Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis

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Osteoporosis is a common age-related disorder characterized by low bone mass and deterioration in bone microarchitecture, leading to increased skeletal fragility and fracture risk. Low BMD is a strong risk factor for osteoporosis, as well as a key indicator for its diagnosis and treatment. BMD is highly heritable, and GWASs have identified common variants at 73 loci associated with the trait, including many that are significantly associated with fracture risk. Recently, deep imputation based on whole-genome sequencing has also identified low-frequency variants of large effect associated with BMD and fracture risk. Despite these advances, common and rare variants explain only 5.8% of the total phenotypic variance in BMD.

In most previous genetic studies of BMD, the data analyzed were derived from dual-energy X-ray absorptiometry (DXA). However, DXA is expensive, and consequently the largest GWAS so far of DXA-derived BMD included only 32,965 individuals, which compromised the researchers’ ability to detect risk loci. An alternative method of analysis of gene expression in mouse osteoblasts, osteocytes and osteoclasts. The results implicate GPC6 as a novel determinant of BMD, and also identify abnormal skeletal phenotypes in knockout mice associated with a further 100 prioritized genes.

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estimating BMD that is quick, safe and relatively inexpensive, and therefore can be used in very large samples of individuals, is derived from ultrasound, typically at the heel calcaneus (referred to here as estimated BMD (eBMD)). Ultrasound-derived eBMD values are highly heritable (on the order of 50% to 80%)5-8, independently associated with fracture risk9,10 and moderately correlated with DXA-derived BMD at the hip and spine (r = 0.4 – 0.6)11. A previous GWAS that used heel ultrasound parameters (N = 15,514) identified variants at nine loci, including seven that had been previously associated with lumbar spine/hip BMD12.

Because genetic loci associated with BMD are strongly enriched for the targets of clinically relevant osteoporosis therapies13,14, the identification of new genetic loci and the biological pathways they implicate may help scientists identify drug targets for the prevention and treatment of fragility fracture. To identify novel genetic determinants of BMD, we investigated genome-wide association in the UK Biobank Study, which has measured eBMD and genome-wide genotypes in 142,487 individuals. We subsequently used three systematic and complementary approaches to prioritize genes for functional validation (Supplementary Fig. 1).

RESULTS

Genome-wide association study of estimated BMD
Quantitative ultrasound of the heel was used to obtain a non-invasive estimate of BMD that predicts fracture9,10. After stringent quality control of both eBMD measurements and genome-wide genotypes (Online Methods, Supplementary Fig. 2), data were available from 142,487 individuals (53% women) (Supplementary Table 1). We tested the additive effect of 17,166,351 SNPs with minor allele frequency (MAF) > 0.1% and imputation quality score > 0.4 on eBMD, controlling for age, sex and genotyping array. In total, 307 conditionally independent SNPs at 203 loci surpassed our revised genome-wide significance threshold (P ≤ 6.6 × 10^{-9}, which accounts for the large number of independent SNPs deeply imputed in the UK Biobank (Online Methods)) and jointly explained ~12% of the variance in eBMD (Supplementary Fig. 3, Supplementary Table 2). Together the 307 SNPs explained about one-third of the eBMD SNP heritability estimated by BOLT-REML (h^2_{SNP} = 0.36). Although there was substantial inflation of the test statistics relative to the null (λ_G = 1.37), linkage disequilibrium (LD) score regression15 indicated that the majority of inflation was due to polygenicity rather than population stratification (LD score regression intercept = 1.05). Of the 203 loci identified, 153 (75%) regions had not been implicated in previous GWASs of BMD5,9,16-22 (Supplementary Table 2, Supplementary Fig. 3). We found it interesting that the list of novel associations included multiple variants (e.g., SNPs at TBX1, ZNRF3) for which there was extremely strong evidence of association with heel eBMD (P < 10^{-30}) but little evidence of association (P > 0.05 for any trait) in a previous GEFOS-seq GWAS of DXA-derived BMD (Supplementary Table 3).

Our study also replicated SNPs in 55 out of 73 regions (>75%) that had been reported as genome-wide significant in previous GWASs of BMD at other body sites (P < 0.05 and consistent direction of effect), and we replicated all loci with genome-wide significance identified in a previous GWAS of ultrasound-derived heel eBMD12 (Supplementary Table 4). Our list of known BMD-associated SNPs is deliberately broad and comprehensive with respect to previous GWASs. This comprehensive inclusion policy, however, called for the

Figure 1 eBMD effect size compared with the effect size from a previous GEFOS meta-analysis of DXA-derived BMD for eBMD-associated SNPs. (a-c) Effect size for heel eBMD (y-axis) from the current UK Biobank study plotted against effect size from the previous GEFOS-seq study4 for BMD at the (a) femoral neck, (b) lumbar spine and (c) forearm (x-axis). Only conditionally independent variants that reached genome-wide significance (P < 6.6 × 10^{-9}) for eBMD in the UK Biobank study are plotted. The −log_{10}(P) value for the (any) fracture analysis of UK Biobank subjects is indicated by the shading of the data points (black indicates robust evidence of association with fracture, and white indicates poor evidence of association). SNPs that reached Bonferroni-corrected significance for fracture (P < 1.6 × 10^{-4}) are labeled. The blue dashed lines show the strong correlation between estimated effect sizes at the heel and at other sites of the body. SNPs at SLC8A1 and AQP1 were significantly related with fracture after correction for multiple testing (P < 1.6 × 10^{-4}) and have not previously been reported as associated with BMD or fracture, although they both reached nominal significance (P < 0.05) in the previous GEFOS-seq analysis.* Multiple conditionally independent variants present at the locus. –The closest gene to the locus (i.e., DEPICT did not detect any region within 1 Mb of the reported SNP).
The incorporation of results from some smaller GWASs that may include false positives. When we restricted our attention to the 64 SNPs reported in the large Genetic Factors for Osteoporosis Consortium (GEFOS) meta-analysis by Estrada et al.3 (which are unlikely to represent type 1 errors), we replicated 54 of the 64 (84%) SNPs. Possible reasons for nonreplicated loci include site specificity, differences in phenotype (ultrasound-derived versus DXA-derived BMD), differences in ancestral population between studies, and type 1 error in the previous, smaller study.

