Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture

Bone mineral density (BMD) is the most widely used predictor of fracture risk. We performed the largest meta-analysis to date on lumbar spine and femoral neck BMD, including 17 genome-wide association studies and 32,961 individuals of European and east Asian ancestry. We tested the top BMD-associated markers for replication in 50,933 independent subjects and for association with risk of low-trauma fracture in 31,016 individuals with a history of fracture (cases) and 102,444 controls. We identified 56 loci (32 new) associated with BMD at genome-wide significance ($P < 5 \times 10^{-8}$). Several of these factors cluster within the RANK-RANKL-OPG, mesenchymal stem cell differentiation, endochondral ossification and Wnt signaling pathways. However, we also discovered loci that were localized to genes not known to have a role in bone biology. Fourteen BMD-associated loci were also associated with fracture risk ($P < 5 \times 10^{-4}$, Bonferroni corrected), of which six reached $P < 5 \times 10^{-8}$, including at 18p11.21 (*FAM210A*), 7q21.3 (*SLC25A13*), 11q13.2 (*LRP5*), 4q22.1 (*MEPE*), 2p16.2 (*SPTBN1*) and 10q21.1 (*DKK1*). These findings shed light on the genetic architecture and pathophysiological mechanisms underlying BMD variation and fracture susceptibility.

Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to increased risk of fracture. The disease accounts for approximately 1.5 million new fracture cases each year, representing a huge economic burden on health care systems, with annual costs estimated to be $17 billion in the United States alone and expected to rise 50% by the year 2025 (ref. 1). Osteoporosis is defined clinically through the measurement of BMD, which remains the single best predictor of fracture[2,3].

Twin and family studies have shown that 50–85% of the variance in BMD is genetically determined[4]. Osteoporotic fractures are also heritable by mechanisms that are partly independent of BMD[5]. Over the past 5 years, genome-wide association studies (GWAS) have revolutionized the understanding of the genetic architecture of common, complex diseases[6]. This approach is providing key insights into the mechanisms of disease, with prospects for the design of effective strategies for risk assessment and the development of new interventions[7].

Previous GWAS have identified 24 loci that influence BMD variation[8–14]. Whereas several variants in these BMD-associated loci have also been nominally associated with fracture risk[15,16], none have shown robust association with genome-wide significance ($P < 5 \times 10^{-8}$). We report here the results of the largest effort to date searching for BMD-associated loci in >80,000 subjects and testing them for association with fracture in >130,000 cases and controls. In addition, we employed bioinformatics tools and gene expression analyses to place the identified variants in the context of pathways relevant to bone biology.

RESULTS

This study was performed across three main stages (**Fig. 1**): (i) the discovery of BMD loci, (ii) follow-up replication and (iii) association of the BMD-associated loci with fracture.

Discovery of BMD loci (stage 1)

We first performed a meta-analysis of multiple GWAS for BMD of the femoral neck (FN-BMD; $n = 32,961$) and lumbar spine (LS-BMD; $n = 31,800$ cases), including ~2.5 million genotyped or imputed autosomal SNPs from 17 studies of populations across North America, Europe, East Asia and Australia, with a variety of epidemiological designs and subject characteristics (Online Methods). We also performed meta-analysis in men and women separately to identify sex-specific associations. The quantile-quantile plots of the discovery meta-analysis showed strong (and not early) deviation of the observed statistics from the null distribution of no association for both BMD traits (**Supplementary Fig. 1**). After double genomic control correction of the overall ($\lambda_{\text{FN-BMD pooled}} = 1.112$; $\lambda_{\text{LS-BMD pooled}} = 1.127$) and sex-stratified ($\lambda_{\text{FN-BMD women}} = 1.091$; $\lambda_{\text{FN-BMD men}} = 1.059$; $\lambda_{\text{LS-BMD women}} = 1.086$; $\lambda_{\text{LS-BMD men}} = 1.061$) analyses, SNPs in 34 loci surpassed genome-wide significance, whereas a total of 82 loci were associated at $P < 5 \times 10^{-6}$ (**Supplementary Figs. 2** and **3**). Thirty-eight loci were associated with FN-BMD, 25 with LS-BMD and 19 with both. The overlap reflects correlation between the femoral neck and lumbar spine measurements (Pearson's correlation = 0.53). Of these 82 loci, 59, 18 and 5 were prioritized from analyses in the sex-combined, female and male sample sets, respectively (**Supplementary Table 1**). The meta-analysis was extended to include the evaluation of 76,253 markers on the X chromosome imputed across 14 of the discovery GWAS, for a total of 31,801 participants (Online Methods). Five loci on the X chromosome were associated at $P < 5 \times 10^{-5}$, with four of these derived from the sex-combined analysis and one identified in the analysis of men only (**Supplementary Table 1**). We further performed genome-wide conditional analyses in all sex-combined stage 1 studies. Each study repeated the GWAS analysis but also adjusted for 82 SNPs
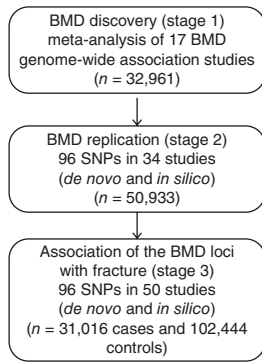
Figure 1 Description of study design. Stage 1: meta-analysis of 17 genome-wide association studies for BMD. Stage 2: 96 top independent SNPs (82 autosomal SNPs with $P < 5 \times 10^{-6}$, 5 SNPs on the X chromosome and 9 SNPs from conditional analysis) were followed up in *de novo* and *in silico* replication of the BMD association in 34 studies. Stage 3: the same 96 SNPs were tested for association with fracture in 50 studies with *de novo* and *in silico* data.

representing the autosomal loci associated at $P < 5 \times 10^{-6}$ (Online Methods). We then performed meta-analysis on these studies in the same way as in the primary GWAS meta-analysis. Nine loci showed at least two independent association signals in this conditional analysis (**Supplementary Fig. 4** and **Supplementary Table 2**), suggesting that allelic heterogeneity underlies BMD variation. We also assessed all possible pairwise interactions of the 82 SNPs, but none were significant after adjusting for the number of tests (**Supplementary Fig. 5** and **Supplementary Table 3**). A total of 96 independent SNPs (82 autosomal SNPs with $P < 5 \times 10^{-6}$, 9 autosomal SNPs from conditional analysis and 5 SNPs on the X chromosome) from 87 genomic loci were selected for further replication (**Fig. 1**).

Follow-up replication (stage 2)
We performed *de novo* genotyping of these 96 SNPs and tested them for association with BMD in up to 50,933 additional participants from 34 studies (Online Methods). Meta-analysis of the 96 SNPs in the discovery and replication studies ($n = 83,894$) yielded 64 replicating SNPs from 56 associated loci. Of these loci, 32 were newly found to show association (**Table 1** and **Supplementary Table 4a**), and 24 were reported previously[8–14] (**Supplementary Table 4b**). Thirty-two SNPs did not reach genome-wide significance after replication (**Supplementary Table 4c**), including 10 markers that remained associated at a suggestive level. Of all the SNPs analyzed, only one (rs9533090 mapping to 13q14.11 near *TNFSF11* (also known as *RANKL*)) showed a high degree of heterogeneity of effects ($I^2 > 50\%$) across studies, despite being the marker that associated with highest significance ($P = 4.82 \times 10^{-68}$) in the fixed-effect meta-analysis (**Supplementary Table 4b**). After applying random-effects meta-analysis, this marker was still associated with genome-wide significance ($P = 3.98 \times 10^{-13}$).

Two of the newly identified loci were discovered in the sex-stratified meta-analysis: 8q13.3 in women and Xp22.31 in men; however, only the association at Xp22.31 showed significant evidence for sex specificity, as reflected by significant heterogeneity of effects across sex strata ($P_{het} = 1.62 \times 10^{-8}$). Yet, we acknowledge that the association at 8q13.3 in women may have been driven by a lower number of men in the discovery and replication data sets (**Table 1** and **Supplementary Table 5**). Furthermore, evidence for BMD site specificity ($P_{het} < 5 \times 10^{-4}$) was observed in a proportion of the loci, including 6 of the 32 new and 4 of the 24 known loci (**Table 1** and **Supplementary**

Fig. 6). Among the newly identified loci, 2q14 (*INSIG2*), 12p11.22 (*PTHLH*) and 16q12.1 (*CYLD*) showed site specificity with FN-BMD, and 8q13.3 (*LACTB2*), 10p11.23 (*MPP7*) and 10q22.3 (*KCNMA1*) showed site-specificity with LS-BMD.

