

Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study

Supplementary methods

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Study samples and clinical characteristics

The TwinsUK discovery cohort consisted of members of the TwinsUK cohort, who were previously shown to be representative of singleton populations and the UK population in general.¹ Participants in the Rotterdam cohort were derived from the Rotterdam study (n=7983), a single-centre prospective population-based study of determinants of chronic disabling diseases in elderly people (aged 55 years and older).^{2,3} The Chingford study was a population-based study of 1003 individuals who were recruited in 1989 for prospective follow-up for osteoarthritis and osteoporosis. It is listed by the NIH as an important epidemiological resource and one of the few such cohorts with wide-ranging musculoskeletal data.

Measurement of bone mineral density

The bone mineral density of all participants in the TwinsUK study was measured at lumbar spine (L1–L4) and femoral neck using dual energy x-ray absorptiometry (QDR 2000W, Hologic, Bedford, MA, USA).⁴ For monozygotic twins in TwinsUK, the genotypic information of one individual per sibling pair was included in the analysis. For these monozygotic twins, the mean bone mineral density for the twin pair was used as the phenotype, since monozygotic twins share identical genetic information. If a single twin had missing data, or was excluded, the remaining sibling was regarded as a singleton in the statistical analysis. In the Rotterdam study, bone mineral density measurements of the lumbar spine and right proximal femur used dual energy x-ray absorptiometry (DXA) with a Lunar DPX-L densitometer (Lunar Radiation Corporation, Madison, WI, USA) and were analysed with DPX-IQ version 4.7d software.⁵ Methods, quality assurance, accuracy, and precision issues for these DXA measurements have been described previously.⁵ In the Chingford study, bone mineral density at the L1–L4 spinal region and femoral neck was measured with DXA on a Hologic QDR 1000 densitometer.

We did not attempt to standardise DXA measurements because we sought replication of genotype dependent differences rather than of absolute bone mineral density values by genotype. However, all bone mineral density measurements were converted to standardised, cohort-specific Z scores.

Genotyping and quality control

For the TwinsUK discovery cohort, all samples were typed with the Infinium assay (Illumina, San Diego, USA) with fully compatible SNP arrays, the Hap300 Duo, Hap300, and Hap550. We pooled the normalised intensity data⁶ for 2820 Twins UK samples typed at Centre National de Génotypage, Duke University, NC,

USA; Helsinki University, Finland; and the Wellcome Trust Sanger Institute, Cambridge, UK, and called genotypes on the basis of the Illuminus algorithm.⁷ No calls were assigned if the most likely call was less than a posterior probability of 0.95. Validation of pooling was done by visual inspection of 100 random, shared SNPs for overt batch effects; none were observed.

We excluded 127 participants for whom the sample call rate was more than 95%; 162 for whom autosomal heterozygosity was more than 37% or less than 33%; and 341 for whom genotype concordance with another sample was more than 97% and the sample was of lesser call rate. We also removed misclassified monozygotic twins. Therefore, we removed 508 samples in total.

We excluded 2704 SNPs because $p \leq 1.0 \times 10^{-4}$ in test for deviation from Hardy–Weinberg equilibrium; 725 because the minor allele frequency was 1% or less; and 733 because the call rate was 90% or less. We retained 314075, 98.7% of all available SNPs for analysis, with a resultant call rate of 99.3%. We also visually inspected all intensity cluster plots of SNPs that showed either an association for overdispersion of the clusters, biased no calling, or erroneous genotype assignment. We discarded SNPs with any of these characteristics.

The Rotterdam study samples were assayed with the same Infinium protocol. Intensity files were analysed with the Beadstudio Genotyping Module software v.3.1.14. A no-call threshold of 0.15 was applied to a custom-generated cluster file derived from the Illumina-provided cluster file. In the custom-cluster file 2308 SNPs with Genecall scores of less than 0.90 were visually checked by two observers and manually reclustered or zeroed accordingly. Samples with a low call rate and 10th percentile Genecall score were excluded before we called genotypes.

We excluded 209 samples with a call rate below 98%. 21 had heterozygosity rates above 37% or below 33% across all autosomal SNPs; six had ambiguous estimates of X chromosome inbreeding (homozygosity) ($0.2 < F < 0.8$); 36 had mismatch between called and phenotypic gender; 102 had outliers (3 SD) identified by the clustering analysis of identity by state; and 129 had outliers identified by identity-by-state probability of greater than 97%. In total, 706 samples were removed. The SNP quality control applied to the TwinsUK cohort was also applied to the Rotterdam cohort. After exclusions, 535 188 (95.3%) of all available SNPs were available for the replication analysis. Replicated SNPs were tested for Hardy–Weinberg equilibrium. We compared genotype accuracy against 22 Taqman SNPs, and recorded less than 0.3% discrepancy across genotyping methods.

Population stratification

In the TwinsUK study, although population stratification has not been evident in other population-based studies of self-reported white Britons, we assessed for possible stratification with STRUCTURE software.⁸ We selected 517 SNPs that were informative for ancestry from the Perlegen database (F_{ST} greater than 0.2 and physical distance greater than 5 Mb). Only one randomly selected twin from each twin pair was analysed with STRUCTURE. Genotype data for unrelated HapMap population (60 from the CEPH group from Utah, 60 Yoruba, and 89 Han Chinese or Japanese) were also included to better estimate ancestral origin for non-white participants. The model allows possible admixture with allele frequencies correlated in three populations. We set so-called burn-ins as 30 000 steps, followed by 100 000 Markov chain Monte Carlo steps. We excluded 14 individuals who lay outside the CEPH cluster with ancestry coefficient of less than 0.6. We therefore removed all people with self-described ethnicity as “not white British” from the sample. Despite controlling for overt population structure, cryptic relatedness and systematic genotyping errors could produce spuriously significant results.⁹

Although TwinsUK is a sibship-based sample, and therefore could be generally robust to population stratification, some singletons and only one monozygote per monozygote pair were genotyped. Therefore we selected all singletons and randomly selected one individual per sibship and controlled for this possible bias by applying genomic control, and found the genomic inflation factor to be 1.02 or less, based on the median χ^2 statistic for both phenotypes of bone mineral density. Therefore, our sample had little evidence of cryptic relatedness. QQ plots for the TwinsUK genome-wide scan are presented in the figure.