Notably, across six loci (RSPO3, LINCO0326, CPED1, MAPP7, KCNMA1 and TME2623), there were SNPs with different directions of effect in the current eBMD study compared with those in previous BMD studies. The SNPs at CPED1 also showed an association with bone strength in the UK Biobank data (discussed below), but in the direction predicted by eBMD rather than the direction predicted by BMD in previous studies (i.e., alleles that predispose subjects to low eBMD are associated with increased risk of fracture). Although these opposite directions of association are difficult to explain, differences in the phenotypes measured by DXA and ultrasound technologies are likely to be responsible. For example, whereas heel ultrasound measures primarily trabecular bone, DXA-based BMD measurements reflect a combination of trabecular and cortical bone. In addition, ultrasound-based measurements are independent of bone size, whereas areal BMD as measured by DXA is not fully size-corrected. In fact, of the six loci that showed opposite associations between DXA BMD and eBMD, three also showed strong associations with height in data from the Genetic Investigation of Anthropometric Traits (GIANT) consortium in the same direction as the DXA BMD data23, which suggests that these three associations may partly have reflected size effects (although it must be noted that several other concordant eBMD and DXA BMD loci also showed associations with height). Whereas bone size and bone mass generally show a strong positive correlation, genetic influences that lead to greater bone size might be inversely related to trabecular bone density at certain sites, owing to reduced mechanical strain as a consequence of a larger and thus stronger skeleton. However, despite these few discrepancies, overall there was a strong positive correlation between estimated effect size for the genome-wide-significant heel eBMD SNPs in the present UK Biobank Study and estimated effect sizes for DXA-derived BMD at other skeletal sites in our previous GEFOS-seq study (femoral neck, Pearson’s r = 0.64 (0.57–0.71); lumbar spine, r = 0.69 (0.62–0.75); forearm, r = 0.49 (0.39–0.58) (Fig. 1)4. Adjusting for weight had little effect on genome-wide significance, save for partially attenuating the strength of the association between eBMD and known adiposity variants (Supplementary Table 5).

Because we had used a large sample size and genotyped and/or imputed low-frequency variants (MAF < 1%), we next assessed the relationship between allelic architecture and eBMD (Fig. 2). We found a strong relationship between MAF and effect size that generally followed the statistical power of our study design. The variants of largest effect (for which each allele increased eBMD by 0.44 s.d.; P = 5 × 10−11) were in the gene IGHMBP2 (within 0.5 Mb of known variants in LRPS) and the known EN1 and WNT16/CPED1 loci. We also detected several rare (MAF < 1%) and low-frequency variants (1% < MAF < 5%)
in previously unreported loci, including rare variants near the genes BMP5 and BMPR2. When we compared the mean absolute effect sizes of genome-wide significant variants, we found a 6.5-fold difference in effects attributed to rare versus common variants.

Sex-specific analyses across the genome and tests of sex heterogeneity at genome-wide significant SNPs revealed a single variant, rs17307280, at FAM9B on the X chromosome that was significantly associated with eBMD in men only (Supplementary Fig. 4).

**Figure 3** Genetic correlations between eBMD as measured in the UK Biobank study (y-axis) and other traits and diseases (x-axis) estimated by LD score regression implemented in LDHub. Genetic correlation ($r_G$) and corresponding 95% confidence intervals (error bars) between eBMD and traits were estimated via LD score regression. The genetic correlation estimates ($r_G$) are color-coded according to their magnitude and direction as defined in the key.

**Figure 4** Increased bone mass and strength in adult Gpc6−/− mice. (a) X-ray microangiography images of femurs and caudal vertebrae from female wild-type (WT) and Gpc6−/− mice at postnatal day 112 (P112). In these pseudocolored grayscale images, green indicates low bone mineral content, and pink indicates high bone mineral content. The graphs at the bottom show reference ranges derived from >250 wild-type mice of identical age, sex and genetic background (C57BL/6). The plots represent the mean (solid center lines), ±1.0 s.d. (dotted lines) and ±2.0 s.d. (gray boxes). Values for parameters from individual Gpc6−/− mice are shown as red dots, and mean values as a thick black line ($n = 2$ animals). (b) Micro-CT images of proximal femur trabecular bone (left) and mid-diaphysis cortical bone (right) from wild-type and Gpc6−/− mice. The graphs below show trabecular bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), cortical thickness (Ct.Th), internal cortical diameter and cortical BMD. Elements of the plots are defined as in a. HA, hydroxyapatite. (c) Representative load-displacement curves from destructive three-point bend testing of femurs from wild-type and Gpc6−/− mice, showing yield load, maximum load, fracture load and gradient of the linear elastic phase (stiffness). The graphs show yield load, maximum load, fracture load, stiffness, and energy dissipated before fracture (toughness). Elements of the plots are defined as in a. P values were generated by permutation analysis as described in the Online Methods. Scale bars (a,b), 1 mm.
Supplementary Table 6) (heterogeneity $P = 1.4 \times 10^{-11}$), thus replicating previous results from Estrada et al.3.

### Effects on fracture

We tested the relationship between eBMD-associated SNPs and fracture. We identified 14,492 individuals (58% women) in UK Biobank who had reported a previous fracture, without giving special consideration to the trauma mechanism, as high-trauma fractures are predicted by low BMD and are predictive of future low-trauma fracture, thus suggesting a shared etiology24,25. In total, we observed that 12 eBMD SNPs were associated with fracture, after controlling for multiple testing ($P \leq 1.6 \times 10^{-4}$). The results of sensitivity analyses including only 8,540 individuals (69% women) who had reported a fracture resulting from a simple fall (i.e., from standing height) were consistent with these findings (Table 1). Of these 12 loci, variants at AQP1 and SLC8A1 had not been associated with BMD or risk of fracture previously (although both SNPs showed nominal association ($P < 0.01$) with DXA-derived BMD values from the GEFOS-seq study4 (Fig. 1, Supplementary Table 3)). We observed an inverse relationship between the effects of genome-wide significant eBMD variants on eBMD and the odds of fracture (Supplementary Fig. 5).

### Shared genetic factors

To test whether eBMD has a shared genetic etiology with 247 other diseases and biomedically relevant traits, we used LD score regression36 as implemented in LDHub27. This method estimates the degree to which genetic risk factors are shared between two diseases or traits, although it says nothing about how this shared genetic etiology arises (i.e., whether one variable causes the other, or whether the relationship between eBMD and the other variable is mediated by an underlying variable such as body mass index (BMI), which is itself partially genetic). Genetically increased eBMD was strongly and negatively correlated with fracture (Fig. 3; $r_g = -0.47$; 95% CI, $-0.59, -0.35$). Further, measures of BMD at other skeletal sites showed moderate positive genetic correlation with eBMD (Fig. 3) in agreement with the concordant directions observed at the genome-wide significant loci (Fig. 1).

We also asked whether eBMD is genetically correlated with a range of other complex traits and diseases (Supplementary Table 7, Fig. 3). We observed weak and negative correlation with HDL cholesterol level, LDL cholesterol level, height, age at menarche and rheumatoid arthritis (Fig. 3). In contrast, eBMD was weakly positively genetically correlated with BMI, waist circumference, waist-to-hip ratio, coronary heart disease and type 2 diabetes. These findings support a shared genetic etiology of several common traits and diseases with eBMD, as has been shown previously for BMD, adiposity and type 2 diabetes through Mendelian randomization28,29.