After replication, the conditional analysis provided significant evidence of association ($P < 5 \times 10^{-8}$) in eight of the nine loci containing secondary signals (**Supplementary Fig. 4** and **Supplementary Table 2**). Three loci had variants located less than 40 kb from the initial main signal, suggesting allelic heterogeneity, including at 1p31.3 (represented by rs17482952 near *WLS*), 6q25.1 (rs7751941 near *ESR1*) and 16q12.1 (rs1564981 near *CYLD*). The secondary signal at 16q12.1 (rs1564981) showed a strong association with LS-BMD, whereas the main signal in this locus (rs1566045) was only associated with FN-BMD. The other five secondary signals were represented by variants localized more than 180 kb from the initial main signal and were located in different candidate genes, including at 1p36.12 (rs7521902 near *WNT4*), 7p14.1 (rs10226308 near *SFRP4*), 7q31.31 (rs13245690 near *C7orf58*), 12q13.13 (rs736825 near *HOXC6*) and 17q21.31 (rs4792909 near *SOST*). The secondary signal mapping to the 13q14.11 locus (rs7326472) did not achieve genome-wide significance after replication.

Association of the BMD loci with fracture (stage 3)
We tested the 96 markers for association with fracture in 31,016 cases and 102,444 controls from 50 studies with fracture information. This collection included 5,411 cases and 21,909 controls tested in the BMD GWAS discovery samples, 9,187 cases and 45,057 controls tested by *in silico* replication and 16,418 cases and 35,478 controls tested by *de novo* genotyping (**Fig. 1** and Online Methods). In this fracture meta-analysis, 14 loci were significantly associated with any type of fracture at Bonferroni-corrected significance ($P = 5 \times 10^{-4}$), of which five were new BMD-associated loci. None of the markers showed large estimates of heterogeneity (**Table 2**, **Supplementary Fig. 7** and **Supplementary Table 6**). Markers at six of these loci reached $P < 5 \times 10^{-8}$, including at 18p11.21 (*FAM210A*; also known as *C18orf19*), 7q21.3 (*SLC25A13*), 11q13.2 (*LRP5*), 4q22.1 (*MEPE*), 2p16.2 (*SPTBN1*) and 10q21.1 (*DKK1*). The proportion of the overall fracture risk explained by BMD ranged between 0.09 and 0.40 across markers (**Supplementary Table 7**) and was estimated in a subset of stage 2 samples (including $n = 8,594$ cases and 23,218 controls) by modeling the effect of BMD-associated SNPs on fracture risk, with and without the inclusion of BMD as a covariate. In general, the effect of these SNPs on BMD was larger than on fracture risk (**Fig. 2a**), except for the most significantly associated locus for fracture at 18p11.21 (**Fig. 2b**). SNPs in genes of the RANK-RANKL-OPG pathway (*TNFRSF11A*, *TNFSF11* and *TNFRSF11B*, respectively), despite being the strongest loci associated with BMD, were not significantly associated with fracture. All 31 BMD-associated loci that had nominal association with fracture risk ($P < 0.05$) showed consistent direction (the allele associated with decreasing BMD was associated with increased risk of fracture). When we performed subgroup analyses using cleaner phenotype definitions generated by limiting subjects to those with clinically validated fractures and stratifying by anatomical site (for example, non-vertebral and vertebral fractures), we did not identify any additional signals (**Supplementary Table 8**). At a nominally significant level ($P < 0.05$), only 3 loci were associated with vertebral fracture, and all 14 BMD-associated loci were associated with non-vertebral fracture, although the difference in effect between fracture sites was not significant. Therefore, the power of our study did not benefit from improving phenotype definitions at the cost of lower sample size.

Table 1 Estimated effects of new genome-wide significant SNPs on FN-BMD and LS-BMD across stages

SNP	Locus	Closest gene/candidate	Functional evidence						A	Freq.	FN-BMD					LS-BMD			P_{het} site[b]
			eQTL	Knockout mouse	OMIM function	GRAIL	Pathway	Tags			β^c	Stage 1 (32,961) P	Stage 2 (50,933) P	Stages 1 and 2 (83,894) P	β^a	Stage 1 (31,800) P	Stage 2 (45,708) P	Stages 1 and 2 (77,508) P	
rs479336	1q24.3	*DNM3*							T	0.74	−0.04	1.1×10^{-7}	**1.3×10^{-8}**	**8.5×10^{-15}**	−0.03	0.01	5.0×10^{-4}	2.1×10^{-5}	0.05
rs7584262	2p21	*PKDCC*		•					T	0.23	0.03	1.4×10^{-7}	3.4×10^{-4}	**1.3×10^{-9}**	0.01	0.13	0.28	0.07	0.01
rs17040773	2q13	*ANAPC1*	•						A	0.76	0.03	4.3×10^{-6}	6.1×10^{-5}	**1.5×10^{-9}**	0.01	0.61	0.21	0.19	5.2×10^{-3}
rs1878526	2q14.2	*INSIG2*							A	0.22	0.00	0.70	0.97	0.79	0.04	7.3×10^{-6}	3.4×10^{-6}	**1.2×10^{-10}**	**8.6×10^{-5}**
rs1026364	3q13.2	*KIAA2018*						•	T	0.37	0.03	2.0×10^{-6}	2.5×10^{-5}	**4.1×10^{-10}**	0.02	0.04	7.3×10^{-3}	7.6×10^{-4}	0.11
rs344081	3q25.31	*LEKR1*							T	0.87	0.03	1.1×10^{-4}	2.5×10^{-3}	2.2×10^{-6}	0.06	2.8×10^{-5}	**3.5×10^{-8}**	**4.5×10^{-12}**	0.12
rs3755955	4p16.3	*IDUA*		•	•			•	A	0.16	−0.05	3.9×10^{-7}	**6.1×10^{-9}**	**1.5×10^{-14}**	−0.05	1.4×10^{-7}	**5.5×10^{-9}**	**5.2×10^{-15}**	0.80
rs11755164	6p21.1	*SUPT3H/RUNX2*		•		•		•	T	0.40	−0.01	0.23	0.12	0.05	−0.03	3.5×10^{-7}	**9.2×10^{-11}**	**5.6×10^{-11}**	2.1×10^{-3}
rs9466056	6p22.3	*CDKAL1/SOX4*				•			A	0.38	−0.03	**1.8×10^{-8}**	1.6×10^{-6}	**2.7×10^{-13}**	−0.03	6.5×10^{-5}	1.1×10^{-4}	**3.6×10^{-8}**	0.34
rs3801387	7q31.31	*WNT16*				•	•	•	A	0.74	−0.08	**4.2×10^{-14}**	**2.0×10^{-27}**	**5.0×10^{-40}**	−0.10	**1.4×10^{-16}**	**1.5×10^{-36}**	**3.2×10^{-51}**	0.09
rs13245690[e]	7q31.31	*C7orf58*							A	0.62	0.00	8.6×10^{-5}	0.69	8.2×10^{-4}	0.03	**1.1×10^{-9}**	1.3×10^{-3}	**6.0×10^{-11}**	0.05
rs7812088	7q36.1	*ABCF2*							A	0.13	0.04	1.2×10^{-6}	4.4×10^{-4}	**7.3×10^{-9}**	0.04	2.9×10^{-5}	1.1×10^{-3}	2.2×10^{-7}	0.86
rs7017914[c]	8q13.3	*XKR9/LACTB2*	•			•			A	0.49	0.02	**4.7×10^{-8}**	7.1×10^{-3}	**1.9×10^{-8}**	−0.01	0.35	0.41	0.98	**9.1×10^{-5}**
rs7851693	9q34.11	*FUBP3*							C	0.64	0.05	**3.1×10^{-8}**	**1.4×10^{-15}**	**3.4×10^{-22}**	0.04	0.06	6.7×10^{-8}	6.1×10^{-8}	0.02
rs3905706	10p11.23	*MPP7*							T	0.22	−0.02	0.63	1.7×10^{-3}	0.03	0.05	**2.9×10^{-9}**	**6.7×10^{-9}**	**2.4×10^{-16}**	**5.8×10^{-11}**
rs1373004	10q21.1	*MBL2/DKK1*					•		T	0.13	−0.04	1.4×10^{-5}	1.5×10^{-4}	**1.5×10^{-8}**	−0.05	5.4×10^{-8}	2.2×10^{-6}	**1.6×10^{-12}**	0.28
rs7071206	10q22.3	*KCNMA1*		•					T	0.78	0.01	0.29	0.26	0.81	−0.05	**1.5×10^{-12}**	**6.2×10^{-9}**	**5.0×10^{-19}**	**5.9×10^{-9}**
rs1084921	10q24.2	*CPN1*							T	0.39	0.04	1.4×10^{-4}	1.6×10^{-6}	**9.0×10^{-10}**	0.03	0.01	1.9×10^{-5}	9.2×10^{-7}	0.58
rs10835187	11p14.1	*LIN7C*	•			•			T	0.55	−0.01	0.17	0.08	0.03	−0.02	3.0×10^{-5}	2.4×10^{-4}	**4.9×10^{-8}**	0.03
rs7953528	12p11.22	*KLHDC5/PTHLH*				•			A	0.18	0.04	5.8×10^{-8}	2.4×10^{-6}	**1.9×10^{-12}**	−0.02	0.94	0.05	0.13	**2.3×10^{-7}**
rs2887571	12p13.33	*ERC1/WNT5B*				•	•		A	0.76	−0.03	1.1×10^{-4}	1.6×10^{-5}	**6.5×10^{-9}**	−0.04	2.2×10^{-7}	2.9×10^{-6}	**5.6×10^{-12}**	0.37
rs12821008	12q13.12	*DHH*				•			A	0.39	0.03	1.9×10^{-4}	5.2×10^{-4}	3.3×10^{-7}	0.05	1.5×10^{-7}	**1.9×10^{-9}**	**1.2×10^{-15}**	0.06
rs1053051	12q23.3	*C12orf23*	•						T	0.52	−0.03	1.4×10^{-5}	1.8×10^{-5}	**9.6×10^{-10}**	−0.02	2.5×10^{-6}	2.4×10^{-3}	7.9×10^{-8}	0.76
rs1286083	14q32.12	*RPS6KA5*							T	0.81	−0.05	**2.9×10^{-8}**	**9.3×10^{-9}**	**2.0×10^{-15}**	−0.04	**1.7×10^{-11}**	7.1×10^{-6}	**1.8×10^{-14}**	0.92
rs4985155	16p13.11	*NTAN1*	•						A	0.67	−0.03	3.5×10^{-4}	1.4×10^{-7}	**1.7×10^{-10}**	−0.03	8.7×10^{-7}	1.8×10^{-4}	**2.2×10^{-9}**	0.98
rs9921222	16p13.3	*AXIN1*		•	•	•	•		T	0.48	−0.03	2.5×10^{-7}	2.4×10^{-6}	**5.2×10^{-12}**	−0.04	**2.2×10^{-8}**	**8.3×10^{-10}**	**1.0×10^{-16}**	0.26
rs13336428	16p13.3	*C16orf38/CLCN7*			•				A	0.43	−0.04	2.9×10^{-7}	**1.1×10^{-10}**	**1.5×10^{-16}**	−0.04	5.9×10^{-5}	**5.8×10^{-10}**	**1.7×10^{-13}**	0.80
rs1566045	16q12.1	*SALL1/CYLD*		•		•			T	0.80	−0.06	**5.0×10^{-12}**	**3.0×10^{-12}**	**1.9×10^{-22}**	−0.01	7.8×10^{-3}	0.55	0.04	**7.5×10^{-6}**
rs1564981[e]	16q12.1	*CYLD*		•					A	0.47	−0.02	1.1×10^{-3}	0.01	4.4×10^{-5}	−0.03	**6.2×10^{-8}**	5.4×10^{-4}	**2.0×10^{-10}**	0.50
rs4790881	17p13.3	*SMG6*							A	0.69	0.05	**1.7×10^{-8}**	**1.2×10^{-11}**	**9.8×10^{-19}**	0.04	6.0×10^{-4}	1.7×10^{-6}	**3.4×10^{-9}**	0.13
rs7217932	17q24.3	*SOX9*		•	•	•		•	A	0.46	0.03	**3.7×10^{-8}**	2.7×10^{-5}	**1.9×10^{-11}**	0.01	0.31	0.15	0.08	3.8×10^{-3}
rs4796995	18p11.21	*FAM210A*							A	0.63	0.02	3.2×10^{-6}	1.1×10^{-3}	**4.9×10^{-8}**	0.01	5.2×10^{-4}	0.11	6.7×10^{-4}	0.29
rs10416218	19q13.11	*GPATCH1*						•	T	0.73	−0.02	5.7×10^{-6}	7.1×10^{-4}	5.5×10^{-8}	−0.03	**9.2×10^{-9}**	1.2×10^{-4}	**6.6×10^{-11}**	0.38
rs5934507[d]	Xp22.31	*FAM9B/KAL1*			•				A	0.74	−0.08	0.01	8.3×10^{-4}	1.6×10^{-4}	−0.09	5.7×10^{-6}	3.2×10^{-4}	**1.2×10^{-8}**	0.17

