GWAS of bone size yields twelve loci that also affect height, BMD, osteoarthritis or fractures

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Bone area is one measure of bone size that is easily derived from dual-energy X-ray absorptiometry (DXA) scans. In a GWA study of DXA bone area of the hip and lumbar spine ($N \geq 28,954$), we find thirteen independent association signals at twelve loci that replicate in samples of European and East Asian descent ($N = 13,608 - 21,277$). Eight DXA area loci associate with osteoarthritis, including rs143384 in $GDF5$ and a missense variant in $COL11A1$ (rs3753841). The strongest DXA area association is with rs11614913[T] in the microRNA $MIR196A2$ gene that associates with lumbar spine area ($P = 2.3 \times 10^{-42}, \beta = -0.090$) and confers risk of hip fracture ($P = 1.0 \times 10^{-8}, \text{OR} = 1.11$). We demonstrate that the risk allele is less efficient in repressing miR-196a-5p target genes. We also show that the DXA area measure contributes to the risk of hip fracture independent of bone density.
Human height and other characteristics of the body’s size and shape have been extensively studied in large genome-wide association (GWA) studies, yielding many associated loci. Studies of parameters of body composition, such as lean mass, fat mass, and bone mineral density (BMD) have also yielded a number of associated loci. An important component of human height and body form is the size and shape of the bones. Not only is height directly related to bone size but the size and shape of bones may contribute to their strength and risk of fracture, and the tendency to develop osteoarthritis. DXA scans are primarily performed to evaluate BMD and fracture risk. The scans assess the area (cm²) and the bone mineral content (BMC, g) of the bone, to produce the BMD (g/cm²). BMD from the intertrochanteric area, three for the trochanter area, and one for the lumbar spine area, is estimated using DXA from quantitative ultrasound of the heel over 1000 independent associations in the large UK Biobank resource. The few GWA studies of bone area of the hip or lumbar spine using DXA have not yielded significant loci, possibly due to small sample sizes. Recently, a study of hip shape models (HSM) derived from statistical shape modeling of DXA scans found eight loci associated with hip shape, where eight for the intertrochanteric area, three for the trochanter area, and one for the lumbar spine area, intersecting with H3K4me1 or H3K27ac marked regions of enhancers, indicative of enhancers. The target genes for these enhancers include SOX9, which encodes hedgehog interacting protein that is involved in an important signaling pathway in osteoarthritis, and COL11A1, a fibril forming collagen of the extracellular matrix. Six loci are found in H3K4me3 regions of which five localize proximal to a transcription start site, indicative of active promoters.

In addition to the SOX9 and HHIP loci, we found association between DXA hip area measures and five of the eight recently reported hip shape loci under FDR of 0.05 (Supplementary Data 5).

**Results**

**Genome wide association study and replication.** To search for sequence variants that contribute to bone size we performed a GWA study of bone area using DXA of the hip (total hip, and the sub-regions femoral neck, trochanter, and intertrochanteric/shaft), and of the lumbar spine (L1–L4, N = 29,059) (Supplementary Fig. 1). We adjusted the bone area measurements for sex, age, height and body mass index (BMI) and tested for association between 33.4 million high quality sequence variants and the DXA bone areas (Methods).

Sixteen variants, all common, at 14 loci satisfied our criteria of genome-wide significance in the association analysis, and after conditional analysis across the loci (Methods); eight for the lumbar spine area, five for the total hip area, four for the intertrochanteric area, three for the trochanter area, and one for the femoral neck area (Table 1, Fig.1, Supplementary Fig. 2, Supplementary Data 1 and 2, Supplementary Table 1). Two of the hip area loci, at 17q24.3 near the SOX9 gene and at 4q31.21 near the HHIP gene, have been reported to associate with hip shape. The SOX9 association is the same signal as reported for hip shape, whereas rs12507427 near the HHIP gene represents an independent signal at that locus.