### Gene prioritization

#### Strategy one: bioinformatic, statistical and functional genomics in humans.

We used several bioinformatics and statistical genetics tools to prioritize likely candidate genes and variants. These included the Variant Effect Predictor software30 to identify deleterious coding variation at genome-wide significant loci (Supplementary Table 8), the FINEMAP software to create configurations of plausible causal SNPs around each conditionally independent lead SNP (Supplementary Table 9), ENCODE maps of DNase I hypersensitivity sites (DHSs)31,32 and contextual analysis of transcription factor occupancy3 to identify SNPs that perturb transcription factor activity, and evidence of cis–expression quantitative trait loci (eQTLs) in human osteoblasts33 (Supplementary Table 10). These results are fully described in Supplementary Note 1.

#### Strategy two: data-driven expression-prioritized integration.

For the second gene-prioritization approach, we used the DEPICT computational tool34. We identified 273 genes as most likely to drive the eBMD association signals (false discovery rate (FDR) < 0.05). Among these 273 genes were several with an established role in bone metabolism, such as BMP2, LRP5, EN1, RUNX2, JAG1, ESR1, COL21A1 and SOST (Supplementary Table 11).

We next tested the DEPICT-prioritized genes for enriched expression in any of 209 Medical Subject Heading (MeSH) tissue and cell-type annotations34. We identified 62 tissue or cell-type annotations (FDR: 5%) among the entries defined from the MeSH tissue and cell annotations (Supplementary Table 12, Supplementary Fig. 6). The strongest evidence of enriched expression of the genes mapping to eBMD loci came from chondrocytes and cartilage, although systems other than the musculoskeletal system were also overrepresented (cardiovascular system, 7/12 significant entries; membrane tissue, 6/7 significant entries; connective tissue cells, 5/7 significant entries).

We also tested the DEPICT-prioritized genes for enriched gene sets, and identified more than 1,000 significantly enriched (FDR: 5%) gene sets. Clustering in 35 ‘meta gene-sets’ showed that most clusters were related to skeletal growth (e.g., regulation of mineralized tissue development, vertebral fusion, abnormal craniofacial development, cartilage development) or signaling pathways involved in bone biology (e.g., mesenchymal stem cell differentiation, BMP or WNT signaling). More global biological processes were also highlighted (e.g., transcription factor binding and regulation, chromatin remodeling, complex, cell development) (Supplementary Fig. 7).

Analysis with the MAGENTA (meta-analysis gene-set enrichment of variant associations) software produced similar results implicating gene sets involved in bone mineralization and development, cadherin, the WNT and Hedgehog signaling pathways, and other pathways worthy of further investigation (oncogenic pathways, melanogenesis, etc.) (Supplementary Table 13).

We tested all genes prioritized by DEPICT for expression in mouse osteoblasts, osteoclasts and osteocytes. Among the 273 genes prioritized, 241 had mouse homologs (the majority that did not have a known homolog were long noncoding RNAs), with 92% expressed in osteoblasts, 66% in osteoclasts and 83% in osteocytes (Supplementary Table 14). In all, 95.4% of these genes were expressed in at least one of the three cell types. This represents a substantial enrichment of genes expressed in osteoblasts, osteocytes and osteoclasts ($P < 0.0001$ for each of osteoblasts, osteocytes and osteoclasts).

We then investigated whether a skeletal phenotype had been reported in the International Mouse Phenotyping Consortium (IMPC; "URLs") or Mouse Genome Informatics ("URLs") databases in knockout mice with deletion of any of the prioritized genes. We found that 189 (78%) of the 241 DEPICT-prioritized genes had mouse knockout phenotype data available, and 62 (33%) of those phenotypes included skeletal abnormalities (Supplementary Table 14).

#### Strategy three: deep phenotyping of knockout of selected genes within 1 Mb of lead SNPs.

The third gene-prioritization approach identified all genes within 1 Mb of lead SNPs at associated eBMD loci. We compared these genes with genes from knockout mice generated at the Wellcome Trust Sanger Institute for the IMPC35. Knockout mice had been generated for 120 of the prioritized genes, and bespoke skeletal phenotyping was undertaken as part of the Origins of Bone
and Cartilage Disease Program. Specifically, we carried out both structural and functional analysis of skeletal samples, using digital X-ray microradiography, micro-CT and biomechanical testing. We compared our results with normal reference data from >250 control mice with an identical C57BL/6 genetic background. We found that 43 (36%) of these 120 prioritized genes were associated with significantly abnormal bone structure, representing twofold enrichment compared with the results of a previous analysis of 100 unselected knockout mice (χ² = 8.359, P = 0.0038) (Supplementary Table 15).

**GPC6 findings**

Using these parallel strategies, we identified 100 genes that, when disrupted, were associated with an abnormal skeletal phenotype in mutant mice (Supplementary Tables 14 and 15). However, all three gene-prioritization strategies identified GPC6, so we selected this gene for further study (Supplementary Table 16).

GPC6 encodes a member of the glycosylphosphatidylinositol-anchored, membrane-bound heparan sulfate proteoglycan protein family. Loss-of-function mutations in GPC6 result in omodysplasia 1 (OMIM 258315), a rare autosomal recessive skeletal dysplasia characterized by short-limbed dwarfism with craniofacial dysmorphism. This indicates a role for GPC6 in skeletal biology, although the gene has not previously been implicated in osteoporosis.

Our bioinformatics pipeline provided evidence for a functional association at the GPC6 locus. A single SNP in GPC6, rs1933784, in high linkage disequilibrium with the conditionally independent lead SNP rs147720516 at this locus (r² > 0.9), was a plausible causal and functional variant. We observed that rs1933784 was a low-frequency SNP (MAF = 0.05) that was significantly associated with eBMD (P = 2.3 × 10⁻¹⁰), with high causal probability (log₁₀ Bayes factor = 2.4), and that it was present within DHSs in several cell types (Supplementary Table 16). The rs1933784 variant also showed some evidence of association with GPC6 expression in osteoblasts (P = 4.7 × 10⁻³) (Supplementary Table 16).

DEPICT identified GPC6 as the gene most likely to be responsible for the association at this locus. GPC6 is expressed in osteoblasts and osteocytes in mice (Supplementary Table 14). In osteocytes, GPC6 had a similar level of enrichment (1.76 log fold-enrichment) as genes known to have key involvement with the skeleton, such as Lrp5 (1.95 log fold-enrichment) (Supplementary Fig. 8), encoding an important receptor that influences bone mass through canonical Wnt signaling, and Runx2 (1.73 log fold-enrichment), encoding a key transcription factor in osteoblast differentiation.