The Rotterdam study data was examined for potential population stratification after excluding outliers detected by the IBS clustering analysis.¹⁰ The genomic control inflation factor⁹ for the distribution of test statistic of bone mineral density in the Rotterdam study was 1.002 and 1.049 across all analyses.

Statistical methods

To calculate the false-positive report probability we used results from previous genome-wide association studies, we estimated that one in 5000 SNPs is a true positive.^{11,12} SNPs with a false-positive report probability of less than 0.5 were considered for replication in the subsample of the Rotterdam cohort, as suggested by Wacholder and colleagues.¹³ The computational power required by the PLINK program (version 1.01) to perform more than 1013 permutations, to control for family structure, was prohibitive. We used the Merlin software package to assess the association between the six SNPs tested for replication in stage two.¹⁴

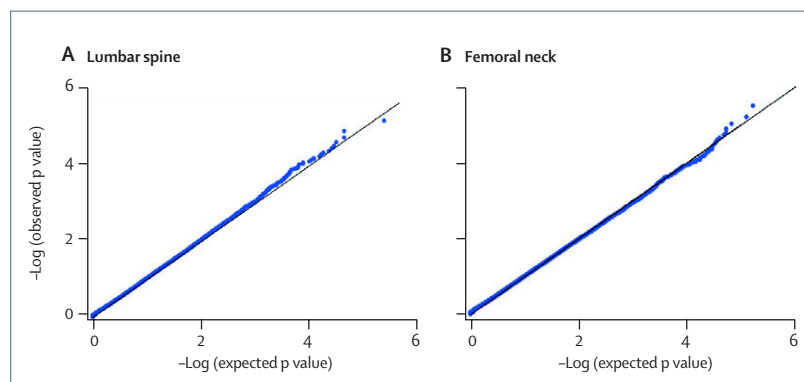


Figure: QQ plots for the TwinsUK discovery cohort

Allelic expression analysis

We used lymphoblastoid cell lines derived from populations of white people, which included 55 unrelated individuals genotyped by the International HapMap Project and 44 lymphoblastoid cell lines derived from an independent panel of unrelated individuals of white European ancestry (both from Coriell Cell Repositories and independent sources). Cells were grown at 37°C and 5% CO₂ in RPMI 1640 medium (Invitrogen, Burlington, Canada) supplemented with 15% heat-inactivated fetal bovine serum (Sigma-Aldrich, Oakville, Canada), 2 mmol/L L-glutamine (Invitrogen, Burlington, Canada) and penicillin/streptomycin (Invitrogen, Burlington, Canada). The cell growth was monitored with a haemocytometer and the cells were harvested when the density reached 0.8×10⁶–1.1×10⁶ cells per mL. Cells were then resuspended and lysed in TRIzol reagent (Invitrogen, Burlington, Canada).

Quantitative sequencing of RT-PCR products for determination of allelic expression

We assessed the extent of allelic imbalance by quantitative sequencing.¹⁵ We assessed RNA quality with an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, USA) before synthesising first strand cDNA with random hexamers (Invitrogen, Burlington, Canada) and Superscript II reverse transcriptase (Invitrogen, Burlington, Canada). For each locus, we designed locus-specific primers to target heteronuclear (unspliced pre-mRNA) at least 50 bp away from the SNP studied. We used PeakPeaker v.2.1¹⁵ with the default settings to quantify the relative amount of the two alleles measured from the chromatogram after peak intensity normalisation.

The normalised heterozygote ratios of genomic DNA samples were used to calculate mean and SD for each SNP. If both heterozygote ratios in two independent RNA samples showed concordant deviation greater than two SDs from the genomic DNA heterozygote ratio mean the sample was called to have allelic imbalance. If one of the two RNA replicates was within two SDs or if the RNA replicates deviated to opposite directions, the sample was

defined as “undetermined”. If two or more marker SNPs had available allelic imbalance data for the same sample, the phase information from HapMap allowed assessment of concordance of allelic expression calls: if a site showed relative overexpression of the transcript derived from one chromosome, the alleles on this chromosome were assigned a “+” sign and the alternate alleles were assigned a “-” sign. If discordant calls were made at independent SNPs (n=2), the sample was set to be informative for qualitative allelic expression mapping. The chromosomal alleles neighbouring a test gene that had an assignment of “+” or “-” were used in the association tests; allele counts for each unequivocally phased site were tabulated in a two-by-two table. If the distribution of alleles deviated significantly (with a two-tailed Fisher’s exact test) between the “+” and “-” chromosomes, a putative association for allelic expression was determined.¹⁶ The quantitative allelic expression assessment was based on average allele ratio for each sample that was heterozygous for rs435580,¹ and even inconsistent allelic expression calls were included in this analysis. We correlated genotypes from HapMap against expression data by linear expression using expression data from two published studies in HapMap CEU lymphoblastoid cell lines^{17,18} to corroborate allelic expression results.