Boldface indicates $P < 5 \times 10^{-8}$ or site-specific $P < 5 \times 10^{-4}$. A, allele; β, effect estimates; freq., allele frequency of A. Effect estimates are expressed as standardized values per copy of the SNP allele from fixed-effects meta-analysis. Black dots in the six functional evidence columns indicate, respectively, that the SNP is an eQTL, there is a knockout mouse with skeletal phenotypes (Mouse Genome Informatics (MGI) database 2011), the candidate gene is involved in a monogenic syndrome with skeletal phenotypes (OMIM 2011), the most significant SNP tags a SNP predicted to have impact on function of the candidate gene, the gene is the best candidate in GRAIL analysis, and the candidate gene is part of a bone-active pathway. Candidate genes from GRAIL and/or the literature are shown if different from the closest gene.
[a]Effect estimates were calculated in the stage 2 samples. [b]Site specificity null hypothesis, $\beta_{LS-BMD} = \beta_{FN-BMD}$. [c]$\beta_{FN-BMD}$; [c]rs7017914 was discovered in the meta-analysis of women only. The effects and P value for this marker are for the meta-analysis of women samples. [d]rs5934507 was discovered in the meta-analysis of men only. The effects and P value for this marker are for the meta-analysis of men samples. [e]rs13245690 and rs1564981 were independently associated to their main signals in conditional analysis.

Table 2 Association of identified BMD-associated loci with risk for any type of low-trauma fracture

SNP	Locus	Closest gene/candidate	eQTL	Knockout mouse	OMIM	Tags function	GRAIL	Pathway	Risk allele	Freq.[b]	OR (95% CI)	P	OR (95% CI)	P	Q$_{het}$ P	I²
											Meta-analysis without studies included in BMD discovery — 25,605 cases, 80,535 controls		Combined meta-analysis results — 31,016 cases, 102,444 controls			
Loci significantly associated with fracture risk at $P < 5 \times 10^{-8}$																
rs4233949	2p16.2	*SPTBN1*				•			G	0.63	1.07 (1.04–1.09)	1.4×10^{-7}	1.06 (1.04–1.08)	**2.6×10^{-8}**	0.36	6
rs6532023	4q22.1	*MEPE/SPP1*	•	•			•	•	G	0.67	1.06 (1.04–1.09)	8.8×10^{-7}	1.06 (1.04–1.09)	**1.7×10^{-8}**	1.00	0
rs4727338	7q21.3	*SLC25A13*							G	0.32	1.08 (1.05–1.10)	1.0×10^{-8}	1.08 (1.05–1.10)	**5.9×10^{-11}**	0.03	31
rs1373004	10q21.1	***MBL2/DKK1***		•				•	T	0.13	1.09 (1.06–1.13)	7.2×10^{-7}	1.10 (1.06–1.13)	**9.0×10^{-9}**	0.64	0
rs3736228	11q13.2	*LRP5*		•	•	•	•	•	T	0.15	1.09 (1.05–1.12)	2.1×10^{-6}	1.09 (1.06–1.13)	**1.4×10^{-8}**	0.78	0
rs4796995	18p11.21	***FAM210A***							G	0.39	1.06 (1.04–1.09)	6.4×10^{-7}	1.08 (1.06–1.10)	**8.8×10^{-13}**	0.12	20
Other significant loci associated with fracture risk at $P < 5 \times 10^{-4}$ (Bonferroni)																
rs6426749	1p36.12	*ZBTB40*							G	0.83	1.06 (1.03–1.09)	2.4×10^{-4}	1.07 (1.04–1.10)	3.6×10^{-6}	0.07	24
rs7521902	1p36.12[a]	*WNT4*	•				•	•	A	0.27	1.10 (1.06–1.14)	3.5×10^{-6}	1.09 (1.06–1.13)	1.4×10^{-7}	0.87	0
rs430727	3p22.1	*CTNNB1*		•			•	•	T	0.47	1.05 (1.03–1.08)	2.4×10^{-5}	1.06 (1.03–1.08)	2.9×10^{-7}	0.93	0
rs6959212	7p14.1	*STARD3NL*							T	0.33	1.04 (1.02–1.07)	1.0×10^{-3}	1.05 (1.02–1.07)	7.2×10^{-5}	0.43	2
rs3801387	7q31.31	***WNT16***				•	•	•	A	0.74	1.08 (1.05–1.11)	4.9×10^{-9}	1.06 (1.04–1.08)	2.7×10^{-7}	0.69	0
rs7851693	9q34.11	***FUBP3***							G	0.37	1.04 (1.01–1.06)	1.9×10^{-3}	1.05 (1.02–1.07)	3.5×10^{-5}	0.65	0
rs163879	11p14.1	*DCDC5*							T	0.66	1.06 (1.03–1.09)	6.4×10^{-6}	1.05 (1.03–1.07)	3.3×10^{-5}	0.05	28
rs1286083	14q32.12	***RPS6KA5***							T	0.81	1.05 (1.02–1.08)	9.8×10^{-4}	1.05 (1.03–1.08)	7.2×10^{-5}	0.01	34
rs4792909	17q21.31[a]	*SOST*		•	•			•	G	0.62	1.07 (1.04–1.11)	4.0×10^{-5}	1.07 (1.04–1.10)	6.9×10^{-6}	0.31	10
rs227584	17q21.31	*C17orf53*	•						A	0.67	1.05 (1.02–1.08)	2.2×10^{-4}	1.05 (1.03–1.07)	4.1×10^{-5}	0.49	0