We followed up these sixteen variant associations in samples with up to 21,277 subjects of European and East Asian descent (Methods, Supplementary Table 2). All but three markers replicated significantly under a false discovery rate (FDR) of 0.05 (Table 1, Supplementary Data 3 and 4). The strongest associations are rs11614913 in the miRNA gene MIR196A2 with reduced lumbar spine area (P = 2.3 × 10⁻¹², β = −0.090), rs143384 in the 5’UTR of GDF5 with increase in both total hip (P = 2.2 × 10⁻²², β = 0.071) and trochanter area (P = 1.1 × 10⁻¹⁸, β = 0.071), rs12601029 at 17q24.3 near the SOX9 gene with increased intertrochanteric area (P = 6.2 × 10⁻¹⁸, β = 0.072), and rs12507427 near HHIP for increase in femoral neck area (P = 8.4 × 10⁻¹⁴, β = 0.054).

We do not confirm the previously reported suggestive association with DXA hip area at the PLCL1 gene (P ≥ 0.70), or lumbar spine area at the HHIGN3 gene (P ≥ 0.56), whereas our association of rs143384 in the 5’UTR of GDF5 with area of both lumbar spine and hip is the same as the previous suggestive association with lumbar spine area (P = 7.2 × 10⁻⁷).

In addition to the SOX9 and HHIP loci, we find association between DXA hip area measures and five of the eight recently reported hip shape loci under FDR of 0.05 (Supplementary Data 5).

**Functional annotation of variants and enrichment analysis.** We carried out functional annotation for the lead variants, along with highly correlated variants (r² > 0.8), using available data for active chromatin marks in trait-relevant cells (Supplementary Data 6); histone H3 mono-methylation and tri-methylation at lysine K4 (H3K4me1 and H3K4me3, respectively) and histone H3 acetylation at lysine K27 (H3K27ac). This analysis identifies ten DXA area loci intersecting with H3K4me1 or H3K27ac marked regions in primary osteoblasts, indicative of enhancers. The target genes for these enhancers include SOX9, at 17q24.3 which is the key-regulator of chondrocyte differentiation, HHIP at 4q31.21 which encodes hedgehog interacting protein that is involved in an important signaling pathway in osteoarthritis, and COL11A1, a fibril forming collagen of the extracellular matrix (Supplementary Data 6). Six loci are found in H3K4me3 regions of which five localize proximal to a transcription start site, indicative of active promoters (Supplementary Data 6).

Enrichment analysis for gene functions using DEPICT highlights genes with predicted functions in ribonucleoprotein complex binding, as well as those in LSm5 and LSm7 protein–protein interaction subnetwork, implicated in splicing and mRNA decay in P-bodies, for lumbar spine area and hip trochanter area association results, respectively (Supplementary Data 7). DEPICT identified significant tissue enrichment (FDR < 0.05) for two out of the five DXA area phenotypes, i.e., for hip total area and hip trochanter area association results, in both cases involving cartilage tissue (Supplementary Data 8). Of note, we find chondrocytes and osteoblasts among the list of top fifteen enriched cell-types ranked according to nominal P values in five and three of the five DXA phenotypes, respectively.

**Overexpression of MIR196A2 in HEK293T cells.** The strongest spine area associated SNP, rs1161491, is located in the miRNA MIR196A2 gene at the HOXC gene cluster of developmental transcription factors. The T allele of rs11614913 introduces a wobble bond to the primary miR-196a hairpin transcript (U to G pairing instead of C to G pairing) without affecting the seed sequences of either the 5p or 3p strands of the miR-196a duplex, and hence, the variant is not expected to influence recognition of target gene mRNAs. However, the variant might affect the efficiency of miR-196-5p target gene suppression either by affecting the thermodynamic stability or processing of the mature miR-196-5p duplex (Supplementary Note 1, Supplementary Fig. 3).