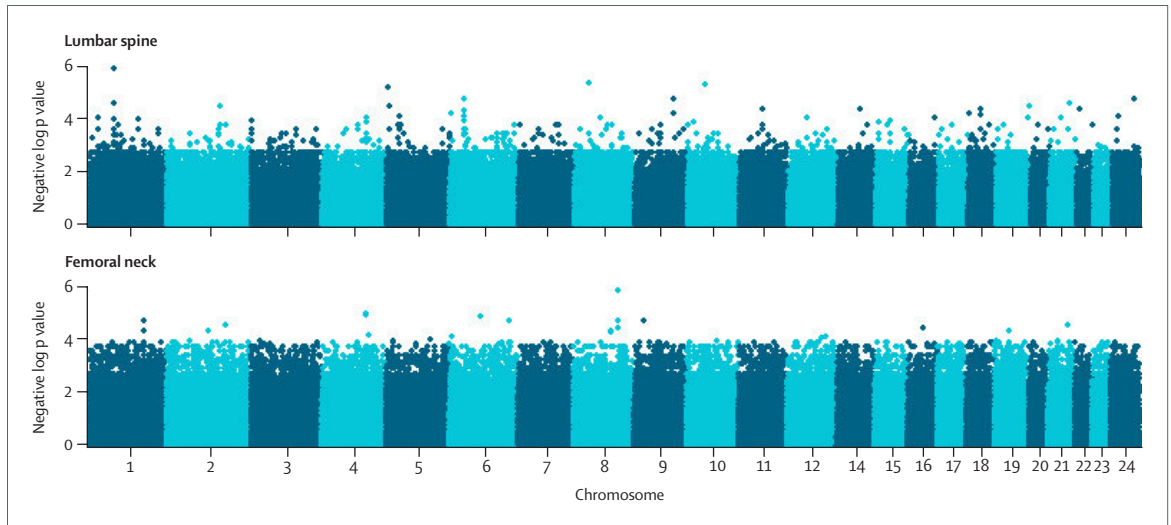
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For the phase information from HapMap see <http://www.hapmap.org>

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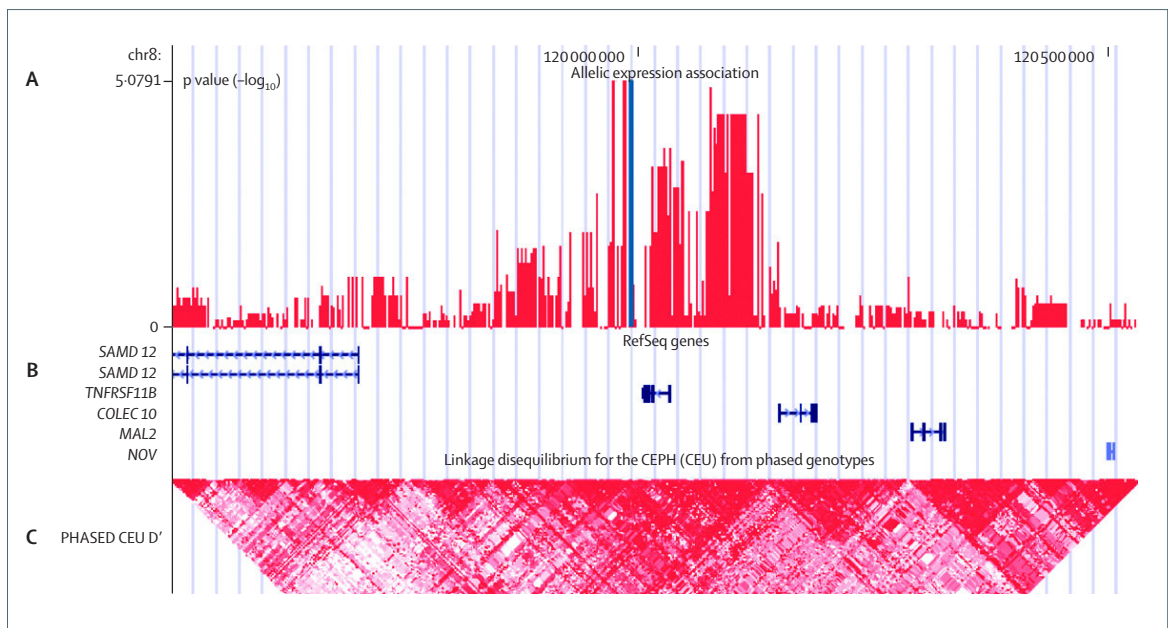
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Webfigure 1: Negative log p value of each single nucleotide polymorphism and the genomic position in the TwinsUK discovery cohort

Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study

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Webfigure 2: Association of allelic expression in lymphoblast cell lines in the region of *TNFRSF11B* in comparison with the likely position of genes and corresponding linkage disequilibrium map

(A) Negative log p value of each single nucleotide polymorphism and genomic position in the allelic expression sample (rs4355801 is coloured in blue).

(B) Annotated reference sequence (RefSeq) genes by chromosomal position (according to the National Centre for Biotechnology Information). (C) Linkage disequilibrium plot of phased CEU HapMap population.