We analyzed adult female Gpc6⁻/⁻ mice and compared the results with data for >250 wild-type control mice of identical C57BL/6 background. Consistent with the phenotype of omodysplasia 1, Gpc6⁻/⁻ mice had femurs and vertebrae that were shorter than those of wild-type mice (−1.95 and −2.17 s.d., permuted P = 0.06 and 0.016, respectively). Gpc6⁻/⁻ mice also had increased femoral bone mineral content (+2.4 s.d., permuted P = 3 × 10⁻⁹) and increased cortical thickness (+2.3 s.d., permuted P = 5 × 10⁻⁸) compared with wild-type mice. The biomechanical consequence of these structural abnormalities was an increase in yield load (+2.1 s.d., permuted P = 8 × 10⁻³) that reflected increased material elasticity (Fig. 4). Although the phenotype of Gpc6⁻/⁻ mice is consistent with human omodysplasia 1, no information is available regarding adult manifestations of the condition. Thus, further studies in Gpc6⁻/⁻ mice are required to characterize the cellular and molecular mechanisms underlying the role of GPC6 in the pathogenesis of osteoporosis.

Finally, we queried 87 separate GWASs using the web utility PhenoScanner, with full genome-wide summary statistics available for the conditionally independent genome-wide significant SNPs for eBMD (rs72635657, rs147720516) at the GPC6 locus, for any associations with a P value of <0.05 (ref. 38). We identified one association, for rs72635657 with femoral neck BMD (P = 0.015). We also searched the NHGRI–EBI catalog of published GWASs for GPC6 (accessed 22 March 2017). SNPs in the region of GPC6 had previously shown evidence of association with attention deficit hyperactivity disorder, FEV1 after bronchodilation, Alzheimer’s disease, neuroticism and lower facial height, although the lead SNPs reported in these scans were not in appreciable LD with the lead conditionally independent SNPs in the present study (all r² < 0.1).

**DISCUSSION**

With this study, we have increased the number of genetic loci associated with BMD in humans almost threefold and doubled the amount of variance explained for this trait. Further, we have demonstrated that several BMD-associated variants also influence the risk of fracture. We have prioritized genes for future study and provided functional evidence that GPC6 has a role in determining BMD and the pathophysiology of osteoporosis.

Our findings provide evidence that the genetic architecture underlying BMD is highly polygenic. The observed effect sizes follow a close relationship with MAF within the limits of the statistical power of the study. This suggests that further low-frequency and rare variants of moderate to large effect will be identified in future studies, which is likely to improve the overall understanding of the cellular and molecular mechanisms involved. Drug targets supported by evidence from human genetics are most likely to result in clinically useful therapies in general, and this has been demonstrated for musculoskeletal conditions. Thus, our findings will be helpful for identifying pathways and proteins amenable to pharmacologic manipulation to decrease the burden of fracture in the population.

GPC6 encodes a glypicin that may serve as a novel drug target for osteoporosis care, as it is a cell-surface protein involved in signaling whose loss of function leads to increased bone mineral content, likely due to increased cortical bone and resultant increased elasticity. GPC6 is a member of the glypican family (GPC1–6) of glycosylphosphatidylinositol-anchored, membrane-bound heparan sulfate proteoglycan core proteins that are involved in cellular growth control and differentiation. Mutations of GPC3, GPC4 and GPC6 result in developmental skeletal abnormalities, but limited or no information is available from affected humans (OMIM 312870, OMIM 258315). The heparan sulfate proteoglycans attached to the GPC6 core protein regulate skeletal signaling pathways involved in bone formation and mineralization, including those mediated by the FGF, VEGF, Hedgehog and BMP pathways. In addition, the adult high-bone-mass phenotype and increased cortical bone thickness identified in Gpc6⁻/⁻ mice in these studies is consistent with the recently identified direct role of GPC6 in the modulation of Wnt signaling, which is the key regulator of osteoblastic bone formation and is associated with BMD in humans. Overall, these findings suggest a number of possible new pharmacological targets that include not only the core protein GPC6, but also the heparan sulfate synthetic (EXT1–2) and modification enzymes (NDST1–4, GLCE, HS2ST and HS6ST1–3) that specifically regulate growth factor binding and activity. The availability of global and tissue-specific Gpc6⁻/⁻ mice now provides the opportunity to test these possibilities directly. However, we caution that although GPC6 and associated proteins seem to be promising targets for pharmacotherapy, other factors (the likelihood of unintended side effects, etc.) will need to be considered before these molecules can be confirmed as suitable candidates for pharmacological manipulation.
There are several limitations to our study. First, despite the high concordance between the loci identified from ultrasound-derived measurements of BMD and those from previous studies that used DXA-derived BMD, there were some notable differences. Our study did not replicate associations at 18 known BMD loci identified in previous studies. Also, our list of genome-wide significant variants included some that were strongly related to eBMD at the heel but were not found in previous studies that used DXA-derived BMD measures at other body sites in considerably smaller samples. For some of these loci, such as TBXI, this may simply be a consequence of the associated variants having been neither genotyped nor tagged well in previous studies. For other loci, it may reflect genetic influences that are specific to the heel (for example, genetic responses of the heel to ground reaction forces) that are not present at other body sites. Interestingly, we identified variants at six loci where the direction of effect was opposite between eBMD at the heel and DXA-derived BMD at other sites, although notably at CPED1 the variants also showed association with risk of fracture in the direction consistent with the heel eBMD association. Although the reason for these differences is unclear, the implication is that ultrasound measurements of the heel capture aspects of bone structure beyond those obtained by central DXA, and this is consistent with previous observations that ultrasound measurements of the heel predict risk of osteoporotic fracture over and above hip BMD.

Second, our study does not provide a definitive biological mechanism through which variants at genome-wide significant loci causally affect eBMD. Our eQTL analyses were not consistent with the mediation of SNP effects through osteoblast expression at a majority of loci. This is probably because at least some of the identified eBMD-associated SNPs may act on cell types other than osteoblasts, such as osteocytes and osteoclasts. Further, the relatively small sample size of 95 individuals in the osteoblast eQTL experiment may have led to uncertain estimates. Also, the expression of genes in culture may reflect different biological processes than those in vivo. Although differences in gene expression are not the only mechanism through which the functional effects of an association can be mediated, we expect that large-scale genomic studies investigating the pattern of genetic association in osteoblasts, osteocytes and osteoclasts will reveal how these eBMD associations are mediated in the not-too-distant future.