To test this we transfected HEK293T cells with MIR196A2 expression plasmids containing either the C or T alleles of...
rs11614913 and assessed, by RNA-sequencing, whether the two MIR196A2 alleles displayed differential effect on mRNA expression (Methods, see Supplementary Note 1 for detailed description of this experiment and results). We first defined a set of seventeen high confidence miR-196a-5p target genes based on conservation scores for predicted binding sites in 3’ UTRs of mRNA sequences using TargetScan v7.2 (Supplementary Fig. 4). Based on gene ontology analysis these direct target genes are highly overrepresented in anterior/posterior pattern specification (25-fold; \( P = 1.7 \times 10^{-5} \)), as well as in skeletal system development (13-fold; \( P = 2.5 \times 10^{-5} \)) (Supplementary Table 3). In the transfected HEK293T cells, 14 of the miR-196a-5p direct target genes are expressed (Supplementary Note 1). In response to overexpression of either alleles of MIR196A2, these 14 direct target genes are significantly downregulated compared to the rest of the MIR196A2 transfected HEK293 expression (\( P \) adjusted < 0.05) (Fig. 2, Supplementary Figs. 5 and 6, Supplementary Table 4). Contrasting the effects of the C and T alleles on suppression of the 14 target genes we observe a subtle difference, with the C allele suppressing more than the T allele (Fig. 2c, d). Permutation-based hypothesis test demonstrates that this suppression bias across the 14 target genes is statistically significant (\( P \) value = 0.042) (Supplementary Note 1), showing that the rs11614913[T] allele of MIR196A2 is less effective in repressing miR-196a-5p target genes. The subtle difference in the suppression of the target genes between the two alleles of MIR196A2 is to be expected given that the alleles are common with small effects on lumbar spine area and fracture risk. When analyzing gene enrichment for the entire set of repressed genes due to experimental induction of MIR196A2 (C-alleles or T-alleles) expression, which represent both primary and secondary targets of miR-196a-5p, we find embryonic skeletal system morphogenesis among the significant enrichment of 31 functional gene ontology terms, supporting the relevance of MIR196A2 for bone morphology (Supplementary Fig. 7).

One of the miR-196a-5p direct target genes is HOXC8, located in the same locus as rs11614913 in MIR196A2. The HOXC gene cluster of developmental transcription factors plays a critical role in limb development and skeletal patterning. In our adipose tissue samples from 746 individuals we find that the rs11614913[C] allele correlates with 4.5% reduction in expression of the HOXC8 gene (\( P = 5.9 \times 10^{-9} \)) (Supplementary Fig. 8, consistent with the effect of rs11614913 on the direct target genes in the MIR196A2 transfected HEK293T cells. We note that another highly correlated variant (\( r^2 = 0.90 \)), and potentially functional as it is in the promoter region of the HOXC8 gene, rs371683123, also correlates with reduced expression of the HOXC8 gene (Supplementary Fig. 8, Supplementary Table 5). It is possible that rs371683123 and other correlated variants also contribute to cis regulation of HOXC8 expression in addition to the effect of rs11614913 on MIR196A2 function.
Association with other bone related phenotypes. To determine whether the DXA bone area variants affect other bone phenotypes we examined association of the sixteen DXA area markers from our discovery analysis with osteoarthritis (Ncases = 25,458–27,321) and vertebral (Ncases = 3293) and hip fractures (Ncases = 7481) in European samples by either direct genotyping or in-silico look-up (Methods, Supplementary Table 2), and with height and BMD in public datasets (GIANT and GEFOS13, respectively).

Eight of the sixteen DXA bone area variants associate significantly with hip or knee osteoarthritis after correcting for FDR (0.05), four associate with hip fractures, six associate with BMD and twelve associate with height (Tables 2, 3). Two of the three variants that did not replicate significantly associate with height, at 15q25.2 and 12q14.1, and one, at 17q23.2, with hip osteoarthritis.

The osteoarthritis associating variants include the known knee osteoarthritis variant rs143384 in the GDF5 gene32,40 (P = 4.2 × 10−23, OR = 0.91), and the known missense variant in the COL11A1 gene32, rs3753841 (p.Pro1284Leu) that associates with hip osteoarthritis (P = 1.2 × 10−11, OR = 0.92). Both variants associate with increased area and increased height (Tables 1, 3). Furthermore, we show that rs3753841[G] in COL11A1 protects against hip dysplasia as shown by higher center-edge angle (β = 0.444, P = 3.4 × 10−4), a measure of joint structure and shape related to dysplasia41 (Supplementary Data 9).