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Chromosome	SNP	Minor allele	Gene	p value	β^*	FPRP
Lumbar spine						
1	rs2769258	G	KCNQ4	1.5×10^{-4}	-0.12	0.48
1	rs983034	A	GPR177	1.8×10^{-4}	-0.13	0.53
1	rs3762371	A	GPR177	1.7×10^{-5}	-0.15	0.10
1	rs1360180	A	GPR177	1.4×10^{-4}	0.20	0.47
1	rs7521837	G	HAX1	9.0×10^{-5}	-0.15	0.36
2	rs4662248	A	ZFHX1B	1.7×10^{-4}	0.12	0.51
2	rs1385363	A	ZFHX1B	1.4×10^{-4}	0.12	0.46
2	rs4664076	A	CACNB4	5.8×10^{-5}	-0.16	0.27
3	rs1400176	G	CCR5	7.6×10^{-5}	0.14	0.32
4	rs11099284	A	PCDH10	1.4×10^{-4}	0.17	0.47
4	rs4075671	C	PCDH10	4.8×10^{-5}	0.23	0.23
4	rs6535028	A	PCDH10	3.7×10^{-5}	0.24	0.19
5	rs594459	C	IRX1	5.3×10^{-5}	0.15	0.25
5	rs4276378	G	ADCY2	1.2×10^{-5}	0.15	0.07
5	rs1423493	A	CTNND2	1.0×10^{-4}	-0.13	0.39
5	rs1818116	G	FLJ14054	1.6×10^{-4}	0.13	0.49
6	rs969931	C	UBD	9.7×10^{-5}	0.13	0.38
8	rs11135929	G	PPP2R2A	3.4×10^{-5}	0.26	0.18
8	rs4355801	G	TNFRSF11B	7.9×10^{-4}	0.11	0.83
8	rs6469792	A	TNFRSF11B	1.4×10^{-4}	0.13	0.47
8	rs6469804	G	COLEC10	1.6×10^{-4}	0.12	0.50
9	rs12353411	A	DOCK8	7.5×10^{-4}	0.22	0.82
9	rs956918	C	DOCK8	1.3×10^{-4}	0.26	0.45
9	rs1452336	G	LRRN6C	2.4×10^{-5}	-0.14	0.13
9	rs1343452	C	LRRN6C	1.1×10^{-4}	-0.14	0.41
9	rs10812748	G	LRRN6C	1.3×10^{-4}	0.13	0.45
9	rs2993008	A	TRPM3	1.3×10^{-4}	-0.21	0.46
9	rs7864018	A	ZNF462	2.1×10^{-5}	0.17	0.12
10	rs11239762	A	BMS1L	2.0×10^{-6}	0.30	0.01
11	rs2306862	A	LRP5	4.8×10^{-5}	-0.18	0.23
11	rs923346	G	LRP5	7.6×10^{-5}	-0.18	0.32
11	rs3736228	A	LRP5	1.9×10^{-5}	-0.20	0.11
13	rs2077779	G	LOC400145	9.2×10^{-5}	-0.13	0.37
13	rs869878	G	LOC400145	1.2×10^{-5}	-0.16	0.07
14	rs7147583	G	STXBP6	1.5×10^{-4}	0.12	0.48
15	rs5025837	A	TMC05	1.4×10^{-4}	0.18	0.47
17	rs10491121	A	CCL4	9.3×10^{-5}	-0.13	0.37
18	rs1561389	G	SALL3	3.1×10^{-5}	-0.14	0.16
20	rs6126343	G	SALL4	1.0×10^{-4}	-0.13	0.38
Femoral neck						
1	rs10489579	G	C1orf26	9.2×10^{-5}	0.12	0.36
1	rs12404187	G	C1orf26	1.6×10^{-4}	0.12	0.50
1	rs6698109	G	C1orf26	2.9×10^{-5}	0.13	0.15
1	rs2378910	A	C1orf26	8.1×10^{-5}	0.12	0.33
1	rs1889976	A	C1orf26	4.3×10^{-5}	0.12	0.21
1	rs12041704	G	C1orf26	3.4×10^{-5}	0.12	0.18
1	rs2073237	G	IVNS1ABP	1.2×10^{-4}	0.11	0.43
1	rs10911730	A	IVNS1ABP	6.8×10^{-5}	0.13	0.30
2	rs1862101	A	DDX1	4.6×10^{-5}	0.12	0.22
2	rs7591368	C	DDX1	1.2×10^{-4}	0.11	0.44
2	rs10496481	A	DPP10	6.5×10^{-5}	-0.14	0.29

(Continues on next page)