Odds ratios (ORs) estimated per risk allele copy for any low-trauma fracture among cases compared with controls. Q_{het} is the Cochran's Q statistic, and I^2 is the measure of heterogeneity. Boldface indicates gene names from new loci and/or those associated with $P < 5 \times 10^{-8}$. Black dots in the six functional evidence columns indicate that, respectively, the SNP is an eQTL, there is a knockout mouse with skeletal phenotypes (MGI database 2011), the candidate gene is involved in a monogenic syndrome with skeletal phenotypes (OMIM 2011), the most significant SNP tags a SNP predicted to have impact on function of the candidate gene, the gene is the best candidate in GRAIL analysis, and the candidate gene is part of a bone-active pathway.
[a]rs7521902 and rs4792909 are secondary independent signals. [b]Freq. is the frequency of the risk allele.

Allele risk modeling for osteoporosis and fracture

The combined effect of all significant autosomal SNPs on BMD, osteoporosis and any type of fracture was modeled in the Prospective Epidemiological Risk Factor (PERF) study ($n = 2,836$), a prospective study in postmenopausal Danish women aged 55–86 years[17]. This study represents an independent validation setting, as it was excluded from the overall meta-analysis for this purpose (**Supplementary Note**). Risk alleles in the score (for example, BMD-decreasing alleles) were weighted by their individual effects on BMD and grouped into five bins (**Supplementary Table 9**). The difference in mean FN-BMD between individuals in the highest bin of risk score (9% of the population; $n = 244$) and those in the middle bin (34% of the population; $n = 978$) was −0.33 s.d. (**Fig. 3a**). This analysis was based on data at 63 SNPs and explained 5.8% (95% confidence interval (CI) = 4.0%–7.6%) of the total genetic variance in FN-BMD.

The ability of this genetic score to predict the risk for osteoporosis (defined by a T score of ≤ −2.5) and for fracture was modeled in the PERF study using the middle bin as reference (odds ratio (OR) = 1). Women in the highest bin had 1.56 (95% CI = 1.12–2.18) increased odds for osteoporosis (**Fig. 3b**), whereas women in the lowest bin were protected from osteoporosis (OR = 0.38, 95% CI = 0.23–0.63). A model based on the 16 BMD-associated SNPs that were also associated with fracture risk showed that women in the highest bin had 1.60 (95% CI = 1.15–2.24) increased odds for fracture, whereas women in the lowest bin had a decreased risk for fracture (OR = 0.54, 95% CI = 0.36–0.83) (**Fig. 3c**). Despite serving as robust proof of the relationship between BMD-decreasing alleles and the risk of osteoporosis and fracture, prediction ability was modest. Receiver operating characteristics (ROC) analysis showed a significant but relatively small discrimination ability of the genetic score alone, with an area
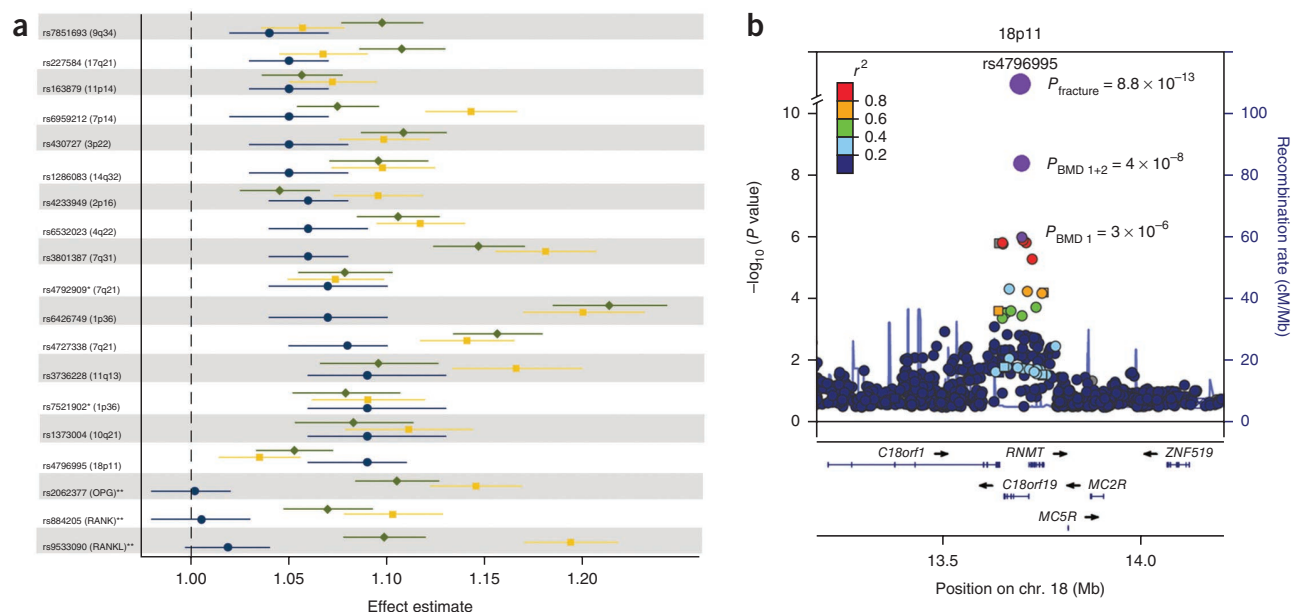
Figure 2 Association of BMD loci with fracture risk. (**a**) Phenotype-wide effects for the BMD loci associated with fracture and those that are part of the RANK-RANKL-OPG pathway. Genetic effect estimates are shown for fracture (blue circles), LS-BMD (yellow squares) and FN-BMD (green diamonds) for the 14 loci associated with fracture risk. Horizontal lines represent 95% confidence limits. Effect estimates are shown after transformation of the standardized mean difference (SMD) in the BMD effect to odds ratio equivalents[34] (for example, a 0.02 SMD in the BMD effect corresponds to an OR of 1.04). Secondary signals for rs227584 and rs6426749 are marked with an asterisk and the signals mapping to the *TNFRSF11B* (also known as *OPG*; rs2062377), *TNFRSF11A* (also known as *RANK*; rs884205) and *TNFSF11* (also known as *RANKL*; rs9533090) genes are marked with a double asterisk. (**b**) Regional association plot for the 18p11.21 locus showing the *P* value for the top SNP associated with fracture (rs4796995) together with *P* values from the BMD discovery set (stage 1) and combined with the BMD replication (stage 1 + 2). SNPs are plotted by position in a 500-kb window of chromosome 18 against association with FN-BMD ($-\log_{10}$ (*P* value)). Estimated recombination rates (from HapMap) are plotted in cyan to reflect the local LD structure. SNPs surrounding the most significant SNP are color-coded according to LD between these markers (pairwise r^2). Genes, exons and transcription direction are derived from the UCSC Genome Browser.

under the curve (AUC) of 0.59 (95% CI = 0.56–0.62) for osteoporosis (**Supplementary Fig. 8**). Adding this score to a model with age and weight alone (AUC = 0.75, 95% CI = 0.73–0.77) did not substantially increase discrimination (AUC = 0.76, 95% CI = 0.74–0.78). A similar pattern was observed for fracture discrimination, with AUCs of 0.57 (95% CI = 0.55–0.59) in a model with the score alone and 0.62 (95% CI = 0.60–0.64) in a model with age, weight and height. A model considering all 63 SNPs did not change the AUC for fracture risk prediction (0.57, 95% CI = 0.54–0.59).