The MIR196A2 variant, rs11614913[T], that associates with reduced lumbar spine area (P = 2.3 × 10−42, β = −0.090) also associates with increased risk of hip fractures (P = 1.0 × 10−8 and OR = 1.11) (Table 2, Fig. 3), while it does not associate with DXA bone area measures of the hip (P ≥ 0.010) (Supplementary Data 1). This is the first sequence variant reported to associate with hip fractures to our knowledge. Furthermore, it associates with reduced lumbar spine BMD in the public GEFOS dataset13 (Table 3), and confers modest risk of vertebral fractures in our study (P = 0.025, OR = 1.07) (Supplementary Table 6).
Twelve of the DXA bone area variants\(^1\) associate with height in the public GIANT dataset under a false discovery rate (FDR) of 0.05 (Table 3). This is despite initially correcting the DXA bone area measurements for height of the individual. Furthermore, of the 697 reported height variants\(^1\), additional 24 associate with DXA bone area measures after correcting for the number of markers tested (\(P_{\text{Bonferroni}} \leq 7.2 \times 10^{-5}\)) (Supplementary Table 7). Although this high proportion of the DXA bone area variants associate with height we find little overall correlation between the effects of reported height\(^1\) variants and their effect on DXA bone area measures in our data \(r^2 \leq 0.037\) (Supplementary Fig. 9). This is likely because some of the alleles that associate with decreased DXA bone area associate with decreased height, while other associate with increased height (Table 3, Fig. 4).

Mendelian randomization did not indicate a causal relationship between height and DXA bone area (Supplementary Table 8).

Six of the DXA bone area variants associate with BMD in the public GEFOS data under a false discovery rate (FDR) of 0.05 (Table 3), and additional eight reported BMD variants\(^13\) associate with DXA area measures after correcting for the number of markers tested (\(P_{\text{Bonferroni}} \leq 0.00071\)) (Supplementary Table 7). We also observe some inconsistency in direction of effect on area and BMD (Table 3, Fig. 4), however, the overall correlation between the effects of reported BMD variants\(^11,13,15\) and their effect on DXA bone area measures is positive for lumbar spine BMD \(r^2 = 0.21, P = 7.4 \times 10^{-5}\), whereas it is negative for hip BMD (femoral neck BMD) \(r^2 = 0.18, P = 0.00029\) (Supplementary Fig. 10). Mendelian randomization confirmed these
associations (Supplementary Table 8). The negative correlation with hip BMD may reflect the periosteal expansion observed with age\(^1\) that is not captured by age adjustments of the measures used. Likewise, the positive correlation between lumbar spine area and lumbar spine BMD may reflect vertebral degenerative changes that are not captured by these adjustments either. Differences in trabecular and cortical compartments of the bones that are not accounted for in the area or BMD measures may also explain these relationships\(^4\).
Genetic correlation between DXA area and related phenotypes.
We examined the genetic correlation between the bone related traits and DXA bone area by cross trait LD score regression across the Icelandic discovery set and the UK Biobank dataset (Table 4, Supplementary Table 9).

Consistent with the lack of correlation between the effect of variants on height and DXA bone area we find little genetic correlation between DXA bone area and height ($r_g = 0.064$ for lumbar spine, and $r_g = 0.14$ for hip). Likewise, we find consistent positive genetic correlation between DXA lumbar spine area and BMD ($r_g = 0.30$), and negative for hip BMD and hip area ($r_g = -0.15$).

For knee osteoarthritis there is no genetic correlation with any of the area measures ($r_g \leq 0.034$), whereas there is a weak positive correlation between hip area and hip osteoarthritis ($r_g = 0.11$–0.18) but none for lumbar spine area and hip osteoarthritis ($r_g = 0.043$). The eight DXA bone area variants that associate with knee or hip osteoarthritis are both lumbar spine (3) and hip area (6) variants.