Chromosome	SNP	Minor allele	Gene	p value	β^*	FPRP
(Continued from previous page)						
2	rs2161917	A	MYO3B	1.4×10 ⁻⁴	-0.11	0.46
2	rs1035629	G	MYO3B	2.8×10 ⁻⁵	-0.13	0.15
3	rs1516321	G	CCR5	1.2×10 ⁻⁴	-0.14	0.42
3	rs7624034	A	NDUFB4	2.2×10 ⁻⁴	-0.14	0.58
3	rs9835250	A	ROBO1	1.3×10 ⁻⁴	0.12	0.44
3	rs1400360	G	DGKG	8.0×10 ⁻⁶	0.22	0.05
4	rs11099284	A	PCDH10	5.7×10 ⁻⁵	0.16	0.26
4	rs4075671	C	PCDH10	2.1×10 ⁻⁵	0.22	0.12
4	rs6535028	A	PCDH10	1.9×10 ⁻⁵	0.23	0.11
5	rs1304732	A	GDNF	1.4×10 ⁻⁴	0.17	0.46
5	rs286810	G	FBXL17	3.3×10 ⁻⁵	-0.13	0.17
5	rs40066	G	FBXL17	9.1×10 ⁻⁶	-0.12	0.36
5	rs40065	G	FBXL17	3.1×10 ⁻⁵	-0.13	0.16
5	rs2445803	A	DRD1	4.3×10 ⁻⁵	-0.13	0.21
5	rs251925	A	DRD1	5.6×10 ⁻⁵	-0.13	0.26
6	rs1003878	A	C6orf10	5.7×10 ⁻⁵	0.14	0.26
6	rs3129943	G	C6orf10	2.5×10 ⁻⁵	0.14	0.14
6	rs1980495	C	C6orf10	5.2×10 ⁻⁵	-0.13	0.25
6	rs2395157	G	C6orf10	1.8×10 ⁻⁴	-0.12	0.53
6	rs3817963	G	BTNL2	5.9×10 ⁻⁴	-0.11	0.79
6	rs3806156	A	BTNL2	1.6×10 ⁻⁴	-0.11	0.51
6	rs10945294	C	OGFRL1	1.9×10 ⁻⁵	0.15	0.11
6	rs851995	G	C6orf97	6.0×10 ⁻⁴	0.10	0.79
6	rs851993	G	C6orf97	4.8×10 ⁻⁵	0.12	0.23
7	rs2892937	G	TWISTNB	4.7×10 ⁻⁵	-0.26	0.23
7	rs6947737	A	TWISTNB	1.3×10 ⁻⁴	-0.24	0.45
7	rs10253828	G	FZD1	1.1×10 ⁻⁴	-0.15	0.41
7	rs7777322	A	NRCAM	4.0×10 ⁻⁵	-0.32	0.20
7	rs11771846	G	SPAM1	1.3×10 ⁻⁴	-0.14	0.45
8	rs1397966	G	CSMD3	6.0×10 ⁻⁶	-0.13	0.04
8	rs4445256	A	CSMD3	5.7×10 ⁻⁵	-0.11	0.26
8	rs12675001	A	CSMD3	3.5×10 ⁻⁵	-0.12	0.18
9	rs10961580	A	ZDHHHC21	3.9×10 ⁻⁵	0.26	0.20
10	rs10508264	A	KLF6	1.4×10 ⁻⁴	0.16	0.47
11	rs1124115	A	SOX6	1.5×10 ⁻⁴	0.11	0.49
12	rs7960594	A	E2F7	1.3×10 ⁻⁴	0.13	0.45
13	rs9590039	G	TGDS	1.4×10 ⁻⁴	0.13	0.47
14	rs8019150	A	PTGDR	7.6×10 ⁻⁵	0.36	0.32
16	rs3927119	G	FAM86A	1.6×10 ⁻⁴	-0.11	0.50
18	rs8086459	A	CDH2	6.8×10 ⁻⁵	-0.25	0.30
18	rs8099735	C	CDH2	1.6×10 ⁻⁴	-0.26	0.50
18	rs11876069	G	CDH2	1.0×10 ⁻⁴	-0.27	0.38
18	rs1834159	A	DCC	1.4×10 ⁻⁴	0.13	0.46
20	rs6067931	A	SALL4	1.5×10 ⁻⁵	0.15	0.09
20	rs6126343	G	SALL4	1.2×10 ⁻⁴	-0.12	0.43
20	rs1293381	G	ZNF218	3.5×10 ⁻⁵	0.12	0.18
22	rs138150	A	ATXN10	8.4×10 ⁻⁵	0.14	0.34
22	rs138203	G	ATXN10	8.9×10 ⁻⁵	0.13	0.36
22	rs135990	G	ATXN10	2.8×10 ⁻⁴	0.12	0.64

* β is the effect of the minor allele on bone mineral density at lumbar spine (measured in standardised standard deviations and controlled for age). FPRP=probability of false positive report.

Webtable 1: SNPs tested for replication stage one: relationship between SNPs and bone mineral density in the TwinsUK discovery cohort (n=2094)

Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study

Gene	p value TwinsUK discovery cohort (n=2094)	p value Rotterdam subsample (n=1586)	β^* at stage one	p value at stage one (n=3680)	p value Chingford cohort (n=690)	p value TwinsUK replication cohort (n=1692)	p value Rotterdam replication cohort (n=2495)	β at stage two	p value at stage two (n=4877)	Combined p value (n=8557)
rs3736228 <i>LRP5</i>	1.9×10^{-5}	3.0×10^{-2}	-0.16	2.2×10^{-5}	0.025	5.3×10^{-3}	8.0×10^{-4}	-0.12	1.2×10^{-6}	6.3×10^{-12}
rs4355801 <i>TNFRSF11B</i>	7.9×10^{-4}	5.0×10^{-4}	-0.11	5.4×10^{-5}	0.032	0.32	3.6×10^{-4}	-0.07	8.8×10^{-5}	7.6×10^{-10}

* β is expressed as the effect of each risk allele on bone mineral density of the lumbar spine (measured in standardised standard deviations and controlled for age).

Webtable 2: Details of SNPs with genome-wide significance for relationship with bone mineral density at lumbar spine