Functional annotations and pathway analyses

For the purpose of fine mapping and identifying additional SNPs with putative functional implication using linkage disequilibrium (LD), a subset of nine discovery studies (FN-BMD, *n* = 21,699; LS-BMD, *n* = 20,835) used 1000 Genomes Project data (Release June 2010) to re-impute genotypes at the 55 autosomal BMD loci (**Supplementary Note**). In 13 of the 55 BMD-associated loci (the SNP on the X chromosome was not included), we identified markers in the surrounding 1-Mb region that were imputed from 1000 Genomes Project data and that were more significant than the original HapMap signals (**Supplementary Tables 10** and **11**), highlighting the benefit of using a denser reference panel of markers. All HapMap markers in LD with variants with functional annotation and showing higher significance in the 1000 Genomes Project meta-analysis are shown (**Supplementary Table 12**). In 14 of the 56 identified BMD-associated loci, a marker from HapMap imputation was highly correlated ($r^2 > 0.8$) with at least one putative functional variant annotated in the 1000 Genomes Project reference. Three of the 14 BMD-associated loci that also associated with fracture contained putative functional variants tagged by

the top SNPs of the BMD meta-analysis. These included the known rs3736228 functional marker in *LRP5* (encoding p.Ala1330Val)[16,18], the intronic marker rs3779381 within a promoter and/or regulatory region of *WNT16* and one intronic marker (rs4305309) within a promoter and/or regulatory region of *SPTBN1*.

Expression profiles at the BMD loci associated with genome-wide significance were analyzed within four data sets (**Supplementary Note**). In transiliac bone biopsies, expression of five genes correlated with LS-BMD and/or FN-BMD of the donors with *P* < 0.001, including *PSME4* (2p16.2), *DKK1* (10q21.1), *MIR22HG* (also known as *C17orf91*; 17p13.3), *SOST* (17q21.31_1) and *DUSP3* (17q21.31_1) (**Supplementary Table 13**). Among these loci, the SNP at *DKK1* (10q21.1) was the most significantly correlated with FN-BMD ($P = 1.3 \times 10^{-5}$) and LS-BMD ($P = 3.2 \times 10^{-4}$). Variants in all these BMD-associated loci (with the exception of *MIR22HG* at 17p13.3) were also associated with fractures.

SNP expression quantitative trait locus (eQTL) analyses were performed across diverse tissues, examining the correlation between marker alleles and transcript levels at the associated BMD loci. Fourteen of the BMD-associated SNPs correlated with the expression of one or more of the nearby genes with $P < 5 \times 10^{-5}$ and were either the strongest *cis* variants or were good surrogates of these for the affected genes (**Supplementary Tables 14** and **15**). The most significant BMD-associated SNP eQTL was observed for rs10835187[T], resulting in reduced expression of the *LIN7C* gene at the 11p14.1 locus ($P = 2.8 \times 10^{-39}$ in adipose tissue). Of particular interest were BMD-associated SNP *cis* variants at three loci that were also associated with fracture, including 1p36.12, 4q22.1 and 17q21.31. At 1p36.12, rs6426749[G] correlated with reduced *WNT4* expression in fibroblasts, osteoblasts
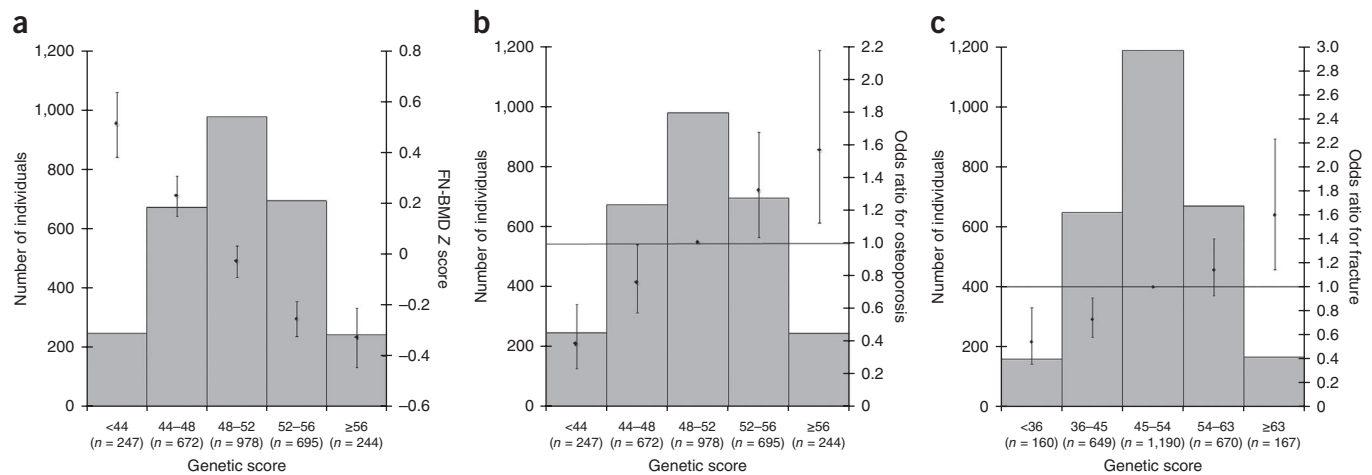
Figure 3 Combined effect of BMD-decreasing alleles and fracture risk–increasing risk alleles modeled in the population-based PERF study ($n = 2,836$ women). (**a**–**c**) Effects are shown for baseline FN-BMD standardized residuals (Z scores) (**a**), risk for osteoporosis (**b**) and risk for any type of fracture (**c**). The genetic score of each individual in **a** and **b** was based on the 63 SNPs showing genome-wide significant association with BMD (55 main and 8 secondary signals) and in **c** was based on the 16 BMD SNPs associated with fracture. Both genetic scores are weighted for relative effect sizes estimated without the PERF study. Weighted allele counts summed for each individual were divided by the mean effect size, making them equivalent to the percent of alleles carried by each individual, and sorted into five bins. Histograms show the numbers of individuals in each genetic score category (left y axis). Diamonds (right y axis) represent mean FN-BMD standardized levels in **a**, risk estimates in the form of odds ratios and osteoporosis (defined as NHANES T score of ≤ -2.5) in **b** and any type of fracture in **c**, using the middle category as reference (OR = 1). Vertical lines represent 95% confidence limits.

and adipose tissue; at 4q22.1, rs6532023[G] correlated with reduced *SPP1* (encoding osteopontin) expression in adipose tissue; and, at 17q21.31, rs227584[A] correlated with increased *C17orf65* expression in monocytes, adipose tissue, whole blood and lymphoblasts.

We applied the Gene Relationships Across Implicated Loci (GRAIL) text-mining algorithm[19] to investigate connections between genes in the 55 autosomal BMD-associated loci. This analysis revealed significant ($P < 0.01$) connections between genes in 18 of the 55 input loci (**Fig. 4** and **Supplementary Table 16**). The strongest connections were seen for members of three key biological pathways: the RANK-RANKL-OPG pathway (encoded by *TNFRSF11A*, *TNFSF11* and *TNFRSF11B*, respectively); mesenchymal stem cell differentiation (*RUNX2*, *SP7* and *SOX9*); and Wnt signaling (*LRP5*, *CTNNB1*, *SFRP4*, *WNT3*, *WNT4*, *WNT5B*, *WNT16* and *AXIN1*), with the ten most frequently connecting terms being bone, catenin, signaling, differentiation, rank, osteoblast, diacylglycerol, kappab, development and osteoclast. To assess the significance of this biological gene connection enrichment, we applied GRAIL to 2,000 randomly matched sets of 55 SNPs (**Supplementary Note**) and did not observe any set with 15 or more loci with significantly enriched connectivity
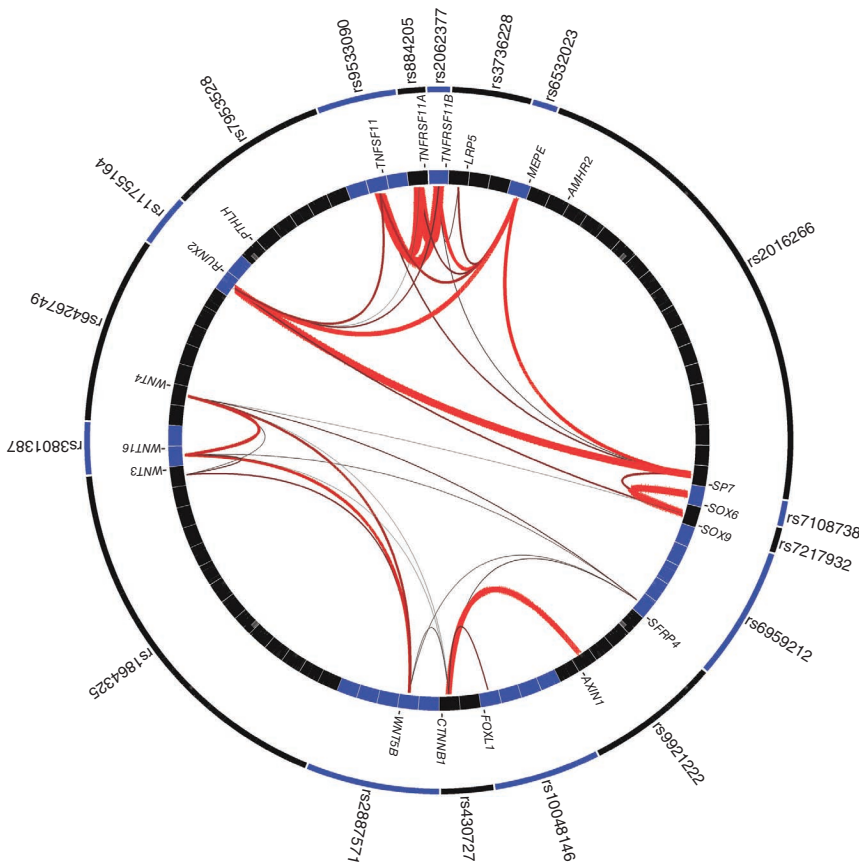
Figure 4 Graphic representation of GRAIL connections between SNPs and corresponding genes for the 18 SNPs, as determined with GRAIL $P < 0.01$. The top ten keywords linking the genes were bone, catenin, signaling, differentiation, rank, osteoblast, diacylglycerol, kappab, development and osteoclast. Thicker redder lines imply stronger literature-based connectivity. Blue and black boxes depict loci boundaries represented for each top-associated marker (outer circle) and for each gene in the region (inner circle).