Hip fractures are substantially correlated genetically with the femoral neck area ($r_g = 0.76$), and even slightly more so than with femoral neck BMD in our data ($r_g = -0.57$). Recently, a large meta-analysis of fractures showed high genetic correlation between fracture and BMD ($r_g = -0.59$), and that BMD has a major causal effect on fracture. Using multivariate logistic regression, we cannot differentiate between contribution through hip BMD, BMC, and the area measure on hip fractures, as each trait showed independent association with hip fractures while
adjusting for the other phenotypes (Table 5, Supplementary Table 10, Supplementary Note 2). This indicates that all three measures, area, BMD and BMC, influence the risk of hip fracture. Of note is that lumbar spine area is associated with hip fractures while adjusting for all the other traits ($P = 0.0056$), while lumbar spine BMD is not significant ($P = 0.15$).

### Discussion

We have found thirteen sequence variants that associate with simple measures of bone size; area measures from bone density DXA scans. The scans assess the area (cm$^2$) and the bone mineral content (g) of the bone to produce the BMD (g/cm$^2$), which is used in evaluation of osteoporosis and risk of fractures. We are here using a component of these commonly performed scans that has not been extensively utilized in GWA studies as the BMD has. Our study is the first to report GWS associations to this trait. By using this simple measure, we also find association with previously reported hip shape loci, derived from statistical modeling of the DXA images. Hence, the DXA bone area associations are likely to capture differences not only in the size of the bones, but also, at least partly, in their shape.

Importantly, we found strong association of two of these DXA bone area variants with osteoarthritis and one with hip fractures, the first significant hip fracture locus reported to our knowledge. These findings are in line with the idea that the size and shape of bones influence their strength, and previous speculations that they would influence the tendency to develop osteoarthritis. We also show that the DXA bone area measure, as well as the BMC measure of the DXA scans, are additional risk factors for hip fracture independent of BMD. The BMD has recently been shown to have a major causal effect on fractures. Hence, all three measures (BMD, BMC, and area) influence the risk of hip fracture. The high genetic correlation that we find between the femoral neck area and hip fractures, which occur mostly at the femoral neck, also reflects the influence of DXA bone area measure on hip fracture. Interestingly, the lumbar spine area also contributes to the risk of hip fractures, independent of BMD and area of the hip. The association of the MIR196A2 variant with lumbar spine area and hip fractures, but not hip area reflects this.

![Table 4 Estimated genetic correlation](image)

![Table 5 Contribution of DXA bone area, BMC and BMD to the risk of hip fractures](image)

Despite the little genetic correlation between the DXA bone area measures and height, most of these DXA area variants also associate with height, although with inconsistent direction of effects. We have recently demonstrated comparable relationship between osteoarthritis and height, with the osteoarthritis risk alleles associating either with increased or decreased height, resulting in little genetic correlation and little overall correlation between the effects of variants on these two traits, similar to what we report here for height and DXA bone area. These results underscore the complex nature of bone longitudinal and radial growth, mineralization, modeling and remodeling. The complex association pattern of the DXA area variants with height, BMD, osteoarthritis and fractures may reflect different aspects of bone biology, such as differences between longitudinal and radial growth, or between trabecular and cortical compartments of the bones, the mineralization process, modeling and remodeling, and the periosteal expansion observed with age.

Finally, we show through overexpression in HEK293T cell line that the variant in MIR196A2, that associates with both decreased lumbar spine area and increased risk of hip fracture, directly influences repression of miR-196a-5p target genes, with the fracture associating allele, rs11614913[T], being less effective than
the rs11614913[C] allele. Many of the mir-196a-5p target genes play roles in development of the skeletal system, such as HOXC8, located within the HOXC gene cluster proximal to MIR196A2, underscoring the biological relevance of mir-196a-5p to bone morphology. Together these data warrants further studies in bone relevant cells.

Methods

Iceland discovery population. The Icelandic samples with area measurements have previously been described in detail 28. The area measures are derived from densitometers at the Landspitali University Hospital’s DXA clinic and the Research Service Center’s Heilsarunnasókn DXXA clinic, both using Hologic DXA machines. Values at the hip (total hip, intertrochanteric, trochanteric and femoral neck) and at lumbar spine (L1-L4) were corrected for machine, and adjusted for age, height and body mass index and standardized in each gender separately to have a mean = 0 and standard deviation = 1.