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Chromosome	Position	SNP	Minor allele	p value	β^*
LRP5					
11	67654059	rs7944372	G	4.6×10 ⁻¹	0.02
11	67662557	rs2511475	G	4.9×10 ⁻¹	0.03
11	67672269	rs1547890	A	8.8×10 ⁻¹	0.01
11	67688337	rs4930561	A	6.1×10 ⁻¹	0.02
11	67690218	rs1114399	A	3.7×10 ⁻¹	0.06
11	67710348	rs10896300	A	8.8×10 ⁻¹	0.01
11	67716183	rs7931502	A	5.4×10 ⁻¹	0.02
11	67728122	rs2129848	G	2.4×10 ⁻¹	-0.06
11	67748900	rs1619377	G	1.4×10 ⁻¹	-0.06
11	67778582	rs2450908	A	1.7×10 ⁻²	-0.08
11	67782043	rs674857	A	1.2×10 ⁻²	-0.10
11	67784557	rs17148984	A	2.8×10 ⁻¹	-0.05
11	67786749	rs3802746	A	1.9×10 ⁻³	0.11
11	67794461	rs1979579	G	1.1×10 ⁻¹	-0.06
11	67797219	rs11600189	A	7.5×10 ⁻¹	-0.01
11	67801380	rs450112	A	3.6×10 ⁻³	0.11
11	67822610	rs312018	A	4.9×10 ⁻³	0.09
11	67845407	rs4988300	A	8.2×10 ⁻¹	-0.01
11	67858576	rs606989	A	5.8×10 ⁻¹	0.04
11	67868248	rs314756	G	1.0	-0.01
11	67875139	rs312783	G	1.0	-0.02
11	67886182	rs160607	A	7.7×10 ⁻¹	-0.01
11	67897990	rs638051	G	1.8×10 ⁻¹	-0.05
11	67900859	rs11602256	A	4.4×10 ⁻¹	-0.03
11	67927589	rs545382	A	1.7×10 ⁻¹	0.07
11	67934086	rs2306862	A	4.8×10 ⁻⁵	-0.18
11	67938951	rs923346	G	7.6×10 ⁻⁵	-0.18
11	67942968	rs546803	G	7.6×10 ⁻³	-0.10
11	67953406	rs608343	G	6.2×10 ⁻²	-0.06
11	67957871	rs3736228	A	1.9×10 ⁻⁵	-0.20
11	67973996	rs676318	G	3.2×10 ⁻²	0.13
11	67987816	rs624003	A	1.6×10 ⁻²	-0.09
11	67992965	rs2472429	G	4.4×10 ⁻¹	-0.03
11	68050121	rs7950900	A	2.3×10 ⁻³	-0.10
11	68055088	rs7106259	C	6.4×10 ⁻⁴	-0.12
11	68055802	rs11228271	A	8.9×10 ⁻¹	-0.01
11	68058677	rs2840367	G	1.8×10 ⁻²	-0.08
11	68074763	rs7946537	G	2.2×10 ⁻¹	0.05
11	68157529	rs2510375	G	1.0	0.01
11	68167923	rs4930585	A	2.4×10 ⁻³	-0.14
11	68178468	rs4930591	G	1.0×10 ⁻¹	-0.06
11	68183820	rs2156464	A	4.5×10 ⁻³	-0.12
11	68186263	rs7935394	G	1.4×10 ⁻¹	-0.05
11	68197773	rs6591348	A	3.1×10 ⁻¹	0.06
11	68203000	rs2187331	G	8.8×10 ⁻⁴	-0.14
11	68210005	rs3136537	C	3.4×10 ⁻¹	-0.08
11	68215046	rs1042577	A	2.8×10 ⁻¹	-0.04
11	68225246	rs6591350	A	1.4×10 ⁻¹	-0.06
11	68250288	rs10791993	A	1.5×10 ⁻¹	-0.05
11	68256450	rs4930243	G	6.2×10 ⁻¹	-0.02
11	68269119	rs12365708	G	6.7×10 ⁻¹	0.02

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Chromosome	Position	SNP	Minor allele	p value	β^*
(Continued from previous page)					
11	68288172	rs4930608	A	8.2×10^{-1}	-0.02
11	68295236	rs2123869	G	8.8×10^{-1}	-0.02
11	68306122	rs2305508	G	3.3×10^{-1}	0.03
11	68313407	rs12364396	A	1.0	-0.01
11	68322480	rs4930248	G	1.3×10^{-1}	-0.05
11	68333527	rs1037488	G	7.5×10^{-1}	0.01
11	68349834	rs11228368	A	8.8×10^{-1}	0.00
11	68353680	rs10896371	G	1.0	-0.03
TNFRSF11B					
8	119900459	rs903612	A	8.8×10^{-1}	0.01
8	119902646	rs1389545	A	3.6×10^{-2}	-0.08
8	119906332	rs3133585	A	3.9×10^{-2}	-0.07
8	119925390	rs3134086	G	6.2×10^{-2}	-0.07
8	119941620	rs2035979	A	4.0×10^{-2}	-0.06
8	119955272	rs3103991	A	4.9×10^{-1}	-0.03
8	119955282	rs3134095	A	5.6×10^{-1}	-0.04
8	119956070	rs16891617	G	3.3×10^{-1}	-0.06
8	119956104	rs2055101	G	1.0×10^{-2}	-0.08
8	119966499	rs4355804	A	2.7×10^{-1}	-0.07
8	119966914	rs4372031	G	5.0×10^{-1}	-0.04
8	119966999	rs4560819	A	3.0×10^{-1}	-0.06
8	119990535	rs4130891	A	2.3×10^{-1}	-0.06
8	119993054	rs4355801	G	7.9×10^{-4}	0.11
8	120014347	rs1564858	A	1.0×10^{-1}	-0.08
8	120015988	rs3102724	A	9.7×10^{-2}	-0.06
8	120018949	rs2875845	G	1.6×10^{-1}	-0.06
8	120020873	rs1905786	G	7.0×10^{-3}	-0.08
8	120020954	rs1032128	A	1.6×10^{-1}	-0.05
8	120022649	rs3134057	G	3.2×10^{-2}	-0.07
8	120023289	rs3134058	A	2.5×10^{-2}	-0.08
8	120025224	rs3134060	G	4.0×10^{-1}	-0.06
8	120028459	rs3134062	G	5.6×10^{-1}	-0.03
8	120028804	rs11573829	A	2.4×10^{-3}	0.11
8	120033024	rs10505346	A	8.0×10^{-2}	-0.08
8	120034251	rs3102735	G	6.0×10^{-2}	-0.09
8	120035568	rs1385505	A	5.7×10^{-2}	-0.09
8	120037914	rs13270766	A	3.6×10^{-2}	-0.12
8	120044770	rs1564859	A	1.1×10^{-1}	-0.06
8	120044851	rs1564860	A	1.3×10^{-2}	-0.09
8	120045325	rs1485302	A	8.8×10^{-3}	-0.09
8	120045437	rs1485303	A	6.6×10^{-3}	-0.09
8	120048278	rs6987559	A	4.1×10^{-1}	-0.04
8	120048430	rs7837123	C	4.0×10^{-2}	-0.11
8	120051022	rs7835846	G	1.0	-0.01
8	120055416	rs4876876	A	8.5×10^{-4}	0.11
8	120077552	rs6469792	A	1.4×10^{-4}	0.13
8	120088833	rs1905776	A	4.9×10^{-1}	-0.03
8	120091667	rs1118342	G	2.6×10^{-1}	-0.05
8	120098890	rs2326193	A	5.9×10^{-4}	0.11
8	120114010	rs6469804	G	1.6×10^{-4}	0.12
8	120120886	rs1385507	A	7.5×10^{-1}	0.04