Third, our study had a limited ability to detect very rare variants (i.e., MAF < 0.1%) or rare variants of small effect (MAF < 1% and effect size < 0.05 s.d.). Finally, we studied the genetic etiology of osteoporosis only in European individuals. It is likely that studies of populations of different ancestry will reveal novel loci that are important in the regulation of BMD, as has been the case for other conditions.

In summary, our findings shed light on the pathophysiological mechanisms that underlie changes in BMD and fracture risk in humans. The proteins identified and prioritized by these studies identify signaling pathways that represent new drug targets for the prevention and treatment of osteoporosis—a major health care priority.

URLs. International Mouse Phenotyping Consortium (IMPC), http://www.mousephenotype.org; Mouse Genome Informatics (MGI), http://www.informatics.jax.org; the Origins of Bone and Cartilage Disease Study (OBCD), http://www.boneandcartilage.com; UK Biobank (UKBB), http://www.ukbiobank.ac.uk/; Genetic Factors for Osteoporosis Consortium (GEfos), http://www.gefos.org/; UK Biobank protocol for measurement of eBMD, https://biobank.ctsu.ox.ac.uk/crystal/docs/Ultrasonicbone densitometry.pdf; UK Biobank document #155580 on genotyping and quality control, http://biobank.ctsu.ox.ac.uk/crystal/docs/genotyping_qc.pdf; Hg19 gene range list, https://www.cog-genomics.org/plink2/.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

S.K., F.R., J.H.T., P.I.C., C.L.A.–B., J.H.D.B., G.R.W., J.B.R. and D.M.E. conceived and designed experiments. J.P.K., J.A.M., C.M.-G., V.F., N.M.W., S.E.Y., J.Z., K.T., E.G., K.M.G., C.X., C.M.T.G., C.L.A.–B., J.H.D.B. and G.R.W. performed statistical analysis. J.P.K., J.A.M., C.M.-G., V.F., N.M.W., S.E.Y., C.L.G., K.T., C.M.T.G., M.T.M., S.K., F.R., J.H.T., P.I.C., C.L.A.–B., J.H.D.B., G.R.W., J.B.R. and D.M.E. wrote the paper. S.E.Y., J.G., J.L., A.S.P., P.C.S., R.A., V.D.L., N.C.B., D.K.-E., A.-T.A., K.F.C., J.W.K., D.J.A., P.I.C., C.L.A.–B., J.H.D.B. and G.R.W. generated mouse models and/or functional experiments. N.C.H. and C.C. generated heel eBMD data. J.P.K., J.A.M. and C.M.-G. were the lead analysts. All authors revised and reviewed the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS
Measurement of eBMD, fracture and weight in UK Biobank. In 2006–2010, the UK Biobank recruited 502,647 individuals aged 37–76 years (99.5% were aged 40–69 years) from across the country. All participants provided information regarding their health and lifestyle via touch screen questionnaires, consented to physical measurements, and agreed to have their health followed. They also provided blood, urine and saliva samples for future analysis. UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee, and informed consent was obtained from all participants. A Sahara Clinical Bone Sonometer (Hologic Corporation, Bedford, Massachusetts, USA) was used for quantitative ultrasound assessment of calcanei in UK Biobank participants. Details of the complete protocol are publicly available on the UK Biobank website (“URLs”). Participants were initially measured at baseline (N = 487,428) and had their left calcaneus (N = 317,815), right calcaneus (N = 4,102) or both calcanei (N = 165,511) measured. A subset of these subjects were followed up at two further time points (N = 20,104 and N = 7,988), during which both heels were measured. A detailed description of the ascertainment procedure is provided in Supplementary Figure 2. Prior to quality control, ultrasound data were available for 488,683 individuals at either baseline and/or follow-up assessment. eBMD (g/cm²) was derived as a linear combination of speed of sound (SOS) and bone ultrasound attenuation (BUA) (eBMD = 0.002592 (BUA + SOS) – 3.687). To reduce the impact of outlying measurements, quality control was applied to male and female subjects separately with the following exclusion thresholds: SOS, ≤1.455 or ≥1.706 m/s for males; BUA, ≤0.12 or ≥0.105 dB/MHz for females; and BUA and SOS (±138 dB/MHz for females). Individuals exceeding the following thresholds for eBMD were excluded: males, ≤0.18 or ≥0.06 g/cm²; females ≤0.12 or ≥0.055 g/cm². Bivariate scatter plots of eBMD, BUA and SOS were visually inspected, and any measurements that did not cluster with the others were removed; this left a total of 483,230 valid measures (476,618 left and 6,612 right calcaneus) for SOS, BUA and BMD (265,057 females and 218,173 males).

Please see Supplementary Figure 2 for a detailed description of the quality control pipeline and Supplementary Table 1 for an overview of descriptive statistics of the cohort after quality control.

We defined 14,492 individuals (8,439 female and 6,053 male) as having a fracture, on the basis of affirmative answers to the question, “Have you fractured/broken any bones in the last 5 years?” at either baseline or first follow-up. Individuals were coded as missing if they responded “Do not know” or “Prefer not to answer” at both baseline and first follow-up; otherwise they were coded as controls (N = 130,563). Self-reported fractures have low false positive and false negative rates. Individuals who stated that they had had a fracture were also asked whether the fracture resulted from a simple fall (i.e., from standing height). We created a second variable using this question, where 8,540 individuals (5,853 female and 2,687 male) had a fracture from a simple fall and 131,333 individuals did not report a fracture. Weight was measured with a Tanita BC418MA body composition analyzer.

Preparation, quality control and genetic analysis in UK Biobank samples. Genotype data from the interim May 2015 release of UK Biobank were available for a subset of 152,729 participants. Data were imputed centrally by UK Biobank Genotype data from the interim May 2015 release of UK Biobank were available for a subset of 152,729 participants. Data were imputed centrally by UK Biobank (UK Biobank document #155580; see “URLs”), we defined a subset of ‘white European’ ancestry samples by using a K-means (K = 4) clustering approach based on the first four genetically determined principal components. A maximum of 142,487 individuals (76,067 females and 66,420 males) with genotype and valid quantitative ultrasound measures were available for the present analyses. We tested genetic variants for association with eBMD, assuming an additive allelic effect, in a linear mixed non-infinite-simale model implemented in BOLT-LMM24 to account for cryptic population structure and relatedness. Genotyping array, age and sex were included as covariates in all models. We also included weight as a covariate in a sensitivity analysis to investigate whether the power to detect association was increased or whether weight mediated the relationship between genotype and eBMD (i.e., some variants may be primarily associated with weight, and their effect on eBMD may be mediated through a causal effect of weight on eBMD25). Only SNPs down to an MAF of 0.1% and with an info-score threshold of >0.4 were analyzed.