Information on osteoarthritis (OA) were derived from a national Icelandic hip or knee arthroplasty registry and from Landspitali University Hospital electronic health records. Fracture assessment was through Landspitali University Hospital’s electronic health records from 1999 to 2016, excluding high trauma fractures according to the NOMESCO Classification of External Causes of Injuries or based on a detailed questionnaire. We only included patients who were 40 years or older at the time of joint replacements and vertebral fractures, and older than 50 years at the time of a hip fracture.

All participants who self-reported data gave informed consent and the National Bioethics Committee of Iceland approved the study (VSN-15-198) which was conducted in agreement with conditions issued by the Data Protection Authority of Iceland. Personal identities of the participant’s data and biological samples were encrypted by a third-party system (Identity Protection System), approved and monitored by the Data Protection Authority.

Replication populations. The Danish samples are postmenopausal women in the age range 55–86 years, taking part in the Prospective Epidemiological Risk Factor (PERF) study 29. Area measures are from DXA-measurement (Hologic QDR2000) at the hip (total hip, femoral neck and intertrochanteric) and lumbar spine. Osteoporotic fractures included self-reported low trauma fractures and vertebral fractures assessed by digital measurements of morphologic changes. Hip and knee joint replacements and OA information were derived from the Landspitali registries, 1996–2014. The study was approved by the Ethical Committee of Copenhagen County and was in accordance with the principles of the Helsinki.

The Swedish samples were from the Swedish Malmo Diet and Cancer (MDC) study of men and women living in the city of Malmo in Sweden who were born between 1923 and 1945 (men) or between 1923 and 1950 (women). The inclusion examination was performed during 1991–1996. The participants (n = 28,449) were followed until first OA surgery, emigration from Sweden, or death until December 31 2005. Information on knee and hip arthroplasty for OA and mortality were from the National Hospital Discharge Register and the Swedish Cause of Death Register. Cases were defined as those who were treated with knee or hip arthroplasty (421 and 551, respectively) during the follow-up time. Controls matched each arthroplasty case for age, gender and BMI. The MDC study was approved by the research ethical committee at Lund University the MDC study (LU 51–99). All participants signed a written informed consent.

The Australian samples were derived from the Dubbo Osteoporosis Epidemiology Study (DOES) 32, including subjects in the age range 60–99 years. All are of Caucasian ethnicity. Area was measured at the lumbar spine and the hip femoral neck by DXA (LUNAR DPX-I) and corrected for gender, age, height and weight. Osteoporotic fractures included low trauma fractures assessed by questionnaire and ascertained by reviewing all radiography reports. The St. Vincent’s Ethics Review Committee (Sydney) approved the study, and all subjects gave written informed consent.

The Dutch samples are from the Rotterdam study, an ongoing population-based prospective cohort comprising 14,926 Dutch individuals aged 45 years and older 44. After its initiation in 1990 (RSI, N = 7983) the cohort had two follow up waves in 2000 (RSII, N = 3011) and 2006 (RSIII, N = 3932). Follow-up examinations were scheduled every 3–5 years. The Rotterdam study was approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare, and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All biochemical and hematological measurements, as well as self-reported medical history and medication use. The United Kingdom Household Longitudinal Study has been approved by the University of Essex Ethics Committee and informed consent was obtained from every participant.

The UK Biobank study is a large prospective cohort study of ~500,000 individuals from across the United Kingdom, aged between 40–69 at recruitment 50. DXA area measures were available for the lumbar spine, for 5075 individuals. The measures were adjusted for age, height and body mass index in each sex separately and standardized to a mean = 0 and standard deviation = 1. Osteoarthritis at the hip and knee was defined as total hip or knee replacement after the age of 40 years and ICD10 codes indicating osteoarthritis. Femoral neck fractures comprised the hip fracture group and lumbar vertebral fracture the vertebral fractured group. We only included individuals of UK European descent. UK Biobank’s scientific protocol and operational procedures were reviewed and approved by the North West Research Ethics Committee (REC Reference Number: 06/MRE08/65), and informed consent was obtained from all participants. This research has been conducted using the UK Biobank Resource under Application Number 25998.