(**Supplementary Fig. 9**), providing strong statistical evidence of the significant clustering of our BMD-associated loci ($P < 0.0005$).

DISCUSSION

In this report of the largest GWAS for osteoporosis traits to date, we identified 32 new genomic loci, bringing the total number of loci robustly associated with BMD variation to 56. Furthermore, we report that six of these BMD-associated loci are also associated with low-trauma fractures at $P < 5 \times 10^{-8}$, an association that has not previously been detected. In terms of other complex traits, our results indicate that hundreds of variants with small effects may contribute to the genetic architecture of BMD and fracture risk[20]. Our hypothesis-free assessment of common variants of the genome provides new insights into biology, implicating several factors that cluster in bone-active pathways.

Our results highlight the highly polygenic nature of BMD variation and the critical role of several biological pathways influencing osteoporosis and fracture susceptibility (**Supplementary Fig. 10**). In addition to the Wnt factors known to be associated with BMD (*CTNNB1*, *SOST*, *LRP4*, *LRP5*, *WLS*, *WNT4* and *MEF2C*), several of the newly discovered loci implicate additional Wnt signaling factors (including *WNT5B*, *WNT16*, *DKK1*, *PTHLH*, *SFRP4* and *AXIN1*). Another clearly delineated pathway is that involved in mesenchymal stem cell differentiation, including the newly identified *RUNX2*, *SOX4* and *SOX9* BMD-associated loci along with the previously known *SP7*. Another bone-relevant pathway includes that of endochondral ossification, which involves essential processes during fetal development of the mammalian skeleton and in which several of our identified BMD-associated loci are implicated, including *SPP1*, *MEF2C*, *RUNX2*, *SOX6*, *PTHLH*, *SP7* and *SOX9*. In addition, the biological relevance of our associations is accentuated by the identification of genes underlying rare monogenetic forms of osteoporosis and/or high bone mass, such as *SOST*, *CLCN7* and *LRP5* (refs. 21–23) (**Supplementary Table 17**), which also contain common variants involved in normal BMD variation at the population level[11,14,16]. This is supportive of a genetic architecture where both common and rare genetic variation may reside in the same locus[24]. Other genes have not been reported to be associated with monogenic forms of osteoporosis but have clear involvement in bone development in animal models. For example, SNPs in the BMD-associated locus at 16q12.1 map near *CYLD*. Human mutations in this gene have been described to cause familial cylindromatosis, a condition without phenotypic skeletal manifestations. However, it has been shown that *Cyld* knockout mice have significant bone loss, leading to a severe osteoporosis phenotype[25] and also that *CYLD* regulates osteoclastogenesis[26]. Moreover, evidence from the GWAS and eQTL analyses also suggests that some loci contain more than one common variant with independent effects on BMD and fracture risk. On the other hand, when no correlation is observed between gene expression and a particular SNP, it is difficult to draw conclusions. A correlation might be missed if the expression of the transcript was not measured in a relevant tissue or if the expression of a particular splice variant was not measured[27].

BMD and fracture genetic effects correlate to some extent, but some important risk variants for fracture may have minimal impact on BMD and vice versa. This is the case for the signal at 18p11.21 (**Fig. 2b**), which, despite a modest effect on BMD (0.02% variance explained), showed the most significant association with fracture risk (OR = 1.08, 95% CI = 1.06–1.10; $P = 8.8 \times 10^{-13}$). This is in contrast to variants that are known to have stronger effects on BMD that were not significantly associated with fracture risk. For example, variants affecting the RANK-RANKL-OPG pathway that has a critical role in osteoclastogenesis had clear associations with BMD but not with fracture risk (**Fig. 2a**). Even though loci discovery was based

on the BMD phenotype, these findings reflect the heterogeneous and complex nature of the mechanistic pathways leading to fracture. Therefore, given our study design, we cannot rule out the possibility that unidentified genetic loci influence risk for fracture independently of BMD. Future well-powered GWAS meta-analyses on fracture risk will address this question, while corroborating the associations with fracture that we report for some of the BMD-associated loci (particularly those not associated with fracture at $P < 5 \times 10^{-8}$).

Our study also provides indication that there is sex and site specificity underlying BMD variation. One of the GWAS signals (Xp22.31) was only significant in the sex-stratified analysis in men and showed significant sex heterogeneity ($P_{\text{het}} = 1.62 \times 10^{-8}$). This is expected, considering the sexual dimorphism of bone[28,29]. In fact, in a recent GWAS, the rs5934507 SNP mapping to Xp22.31, which is associated with BMD in the current study, was previously associated with male serum testosterone levels[30]. Thus, it is likely that rs5934507 affects serum testosterone, which in turn regulates BMD. In line with the different types of bone composition at different skeletal sites (predominantly trabecular at the lumbar spine and cortical at the femoral neck), we observed some indication of site specificity in 10 of the 56 BMD loci, suggesting differential genetic influences on BMD determination across skeletal sites. As has been previously shown[31], we did not find in our results major differences in effect sizes between individuals of European and east Asian ancestry (**Supplementary Fig. 7**). However, this may be due to reduced power, given the smaller number of individuals of east Asian ancestry. We tested a genetic risk score to identify individuals at risk for osteoporosis and fracture and showed that, cumulatively, the identified variants generate a gradient of risk. These gradients reach ORs of 1.56 for osteoporosis and 1.60 for fractures, when comparing participants with the highest risk scores to those having the mean score. Yet, at present, there is limited clinical usefulness for this score, as evidenced by its non-significant contribution to case discrimination when considering clinical risk factors with strong effects on osteoporosis and fracture risk (like age and weight). This is not unexpected, given the small fraction of genetic risk for either BMD or fracture that has been identified thus far.

Our study has limitations. The identified SNPs are probably not the causal variants; it is more likely that these markers are in LD with the underlying causal variants. Additional analyses on potential functional SNPs identified in this study will be required to determine whether they are causal in these relationships with BMD. Moreover, the causal genes underlying the GWAS signals may be different from the candidate genes we describe, considering that our understanding of the role of these candidate genes in bone biology is limited. Further exploration of these loci with more detailed sequencing, gene expression and translational studies will be required. Such studies can also disentangle the diverse types of complex relationships we currently cannot distinguish in the BMD-associated loci with secondary signals to determine whether these are the result of true allelic heterogeneity or if they are driven by a second gene in the same region[32]. Similarly, despite our large sample size, power limitations still influence the detection of additional associations with smaller effect sizes and/or those arising from rarer variants. Finally, given the different levels of data availability and the difficulty of standardization across studies, we did not evaluate the effect of additional risk factors for osteoporosis, such as menopausal status and smoking, which can influence genetic associations with BMD. Nonetheless, despite these limitations, we have identified many new and previously unsuspected associations with BMD variation and fracture risk.