We additionally analyzed the association between eBMD and directly genotyped SNPs on the X chromosome, adjusting for genotyping array, age, sex and the first four ancestry principal components, using Plink v1.09 beta 3.38 (7 June 2016) software48 and a nested sample of unrelated subjects (N = 135,729). Because the analyses for the X chromosome data were based on observed genotypes, our quality control was slightly different. We excluded SNPs with evidence of deviation from Hardy–Weinberg equilibrium (1 × 10⁻⁶), MAF < 0.1% and overall missing rate > 5%, which yielded 15,552 X chromosome SNPs for analysis. Heterogeneity between sexes in effect size coefficients was tested with EasyStrata49. Manhattan and Miami plots of our genome-wide association scans were generated by EasyStrata version 15.3. Regional association plots were generated with LocusZoom (v1.3)50, using LD information estimated from our reference UK Biobank sample, together with the December 2016 release of the NHGRI–EBI catalog of published GWASs. SNPs that were associated with eBMD at genome-wide significance levels were additionally tested for association with fracture using BOLT-LMM, including age, sex, BMI and the time of reporting the fracture as fixed effects51.

Estimation of genome-wide significance threshold. Traditional estimates of the genome-wide significance threshold for common variants (MAF > 5%) in European populations (i.e., α = 5 × 10⁻⁶) are based on a Bonferroni correction of α = 0.05/10⁵, as there are an estimated 1 million statistically independent SNPs above this MAF threshold. However, in the case of UK Biobank, we assessed SNPs down to an MAF of 0.1% in 142,487 individuals and applied an info-score threshold of >0.4, which resulted in 17.17 million SNPs. Thus, we defined a new and more conservative threshold to declare genome-wide significance, accounting for the number of independent statistical tests performed in our data. To do this, we applied the method we used previously in the UK10K sequencing consortium5, which assesses the correlation between nearby test statistics empirically. Analysis of permuted data derived from a small proportion of all tested variants allows assessment of the correlation patterns. Thus we were able to estimate, in subsets of the genome of varying size, the relationship between the Bonferroni significance threshold and the empirical significance threshold that corrects for correlations, and thereby extrapolate to the whole genome. Specifically, when assessing all 740,018 variants that met our filtering criteria across chromosome 9 (Supplementary Fig. 9), we saw a good linear fit between family-wise error rate (α = 0.05), divided by the number of tests and the empirical significance thresholds. Our estimated genome-wide significance threshold then, accounting for all SNPs with MAF ≥ 0.1% and info-score > 0.4, was α = 6.6 × 10⁻⁸.

Approximate conditional association analysis. To detect multiple independent association signals at each of the genome-wide significant eBMD loci, we carried out approximate conditional and joint genome-wide association analysis using the software package GCTA51. SNPs with high multicollinearity (multiple regression R² > 0.9) were ignored, and those situated more than 20 Mb away were assumed to be in complete linkage equilibrium. A reference sample of 15,000 unrelated (pairwise relatedness < 0.025) individuals of white British origin randomly selected from UK Biobank was used to model patterns of LD between variants. The reference genotyping data set consisted of the same 17 million variants assessed in our GWAS, but with an additional quality control step to exclude SNPs that deviated from Hardy–Weinberg equilibrium (1 × 10⁻⁶). Conditionally independent variants that reached GWAS significance were annotated to the physically closest gene with bedtools52 v2.26.0 and the Hg19 Gene range list available online (see “URLs”).

Estimation of variance explained by significant variants and SNP heritability. We estimated the proportion of phenotypic variance tagged by all SNPs on the genotyping array (i.e., the SNP heritability) with BOLT-REML53. To calculate the variance explained by all genome-wide significant SNPs, we first used the method of Bigdelli et al.14 to shrink the effect sizes of SNPs likely to suffer from ‘winner’s curse’. Briefly, the method works by shrinking the effect sizes of SNPs that just reach significance while having a negligible effect on SNPs that are more robustly significant (and consequently more accurately and precisely estimated). After calculating the corrected effect sizes, we removed the combined effect of the SNPs on the individual’s eBMD and recalculated the total expected variance in BOLT-LMM. The difference between this estimate
and the total expected variance calculated on the original data without the SNP correction was an estimate of the variance explained by all SNPs.

### Linkage disequilibrium score regression
To estimate the amount of genomic inflation in the data due to residual population stratification, cryptic relatedness and other latent sources of bias, we used LD score regression\(^{13}\). LD scores were calculated for all high-quality SNPs (i.e., INFO score > 0.9 and MAF > 0.1%) from a data set consisting of 15,000 unrelated individuals from the UK Biobank. To estimate the genetic correlation between eBMD and other complex traits and diseases, including those related to osteoporosis, we used a relatively new method based on LD score regression as implemented in the online web utility LDHub\(^{26,27}\). This method uses the cross-products of summary test statistics from two GWASs and regresses them against a measure of how much variation each SNP tags (its LD score). Variants with high LD scores are more likely to contain more true signals and thus provide a greater chance of overlap with genuine signals between GWAs. The LD score regression method uses summary statistics from the GWAS meta-analysis of eBMD and the other traits of interest, calculates the cross-product of test statistics at each SNP, and then regresses the cross-product on the LD score. The slope of the regression is a function of the genetic covariance between traits:

\[
E(z_1 | z_2) = \frac{\sum [N_i N_j \rho_{ij} - \rho_{i} \rho_{j}]}{N_i N_j} + \rho_{\text{NS}} \frac{\sum N_i N_j}{\sqrt{N_i N_j}}
\]

where \(N_i\) is the sample size for study \(i\), \(\rho_{ij}\) is the genetic covariance, \(M\) is the number of SNPs in the reference panel with MAFs between 5% and 50%, \(l_j\) is the LD score for SNP \(j\), \(N_i\) quantifies the number of individuals that overlap both studies, and \(\rho\) is the phenotypic correlation among the \(N_i\) overlapping samples. Thus, if there is sample overlap (or cryptic relatedness between samples), it will affect only the intercept from the regression (i.e., the term \(\rho_{i} \rho_{j}\)) and not the slope, and hence estimates of the genetic covariance will not be biased by sample overlap. Likewise, population stratification will affect the intercept but will have a minimal effect on the slope (i.e., intuitively, as population stratification does not correlate with LD between nearby markers).

### Gene prioritization and pathway analysis
To establish functional connections, we conducted three different analyses implemented in the DEPICT v1 tool\(^3\). First, to prioritize genes with relevant biological roles in the eBMD-associated loci, we tested functional similarities among genes from different associated regions where genes with high functional similarity across regions obtained lower prioritization \(P\) values. Second, we analyzed expression enrichment across particular tissues or cell types by testing whether genes in the associated eBMD loci had high expression in any of the 209 MeSH annotations, using data from 37,427 expression arrays. Third, we performed a gene set enrichment analysis to test whether the genes in the associated eBMD loci were enriched in reconstructed gene sets. The 10,968 gene sets tested were generated from diverse databases, including Gene Ontology, KEGG, REACTOME, the InWeb database (high-confidence protein–protein interaction), and the Mouse Genetics Initiative (phenotype–genotype relationships). In all three analyses we used the FDR to adjust for multiple testing; significance was defined at FDR = 5%.

