	rs11898505	54538061	Unknown	2p16.2	SPTBN1	–	1.7×10^{-6}	–	5.1×10^{-3}	[a] 1.7×10^{-7}
	rs4276378	7297491	Unknown	5p15.31	ADCY2	–	–	1.2×10^{-5}	4.5×10^{-2}	[b] 8.3×10^{-6}
	rs3020331	152050473	Unknown	6q25.1	ESR1	–	3.4×10^{-6}	–	2.0×10^{-2}	[a] 1.2×10^{-6}
	rs4870044	151943102	Intron	6q25.1	ESR1, C6orf97	–	4.1×10^{-6}	–	5.7×10^{-4}	[a] 4.9×10^{-8}
	rs11239762	42582267	Unknown	10q11.21	BMS1L	–	–	2.0×10^{-6}	8.9×10^{-3}	[b] 3.4×10^{-7}
	rs3736228	67957871	Coding exon	11q13.2	LRP5	–	6.5×10^{-4}	6.3×10^{-12}	2.8×10^{-2}	[b] 5.3×10^{-12}
	rs2306862	67934086	Coding exon	11q13.2	LRP5	–	4.9×10^{-4}	4.8×10^{-5}	7.9×10^{-3}	[b] 6.0×10^{-6}
HIPBMD	rs6696981	22575445	Unknown	1p36.12		–	1.2×10^{-6}	–	3.5×10^{-2}	[a] 7.6×10^{-7}
	rs12437971	98662088	Intron	15q26.3	ADAMTS17	–	2.1×10^{-5}	–	4.9×10^{-2}	[a] 1.6×10^{-5}
FNBMD	rs1823926	119882620	Intron	5q23.1	LOC51334	1.3×10^{-5}	–	–	3.3×10^{-2}	[c] 6.7×10^{-6}

[a] Combined *p* value from Icelandic deCODE and replication study

[b] Combined *p* value from UK-NL and replication study

[c] Combined *p* value from FHS100K and replication study

"–" data is not available from the original report; *SPNBMD* combined value at the L2–L4 in FHS100K and Icelandic deCODE and the combined value at the L1–L4 in UK-NL and current study; *HIPBMD* combined BMD at the femoral neck, trochanter, and intertrochanter region; *FNBMD* BMD at the femoral neck