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Chromosome	Position	SNP	Minor allele	p value	β^*
(Continued from previous page)					
8	120121419	rs6993813	A	1.8×10^{-4}	0.12
8	120133224	rs16891916	C	8.5×10^{-1}	-0.01
8	120134430	rs2465384	A	8.8×10^{-1}	-0.01
8	120141079	rs1485315	A	2.6×10^{-1}	-0.05
8	120148425	rs2450058	G	8.8×10^{-1}	0.03
8	120149296	rs1385498	G	8.8×10^{-1}	-0.01
8	120158016	rs1905775	A	2.4×10^{-1}	0.04
8	120180592	rs17179583	G	1.9×10^{-1}	-0.06
8	120182163	rs1351953	G	4.3×10^{-1}	0.04
8	120189610	rs6469809	G	2.1×10^{-1}	0.07
* β is the effect of the minor allele on bone mineral density at lumbar spine (measured in standardised standard deviations and controlled for age).					
Webtable 3: SNPs near the LRP5 and TNFRSF11B genes and bone mineral density at lumbar spine in the TwinsUK discovery cohort (n=2094)					

Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study

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Chromosome	Position	SNP	Minor allele	p value	β^*
LRP5					
11	67654059	rs7944372	G	4.6×10 ⁻¹	0.02
11	67662557	rs2511475	G	4.9×10 ⁻¹	0.03
11	67672269	rs1547890	A	8.8×10 ⁻¹	0.01
11	67688337	rs4930561	A	6.1×10 ⁻¹	0.02
11	67690218	rs1114399	A	3.7×10 ⁻¹	0.06
11	67710348	rs10896300	A	8.8×10 ⁻¹	0.01
11	67716183	rs7931502	A	5.4×10 ⁻¹	0.02
11	67728122	rs2129848	G	2.4×10 ⁻¹	-0.06
11	67748900	rs1619377	G	1.4×10 ⁻¹	-0.06
11	67778582	rs2450908	A	1.7×10 ⁻²	-0.08
11	67782043	rs674857	A	1.2×10 ⁻²	-0.10
11	67784557	rs17148984	A	2.8×10 ⁻¹	-0.05
11	67786749	rs3802746	A	1.9×10 ⁻³	0.11
11	67794461	rs1979579	G	1.1×10 ⁻¹	-0.06
11	67797219	rs11600189	A	7.5×10 ⁻¹	-0.01
11	67801380	rs450112	A	3.6×10 ⁻³	0.11
11	67822610	rs312018	A	4.9×10 ⁻³	0.09
11	67845407	rs4988300	A	8.2×10 ⁻¹	-0.01
11	67858576	rs606989	A	5.8×10 ⁻¹	0.04
11	67868248	rs314756	G	1.0	-0.01
11	67875139	rs312783	G	1.0	-0.02
11	67886182	rs160607	A	7.7×10 ⁻¹	-0.01
11	67897990	rs638051	G	1.8×10 ⁻¹	-0.05
11	67900859	rs11602256	A	4.4×10 ⁻¹	-0.03
11	67927589	rs545382	A	1.7×10 ⁻¹	0.07
11	67934086	rs2306862	A	4.8×10 ⁻⁵	-0.18
11	67938951	rs923346	G	7.6×10 ⁻⁵	-0.18
11	67942968	rs546803	G	7.6×10 ⁻³	-0.10
11	67953406	rs608343	G	6.2×10 ⁻²	-0.06
11	67957871	rs3736228	A	1.9×10 ⁻⁵	-0.20
11	67973996	rs676318	G	3.2×10 ⁻²	0.13
11	67987816	rs624003	A	1.6×10 ⁻²	-0.09
11	67992965	rs2472429	G	4.4×10 ⁻¹	-0.03
11	68050121	rs7950900	A	2.3×10 ⁻³	-0.10
11	68055088	rs7106259	C	6.4×10 ⁻⁴	-0.12
11	68055802	rs11228271	A	8.9×10 ⁻¹	-0.01
11	68058677	rs2840367	G	1.8×10 ⁻²	-0.08
11	68074763	rs7946537	G	2.2×10 ⁻¹	0.05
11	68157529	rs2510375	G	1.0	0.01
11	68167923	rs4930585	A	2.4×10 ⁻³	-0.14
11	68178468	rs4930591	G	1.0×10 ⁻¹	-0.06
11	68183820	rs2156464	A	4.5×10 ⁻³	-0.12
11	68186263	rs7935394	G	1.4×10 ⁻¹	-0.05
11	68197773	rs6591348	A	3.1×10 ⁻¹	0.06
11	68203000	rs2187331	G	8.8×10 ⁻⁴	-0.14
11	68210005	rs3136537	C	3.4×10 ⁻¹	-0.08
11	68215046	rs1042577	A	2.8×10 ⁻¹	-0.04
11	68225246	rs6591350	A	1.4×10 ⁻¹	-0.06
11	68250288	rs10791993	A	1.5×10 ⁻¹	-0.05
11	68256450	rs4930243	G	6.2×10 ⁻¹	-0.02
11	68269119	rs12365708	G	6.7×10 ⁻¹	0.02