Finally, the relatively weak effects of the variants discovered by GWAS do not undermine the biological relevance of the genes identified,

as exemplified by the identification of genetic signals at genes coding for proteins currently targeted by new osteoporosis treatments (**Supplementary Fig. 10**). The new genes identified in our study may represent new candidates to target for osteoporosis drug discovery. Most established treatments for osteoporosis focus on curtailing bone resorption (for example, bisphosphonates and RANKL inhibitors), whereas only a few anabolic treatments are currently approved for the treatment of osteoporosis (recombinant truncated or altered PTH). Other anabolic compounds undergoing Phase 2 development include PTHrP fragments and Wnt signaling enhancers, such as antibodies to sclerostin[33]. Several of the variants robustly associated with BMD map in or close to genes that encode proteins involved in these pharmacologic pathways, namely *TNFRSF11B* (encoding osteoprotegerin), *TNFRSF11A* (encoding RANK), *TNFSF11* (encoding RANKL), *PTHLH* (encoding PTHrP), *LRP5* (encoding low-density lipoprotein receptor–related protein 5), *SOST* (encoding sclerostin) and *DKK1* (encoding Dickkopf-1).

In conclusion, these findings highlight the highly polygenic and complex nature of BMD variation, shed light on the pathophysiological mechanisms underlying fracture susceptibility and may contribute to the identification of future drug targets for the treatment of osteoporosis.

URLs. GEFOS Consortium, http://www.gefos.org/; GENOMOS Consortium, http://www.genomos.eu/; HapMap Project, http://hapmap.ncbi.nlm.nih.gov/; 1000 Genomes Project, http://www.1000genomes.org/; LocusZoom, http://csg.sph.umich.edu/locuszoom/; METAL, http://www.sph.umich.edu/csg/abecasis/Metal/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS

This work was done under the auspices of the European Commission–sponsored Genetic Factors for Osteoporosis (GEFOS) consortium.

Study-specific design and management were performed by U.S., M.A., L.M., J.P., S.B., M.L.B., B.M.B., C. Christiansen, C. Cooper, G.D., I.F., M.F., D.G., J.G.-M., M. Kähönen, M. Karlsson, J.-M.K., P.K., B.L.L., W.D.L., P.L., Ö.L., R.S.L., J.M., D.M., J.M.O., U.P.-K., J.A.R., P.M.R., F. Rousseau, P.E.S., N.L.S.T., R.U., W.V.H., J.V., M.T.Z., K.M.G., T.P., D.I.C., S.R.C., R.E., J.A.E., V.G., A.H., R.D.J., G.J., J.W.J., K.-T.K., T.L., M. Lorentzon, E.M., B.D.M., G.C.N., M.P., H.A.P.P., R.L.P., O.R., I.R.R., P.N.S., P.C.S., A.R.S., F.A.T., C.M.v.D., N.J.W., L.A.C., M.J.E., T.B.H., A.W.C.K., B.M.P., J. Reeve, T.D.S., E.A.S., M.C.Z., U.T., C.O., J.B.R., M.A.B., K. Stefansson, A.G.U., S.H.R., J.P.A.I., D.P.K. and F. Rivadeneira. Study-specific genotyping was performed by K.E., U.S., E.L.D., L.O., L.V., S.-M.X., A.K.A., D.J.D., S.G., R.K., C.K., A.Z.L., J.R.L., S.M., S.M.-B., S.S., S.T., O.T., S.C., E.K., J.M., B.O.-P., Y.S.A., E.G., L.H., H.J., T. Kwan, R. Luben, C.M.-G., S.T.P., S. Reppe, J.I.R., J.B.J.v.M., D.V., K.M.G., D.I.C., G.R.C., P.D., R.D.J., T.L., Y.L., M. Lorentzon, R.L.P., N.J.W., L.A.C., C.O., M.A.B., A.G.U. and F. Rivadeneira. Study-specific phenotyping was performed by U.S., E.L.D., O.M.E.A., A.M., S.-M.X., N. Alonso, S.K.K., S.G.W., A.K.A., T.A., J.R.C., Z.D., N.G.-G., S.G., G.H., L.B.H., K.A.J., G.K., G.S.K., C.K., T. Koromila, M. Kruk, M. Laaksonen, A.Z.L., S.H.L., P.C.L., L.M., X.N., J.P., L.M.R., K. Siggeirsdottir, O.S., N.M.v.S., J.W., K.Z., M.L.B., C. Christiansen, M.F., M. Kähönen, M. Karlsson, J.-M.K., Ö.L., J.M., D.M., B.O.-P., J.M.O., U.P.-K., D.M.R., J.A.R., P.M.R., F. Rousseau, W.V.H., J.V., M.C.-B., E.G., T.I., R. Luben, S. Reppe, G.S., J.B.J.v.M., D.V., F.M.K.W., K.M.G., J.A.C., D.I.C., E.M.D., R.E., J.A.E., V.G., A.H., R.D.J., G.J., Y.L., M. Lorentzon, E.M., G.C.N., B.A.O., M.P., H.A.P.P., R.L.P., O.R., I.R.R., J. Robbins, P.N.S., C.M.v.D., M.J.E., J. Reeve, E.A.S., M.C.Z., C.O., M.A.B., A.G.U., D.P.K. and F. Rivadeneira. Study-specific data analysis were performed by K.E., U.S., E.E., Y.-H.H., E.L.D., E.E.N., L.O., O.M.E.A., N. Amin, J.P.K., D.L.K., G.L., C.L., R.L.M., A.M., L.V., D.W., S.-M.X., L.M.Y.-A., H.-F.Z., J.E., C.M.K., S.K.K., P.J.L., G.T., J.F.W., V.A., A.K.A., T.A., J.R.C., G.H., L.J.H., C.K., T. Koromila, A.Z.L., S.M.-B., T.V.N., M.S.P., J.P., L.M.R., A.V.S., O.S., S.T., S.C., J.M., B.O.-P., U.P.-K., R. Li, R. Luben, S. Reppe, J.I.R., A.R.W., Y.Z., S. Raychaudhuri, D.I.C., J.A.E., R.D.J., T.L., K.N., O.R., D.M.E., D.K., J.B.R., M.A.B., J.P.A.I., D.P.K. and F. Rivadeneira. Analysis plan design was performed by K.E., E.E., U.S., D.K., D.P.K., J.P.A.I. and F. Rivadeneira. K.E., E.E., Y.-H.H. and E.E.N. carried out meta-analyses. K.E., E.E. and A.R.W. determined gene-by-gene interaction. Risk modeling and analysis of secondary signals were performed by K.E. and F. Rivadeneira. Expression QTLs were analyzed by U.S., G.T., E.G., S. Reppe, K.M.G. and T.P. Y.-H.H. performed functional SNP prediction. GRAIL was carried out by K.E., E.L.D., D.W. and S. Raychaudhuri. Standardization of phenotype and genotype replication data sets was performed by K.E., U.S., E.E., E.L.D., L.O., G.T., L.H. and C.M.-G. Interpretation of results was carried out by K.E., U.S., E.E., Y.-H.H., E.L.D., E.E.N., L.O., O.M.E.A., N. Amin, D.L.K., C.-T.L., R.L.M., A.M., L.V., D.W., S.-M.X., L.M.Y.-A., J.E., C.M.K., S.K.K., A.W.C.K., J. Reeve, M.C.Z., C.O., D.K., J.B.R., M.A.B., A.G.U., S.H.R., J.P.A.I., D.P.K. and F. Rivadeneira. The manuscript draft was prepared by K.E., U.S., E.E., Y.-H.H., E.L.D., E.E.N., L.O., O.M.E.A., A.M., C.O., D.K., J.B.R., M.A.B., A.G.U., S.H.R., J.P.A.I., D.P.K. and F. Rivadeneira. The steering committee for GEFOS includes U.S., E.E., U.T., A.G.U., S.H.R., J.P.A.I. and F. Rivadeneira.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Study design. This study was conducted as part of the GEFOS Consortium, a coalition of teams of investigators dedicated to identifying the genetic determinants of osteoporosis. The discovery samples comprised 17 GWAS ($n = 32,961$) from populations across North America, Europe, East Asia and Australia, with a variety of epidemiological designs (**Supplementary Table 18a**) and clinical characteristics of individuals (**Supplementary Table 18b**); a subset of these had fracture information available (**Supplementary Table 18c**). Subjects from 34 additional studies with BMD data ($n = 50,933$) were used for replication, and association with fracture was tested across 50 studies with fracture information, most of which were also used for the BMD analysis ($n = 31,016$ cases and 102,444 controls) (**Fig. 1** and **Supplementary Tables 19a–c** and **20a–c**). All studies were approved by their institutional ethics review committees, and all participants provided written informed consent.