The DEPICT analyses were based on independent lead SNPs (\(r^2 < 0.1\); European populations, 100 Genomes reference panel) with \(P\) values below the genome-wide significance threshold (\(P < 6.64 \times 10^{-8}\)). Because many of the gene sets tested came from different repositories, they overlapped; hence significantly enriched gene sets were further grouped into ‘meta gene sets’ through similarity clustering, as previously described\(^{13}\). The visualization of these meta gene sets was performed in Cytoscape\(^{30}\), filtering at FDR < 1%.

We also compared the DEPICT gene set enrichment results to analyses with the MAGENTA software\(^{28}\). Briefly, MAGENTA maps each gene in the genome to a single index SNP with the lowest \(P\) value within a 110-kb upstream and 40-kb downstream window (excluding genes in the HLA region owing to complex patterns of LD). This \(P\) value is then corrected for confounding factors (SNP density, gene size, etc.) in a linear regression model, and each gene is ranked by its adjusted gene score. The observed number of gene scores in a given pathway, with a ranked score above a specified threshold (i.e., 95th and 75th percentiles of all gene scores), is then calculated. This observed statistic

### Prioritizing candidate genes and possible causal variants at each eBMD locus
We combined a number of approaches to identify possible causal SNPs at each eBMD signal (defined here as all SNPs within 500 kb of a conditionally independent lead SNP that attained genome-wide significance). First, we used the Variant Effect Predictor (VEP)\(^{36}\) to annotate all SNPs within a locus (defined as ±500 kb from a conditionally independent lead SNP) for deleterious coding variation annotation if they were significantly associated with eBMD (\(P < 6.6 \times 10^{-8}\)). Deleterious SNPs were classified as such if they had one of the following sequence ontology terms: frameshift_variant, inframe_deletion, inframe_insertion, initiator_codon_variant, missense_variant, splice_acceptor_variant, splice_donor_variant, stop_gained, or stop_lost.

Next, using FINEMAP\(^{37}\), we identified 305 autosomal lead SNPs and further defined sets of plausible causal SNPs within each locus. For each locus, FINEMAP implements a shotgun stochastic search algorithm to test multiple causal configurations of SNPs, calculating within a Bayesian framework the posterior probabilities of each configuration to identify the number of likely causal SNPs. We note that this approach assumes that the true causal variants have been included in the analysis and have been well imputed. We also emphasize that approaches such as this that are based solely on association test statistics and LD are unlikely to be definitive with respect to the identification of causal variants/gene sets. Thus, we regard these fine-mapping analyses as one of several approaches that can be used to implicate specific variants/gene sets in osteoporosis etiology. When the same variant/gene is implicated by multiple independent approaches (for example, mouse knockout, human knockout, gene expression and eQTL studies), there is greater confidence of the identity of the gene/variant(s) underlying the statistical association.

For a given number of plausible causal SNPs, FINEMAP will calculate for each SNP the Bayes factor, which quantifies the evidence that the particular SNP is causal. We retained only SNPs with Bayes factors greater than 100, or \(\log_{10}\) Bayes factors greater than 2, as our plausible causal SNPs for each locus.

We then annotated each set of plausible causal SNPs for overlap with DHs, using a master list derived from 115 cell types\(^3\). DHs are local sites of open chromatin comprising the collective transcription factor binding sites in a given cell type. We further annotated each SNP inhabiting a DHS with Contextual Analysis of Transcription Factor Occupancy (CATO) scores. CATO, previously described by Maurano et al.\(^4\), scores the likelihood that a variant will cause allelic imbalance of a DHS by modeling both local sequence context and direct effects on the transcription factor recognition sequences for 44 transcription factor motif families. CATO scores range between 0 and 1, and we considered SNPs with CATO scores greater than 0.1 as having very strong functional evidence (corresponding to a 51% positive predictive rate in the initial training set\(^4\)).

### Genetically modified animals used for functional validation
The IMPC (“URLs”)\(^{38}\) and the International Knockout Mouse Consortium (IKMC) are generating null alleles for all protein-coding genes in mice on a C57BL/6 genetic background\(^{39}\). These mice are phenotyped through a broad-based phenotyping screen\(^{40}\). This approach can be used for functional investigation of candidate genes identified by a GWAS of human disease or traits, and studies have already ascribed novel functions for poorly annotated or previously unpublished genes. The Origins of Bone and Cartilage Disease (OBCD) study (“URLs”) is undertaking a validated multiparameter skeletal phenotyping screen\(^{36}\) of mutant mouse lines generated by the Wellcome Trust Sanger Institute as part of the IKMC and IMPC effort.

### OBCD methods
Samples from 16-week-old female wild-type and knock-out mice were stored in 70% ethanol, anonymized and randomly assigned to batches for rapid-throughput analysis in an unscreened fashion. The relative bone mineral content (BMC) and length of the femur and caudal vertebrae.
were determined at 10-µm pixel resolution by digital X-ray microangiography (Faxitron MX20). Micro-CT (Scanco uCT50, 70 kV, 200 µA, 0.5-mm aluminum filter) was used to determine cortical bone parameters (thickness, BMD, medullary diameter) at 10-µm voxel resolution in a 1.5-mm region centered on the mid-shaft region 56% of the way along the length of the femur distal to the femoral head, and trabecular parameters (bone volume, trabecular number, thickness, spacing) at 5-µm voxel resolution in a 1-mm region beginning 100 µm proximal to the distal growth plate. Biomechanical variables of bone strength and toughness (yield load, maximum load, fracture load, the percentage of energy dissipated before fracture) were derived from destructive three-point bend testing of the femur and compression testing of caudal vertebrae 6 and 7 (Instron 5543 load frame, 100-N load cell)69. Overall, 19 skeletal parameters were reported for each individual mouse studied and compared to reference data obtained from >250 16-week-old wild-type C57BL/6 female mice. Coefficients of variation for each skeletal parameter were as follows: femur BMC (2.0%) and length (2.1%); vertebra BMC (2.1%) and length (2.3%); trabecular bone volume/tissue volume (18.5%), trabecular number (7.3%), trabecular thickness (7.9%) and trabecular spacing (8.3%); cortical bone thickness (4.3%), internal diameter (6.0%) and BMD (4.0%); femur yield load (13.2%), maximum load (10.0%), fracture load (29.0%), stiffness (15.3%) and energy dissipated before fracture (26.7%); and vertebra yield load (13.0%), maximum load (10.3%) and stiffness (13.3%).