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Chromosome	Position	SNP	Minor allele	p value	β^*
(Continued from previous page)					
11	68288172	rs4930608	A	8.2×10^{-1}	-0.02
11	68295236	rs2123869	G	8.8×10^{-1}	-0.02
11	68306122	rs2305508	G	3.3×10^{-1}	0.03
11	68313407	rs12364396	A	1.0	-0.01
11	68322480	rs4930248	G	1.3×10^{-1}	-0.05
11	68333527	rs1037488	G	7.5×10^{-1}	0.01
11	68349834	rs11228368	A	8.8×10^{-1}	0.00
11	68353680	rs10896371	G	1.0	-0.03
TNFRSF11B					
8	119900459	rs903612	A	8.8×10^{-1}	0.01
8	119902646	rs1389545	A	3.6×10^{-2}	-0.08
8	119906332	rs3133585	A	3.9×10^{-2}	-0.07
8	119925390	rs3134086	G	6.2×10^{-2}	-0.07
8	119941620	rs2035979	A	4.0×10^{-2}	-0.06
8	119955272	rs3103991	A	4.9×10^{-1}	-0.03
8	119955282	rs3134095	A	5.6×10^{-1}	-0.04
8	119956070	rs16891617	G	3.3×10^{-1}	-0.06
8	119956104	rs2055101	G	1.0×10^{-2}	-0.08
8	119966499	rs4355804	A	2.7×10^{-1}	-0.07
8	119966914	rs4372031	G	5.0×10^{-1}	-0.04
8	119966999	rs4560819	A	3.0×10^{-1}	-0.06
8	119990535	rs4130891	A	2.3×10^{-1}	-0.06
8	119993054	rs4355801	G	7.9×10^{-4}	0.11
8	120014347	rs1564858	A	1.0×10^{-1}	-0.08
8	120015988	rs3102724	A	9.7×10^{-2}	-0.06
8	120018949	rs2875845	G	1.6×10^{-1}	-0.06
8	120020873	rs1905786	G	7.0×10^{-3}	-0.08
8	120020954	rs1032128	A	1.6×10^{-1}	-0.05
8	120022649	rs3134057	G	3.2×10^{-2}	-0.07
8	120023289	rs3134058	A	2.5×10^{-2}	-0.08
8	120025224	rs3134060	G	4.0×10^{-1}	-0.06
8	120028459	rs3134062	G	5.6×10^{-1}	-0.03
8	120028804	rs11573829	A	2.4×10^{-3}	0.11
8	120033024	rs10505346	A	8.0×10^{-2}	-0.08
8	120034251	rs3102735	G	6.0×10^{-2}	-0.09
8	120035568	rs1385505	A	5.7×10^{-2}	-0.09
8	120037914	rs13270766	A	3.6×10^{-2}	-0.12
8	120044770	rs1564859	A	1.1×10^{-1}	-0.06
8	120044851	rs1564860	A	1.3×10^{-2}	-0.09
8	120045325	rs1485302	A	8.8×10^{-3}	-0.09
8	120045437	rs1485303	A	6.6×10^{-3}	-0.09
8	120048278	rs6987559	A	4.1×10^{-1}	-0.04
8	120048430	rs7837123	C	4.0×10^{-2}	-0.11
8	120051022	rs7835846	G	1.0	-0.01
8	120055416	rs4876876	A	8.5×10^{-4}	0.11
8	120077552	rs6469792	A	1.4×10^{-4}	0.13
8	120088833	rs1905776	A	4.9×10^{-1}	-0.03
8	120091667	rs1118342	G	2.6×10^{-1}	-0.05
8	120098890	rs2326193	A	5.9×10^{-4}	0.11
8	120114010	rs6469804	G	1.6×10^{-4}	0.12
8	120120886	rs1385507	A	7.5×10^{-1}	0.04

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Chromosome	Position	SNP	Minor allele	p value	β^*
(Continued from previous page)					
8	120121419	rs6993813	A	1.8×10^{-4}	0.12
8	120133224	rs16891916	C	8.5×10^{-1}	-0.01
8	120134430	rs2465384	A	8.8×10^{-1}	-0.01
8	120141079	rs1485315	A	2.6×10^{-1}	-0.05
8	120148425	rs2450058	G	8.8×10^{-1}	0.03
8	120149296	rs1385498	G	8.8×10^{-1}	-0.01
8	120158016	rs1905775	A	2.4×10^{-1}	0.04
8	120180592	rs17179583	G	1.9×10^{-1}	-0.06
8	120182163	rs1351953	G	4.3×10^{-1}	0.04
8	120189610	rs6469809	G	2.1×10^{-1}	0.07
* β is the effect of the minor allele on bone mineral density at lumbar spine (measured in standardised standard deviations and controlled for age).					
Webtable 3: SNPs near the LRP5 and TNFRSF11B genes and bone mineral density at lumbar spine in the TwinsUK discovery cohort (n=2094)					

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Top allelic expression SNPs	p value for allelic expression	Overexpressed allele in allelic expression test
rs10955908	8.3×10^{-6}	A
rs13439134	8.3×10^{-6}	C
rs13250753	8.3×10^{-6}	G
rs4407910	8.3×10^{-6}	G
rs13277230	8.3×10^{-6}	T
rs10101385	8.3×10^{-6}	G
rs4355801	8.3×10^{-6}	G
rs4567065	1.3×10^{-5}	C
rs7842942	1.3×10^{-5}	T
rs4354338	4.5×10^{-5}	A

Webtable 4: Association between SNPs and *TNFRSF11B* allelic expression