BMD measurements and fracture definition. LS-BMD and FN-BMD were measured in all cohorts using dual-energy X-ray absorptiometry, following standard protocols (**Supplementary Tables 18b**, **19b** and **20b**). Three clinically distinct fracture definitions were used: (i) any type, consisting of low-trauma fractures at any skeletal site (except fingers, toes and skull) occurring after age 18 years, assessed by X-ray, radiographic report, clinical record, clinical interview and/or questionnaire, (ii) validated non-vertebral, consisting of fractures occurring after age 50 years, with diagnosis confirmed by hospital records and/or radiographs, and (iii) radiographic vertebral fractures, from lateral morphometry scored on X-rays. The first definition is most-inclusive, whereas the latter two are more stringent fracture definitions that are commonly used in randomized trials[35,36]. Controls were defined as individuals without a history of fracture, using for each fracture type the same age limit categories as for the cases.

Stage 1 genome-wide association analysis. *Genotyping and imputation.* GWAS genotyping was performed by each study following standard protocols, and imputation was then carried out on ~2.5 million SNPs from HapMap[37] Phase 2 release 22 using Genome Build 36. Quality control was performed independently for each study. To facilitate meta-analysis, each group performed genotype imputation with BIM-BAM[38], IMPUTE[39] or MACH[40] software using genotypes from HapMap Phase 2 release 22 (CEU or Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT) as appropriate). HapMap release 21 was used as a reference for SNPs residing on the X chromosome, and IMPUTE software was used for imputation. Overall, imputation quality scores for each SNP were obtained from IMPUTE (proper_info) and MACH (rsq_hat) statistics. Details of the genotyping platform, genotype quality control procedures and software for imputation that were used by each study are presented (**Supplementary Tables 18d** and **19d**).

Association analysis with BMD. Each study performed genome-wide association analysis for FN-BMD and LS-BMD, using sex-specific and age-, weight- and principal component–adjusted standardized residuals analyzed under an additive (per allele) genetic model. Analyses of autosomal and X-chromosome markers were performed separately. The analysis of imputed genotype data accounted for uncertainty in each genotype prediction by using either the dosage information from MACH or the genotype probabilities from IMPUTE and BIM-BAM. Studies used MACH2QTL[40] directly or via GRIMP[41] (which uses genotype dosage value as a predictor in a linear regression framework), SNPTEST[39], Merlin[42], BIM-BAM or the linear mixed-effects model of the Kinship and ProbABEL[43] (**Supplementary Tables 18d** and **19d**). For analysis of the X chromosome, either SNPTEST or R software was used in each participating study. We coded 'effect allele homozygous genotype' as 2 and 'other allele homozygous genotype' as 0 in the genotyped SNPs in men on the X chromosome. The imputed genotypes were coded as continuous variables from 0 to 2 to take into account imputation uncertainty. The genomic control method[44] was used to correct the standard error (SE) by the square root of the genomic inflation factor (λ): $SE_{corrected} = s.e.m. \times \sqrt{\lambda}$.

Meta-analysis of the GWAS. Before performing meta-analysis on the genome-wide association data, SNPs with poor imputation quality scores (rsq_hat of <0.3 in MACH, proper_info of <0.4 in IMPUTE or a ratio of observed-to-expected dosage variance of <0.3 in BIM-BAM) and markers with a minor allele frequency (MAF) of <1% were excluded from each study. All individual GWAS were genomic control corrected before meta-analysis[44]. Individual study-specific genomic control values ranged from 0.98 to 1.08 (**Supplementary Table 18d**). A total of 2,483,766 autosomal SNPs were included in meta-analysis across 17, 16 and 13 studies for FN-BMD (pooled, women-only and men-only analyses, respectively) and 16, 13 and 12 studies for LS-BMD (pooled, women-only and men-only analyses, respectively). A total of 76,253 X-linked SNPs were included in meta-analysis across 14, 13 and 10 studies for LS-BMD and FN-BMD (pooled, women-only and men-only analyses, respectively). In our discovery analysis, we chose to implement a fixed-effects model, as it is generally preferable for the purposes of initial discovery, where the aim is to screen and identify as many of the true variants as possible[45,46]. SNPs present in less than three studies were removed from the meta-analysis, yielding ~2.2 million SNPs in the final results. The genomic inflation factors (λ) were 1.11, 1.09 and 1.06 for FN-BMD (pooled, women-only and men-only analyses, respectively) and 1.13, 1.09 and 1.06 for LS-BMD (pooled, women-only and men-only analyses, respectively). A second genomic control correction was applied to the overall meta-analysis results, although such a second correction is considered overly conservative[47]. Significance for BMD association was set at $P < 5 \times 10^{-8}$, and a Bonferroni correction was used for association with fracture[48].

Selection of SNPs for replication. We took forward the most significant 96 SNPs for replication. With respect to power estimations, after adding 30,000 samples in stage 2, these variants had a priori power of ≥85% to reach $P = 5 \times 10^{-8}$ in the meta-analysis. Loci were considered independent when separated by at least 1 Mb from a top GWAS signal. The 96 variants included the 82 index SNPs representing each of the 82 loci reaching $P < 5 \times 10^{-6}$ in stage 1, 9 SNPs that were within the same 2-Mb windows as the 82, which were independent from the main signals (secondary signals), and the top 5 most-associated SNPs on the X chromosome (with $P < 5 \times 10^{-5}$).

Association analyses with fracture risk. Effect estimates (odds ratios) for association of allele dosage of the top signals with fracture risk were obtained from logistic regression models adjusted for age, age[2], weight, sex, height and four principal components. The proportion of the fracture risk explained by FN-BMD was calculated from the regression coefficients as ($\beta_{unadjusted} - \beta_{BMDadjusted}$) / $\beta_{unadjusted}$ in a subset of replication samples for which both FN-BMD and complete fracture information was available.

Stage 2 replication. *Samples and genotyping.* Fracture association results were also obtained for the 82 most-significant SNPs from 54,244 individuals of European ancestry from 7 GWAS (*in silico* genotyping) that had not been included in the stage 1 analyses (**Supplementary Table 19a–c**). Subjects from 34 studies of the GENOMOS Consortium with BMD and/or fracture information were studied in replication analysis (**Supplementary Table 3a–c**). *De novo* replication genotyping was performed in the UK (Kbiosciences), Iceland (deCODE Genetics), Australia (University of Queensland Diamantina Institute) and the United States (WHI GeCHIP) using KASPar, Centaurus, OpenArray and iSelect assays, respectively (**Supplementary Note**). Minimum genotyping quality control criteria were defined as sample call rate of >80%, SNP call rate of >90%, Hardy-Weinberg Equilibrium P value of >1×10^{-4} and MAF of >1%.

Association analyses and meta-analysis. We tested the association between the 96 SNPs and BMD and fracture risk in each *in silico* and *de novo* stage 2 study separately, as described for the stage 1 studies. We subsequently performed meta-analysis of effects and standard errors from the stage 2 studies and then carried out a meta-analysis of the summary statistics of stages 1 and 2 combined using the inverse-variance method in METAL. At the replication stage, where more than 30 studies were synthesized, we chose to first assess the underlying heterogeneity, considering both the Cochran's Q statistic and the I^2 metric. If the heterogeneity was not significant, fixed-effects model were applied. If the Cochran's Q P value was <0.0005 and I^2 was >50%, we used the more conservative random-effects model.

Additional analyses. Further analyses were performed for the SNPs carried forward for replication. Each of these analyses is described in detail in the **Supplementary Note**.

In brief, we performed (i) a conditional genome-wide association analysis to determine whether any of the 82 BMD loci harbored additional independent signals, (ii) tested gene-by-gene pairwise interactions between these BMD loci, (iii) assessed within the independent setting of the PERF study (for details on study design see **Supplementary Table 20a–c**) the predictive ability derived from the cumulative effect of the 63 autosomal SNPs associated with BMD with genome-wide significance in relation to BMD levels and osteoporosis risk and that of the 16 BMD SNPs also associated with fracture risk in relation to fracture risk, (iv) identified SNPs with r^2 of ≥0.80 with the lead SNP that were potentially functional (for example, nonsense, non-conservative nonsynonymous, synonymous, exonic splicing, transcription factor binding), using regional imputation with 1000 Genomes Project data (June 2010 release), (v) tested the relationship between gene expression profiles from transiliac bone biopsies and BMD in 84 unrelated postmenopausal women[49] and examined *cis* associations between each of the 55 significant BMD SNPs and expression of nearby genes in different tissues, including lymphoblastoid cell lines[50–52], primary human fibroblasts and osteoblasts[53], adipose tissue[54], whole blood[54] and circulating monocytes[55], and (vi) evaluated the connectivity and relationships between identified loci using literature-based annotation with the GRAIL[19] statistical strategy.

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