In Supplementary Table 15, we highlight knockout mice with phenotypes greater than 2 s.d. away from the mean of wild-type mice. We generated P values for the reported Gpc6−/− mouse phenotypes through permutation. To do so we first identified the least extreme phenotype for the Gpc6−/− mice tested. We then permuted the knockout labels 100,000 times to observe the number of times we observed two knockout animals with both phenotypes as extreme as the least extreme Gpc6−/− mouse phenotype. The P value was then calculated as the number of extreme permutations divided by 100,000. All mouse studies were undertaken by the Wellcome Trust Sanger Institute Mouse Genetics Project as part of the IKMC and licensed by the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986 and the recommendations of the Weatherall report.

Gene expression in primary human and mouse osteoblasts. To study human osteoblasts, we undertook cis-eQTL analyses of plausible causal regulatory SNPs in 95 primary human osteoblasts as previously described by Grundberg et al.33, performed with an updated imputation panel, the combined UK10K and 1000 Genomes phase 1 v3 reference panel40. We used an α level of 0.05 to identify possible gene targets of plausible causal SNPs. We investigated the possibility that heel eQTL associations and cis-eQTL effects in osteoblasts represent different signals (as opposed to a causal effect of osteoblast expression on eBMDD) by performing two sample summary Mendelian randomization analyses on osteoblast eQTL and heel eBMD GWAS summary results62,63. A HEIDI (heterogeneity in dependent instruments) test was used to identify situations in which the lead cis-eQTL was likely to be in LD with two distinct causal variants (one affecting gene expression, and the other affecting eBMD variation), as opposed to expression of the relevant gene mediating the relationship between the SNP and eBMD. Intuitively the test works by comparing estimates of the putative causal effect of gene expression on eBMD obtained by Mendelian randomization analysis of each variant while taking into account dependencies between the SNPs. Under a causal model, different SNPs should produce the same causal estimate (subject to sampling error), whereas under a model of linkage (i.e., two separate signals in the region, one affecting gene expression in osteoblasts and the other affecting eBMD), the estimates from the Mendelian randomization analysis may significantly differ. In the context of our study, a significant HEIDI test suggested that expression of the relevant gene in osteoblasts does not mediate the SNP–eBMD association. We therefore performed HEIDI tests for all the probes listed in Supplementary Table 10 that were implicated in our gene expression analyses. To prevent weak SNP instruments from potentially affecting our results, we included only SNPs that exhibited strong evidence of association (i.e., F statistic > 10) in the eQTL analysis63.

Gene expression profiles of candidate genes were examined in primary mouse osteoblasts undergoing differentiation. These data have been described in detail previously64 and are publicly available from the Gene Expression Omnibus (GSE54461). To study mouse osteoblasts, we obtained pre-osteoblast-like cells from calvaria collected from neonatal C57BL/6 mice carrying a transgene expressing cyan fluorescent protein (CFP) under the control of the Col 3.6-Kb promoter. The cells were plated into culture for 4 d in growth media, and cells that did not express CFP at the end of that culture period were removed by FACS. The remaining pre-osteoblast cells were re-plated and exposed to an osteoblast-differentiation cocktail, and RNA was collected every 2 d from day 2 to 18 d post-differentiation. We used RNA-seq to evaluate the transcriptome at each time point with an Illumina HiSeq 2000. Three technical replicates per samples were sequenced. The alignments for abundance estimation of transcripts were created with Bowtie version 0.12.9, using the NCBI-C37 reference genome. We calculated the expression level per gene with RSEM version 1.2.0 with parameters of --fragment-length-mean 280 and --fragment-length-sd 50, and the expression level for each sample was normalized relative to the per-sample upper quartile.

Gene expression in mouse osteocytes. We determined osteocyte expression by analyzing whole-transcriptome sequences derived from four different mouse bones: theibia, femur, humerus and calvaria (marrow removed; n = 8 per bone). A threshold of expression was determined on the basis of the distribution of normalized gene expression for each sample, using a modified statistical approach from Hart et al.44. ‘Expressed’ genes were above this threshold for eight of eight replicates in any bone type. We determined the specificity of these genes’ expression in the skeleton by comparing transcriptome-sequencing data from bone samples with osteocytes isolated to data from bones with the marrow left intact (n = 5 per group) (S.E.Y., J.H.D.B., G.R.W., and P.I.C., manuscript in preparation).

Gene expression in mouse osteoclasts. Expression of genes in mouse osteoclasts was determined from publically available data obtained via RNA-seq of bone-marrow-derived osteoclasts obtained from 6–8-week-old C57BL/6 mice (GEO accession GSM1873361).

Data availability. The human genotype and phenotype data on which the results of this study are based are available upon application from UK Biobank ("URLs"). GWAS summary statistics from this study are available via the GEFS+ website ("URLs"). No new data sets or related accession codes were generated as part of this study. Mouse phenotype data are available online from the IMPC ("URLs") and OBCD ("URLs").

A Life Sciences Reporting Summary for this paper is available.
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Experimental design

1. Sample size
   Describe how sample size was determined.
   
   Skeletal phenotyping of knockout mice. The reference ranges for each skeletal parameter were derived from >250 female 16 week old C57BL/6 wild-type mice. Using these data together with coefficients of variation for each test, power calculations indicate an 80% power to detect outlier phenotype of greater or equal to 2SD with a sample size of n=2

2. Data exclusions
   Describe any data exclusions.
   
   If skeletal samples were damaged or incomplete on receipt they were excluded from the analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   
   No replication of knockout mouse lines.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   
   Not applicable

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   
   All skeletal samples from knockout mice generated by the Wellcome Trust Sanger Institute were bar coded. Samples were sent to Imperial College in anonymized batches and all skeletal phenotyping and analysis was performed blind to genotype.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑️ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☑️ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑️ | A statement indicating how many times each experiment was replicated |
| ☑️ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑️ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑️ | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| ☑️ | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☑️ | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

Software

Describe the software used to analyze the data in this study.

NA

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All knockout lines and primary phenotype data are available on line at the IMPC http://www.mousephenotype.org/ and OBCD http://www.boneandcartilage.com/NA

Antibodies

NA

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

NA

b. Describe the method of cell line authentication used.

NA

c. Report whether the cell lines were tested for mycoplasma contamination.

NA

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

NA
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Knockout mice were generated at the Wellcome Trust Sanger Institute for the International Mouse Phenotyping Consortium. Skeletal samples from female 16 week old C57BL/6 wild type and mutant mice in an identical genetic background were analysed.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

NA