The Chinese Hong Kong samples are comprised of two samples of different sex, the Mr Os and Ms OS studies, aged 65 years and above 25. The DXA bone area measures were derived using Hologic QDR-4500W densitometer and corrected for gender, age, height, and weight. All participants provided informed consent. The Clinical Research Ethics Committee of the Chinese University of Hong Kong approved the study.

The Korean samples are postmenopausal women who visited the Osteoporosis Clinic of Ansan Medical Center (AMC, Seoul, Korea)25. Bone area was measured at the femoral neck and the lumbar spine (L1-L4) using Lunar-Expert XL, and Hologic QDR 4500-A absorptiometers. The DXA area measures were corrected for machine, gender, age, height and weight. All participants provided informed consent. The AMC Ethics Review Committee (Seoul) approved the study.

The Vietnamese samples are from the Vietnam Osteoporosis Study (VOS), a population study in Ho Chi Minh City to map genome and exposures factors for predicting chronic diseases, including osteoporosis and fracture 32. DXA bone area data was derived using Hologic QDR-4500W densitometer and corrected for gender, age, height and weight. The study’s procedure and protocol were approved by the research and ethics committee of the People’s Hospital 115 on August 6, 2015 (Approval number 297/BV/NCCH).

All participants in these studies provided written informed consent, and we obtained approval from all institutional review board to carry out the study.

DXA area measures. Measurement of the different parameters is automated within the DXA machine software. Each machine is calibrated every morning before operation using a phantom. The two DXA machines used in Iceland were fully calibrated by the manufacturer of the same machines. There are two main manufacturers of DXA machines, Hologic (https://www.hologic.com/hologic-products/breast-skeletal/horizon-dxa-system) and Lunar...
we believe most of those differences are accounted for in our analyses. Supplementary Table 1 summarizes the differences. The Hologic manufacturers refer to the intertrochanteric/shaft area as intertrochanteric, and the Lunar manufacturers as shaft. This includes also the lesser trochanter. The femoral head is not included in the output from the DXA machines.

Association analysis in the Icelandic samples. We sequenced the whole genomes of 15,220 Icelanders using Illumina technology to a mean depth of at least 10 × (median 32 ×)29,54. SNPs and indels were identified and their genotypes called using joint calling with the Variant Effect Predictor (version 84)35. We improved genotype calls by using information about haplotype type, taking advantage of the fact that all the sequenced individuals had also been chip-typed and long range phased. We then imputed the 33.4 million variants that passed high quality threshold into 151,677 Icelanders who had been genotyped with various Illumina SNP chips and their genotypes phased using long-range phasing. Using genealogic information, the sequence variants were imputed into un-typed relatives of the chip-typed to further increase the sample size for association analysis and increased the power to detect associations. All of the variants that were tested had imputation information over 0.8.

Quantitative traits were tested using a linear mixed model implemented in BOLT-LMM56. The area measures were corrected for age, height, and body mass index in each sex separately prior to regression analysis. The case-control analysis were corrected for age, sex, and country of birth.

We applied the method of LD score regression57 to account for inflation in test statistics due to cryptic relatedness and stratification. We regressed the χ2 statistics from our GWAS scan against LD score with a set of 1.1 M variants and used the intercept as a correction factor. We downloaded the LD scores from a LD score database containing data from all 66 International HapMap reference populations (GBR, CHB, JPT, YRI, ASJ, sex-assorted), HapMap, and CEPH populations, plus an additional 200,000 HapMap reference individuals (ftp://atguftp.mgh.harvard.edu/brendan/1k_eur_r2_hm3snps_se_weights.npz). The server uses SHAPEIT2 (v2.r790) to phase the data and assigns the individuals to 179 populations. We used the method of LD score regression on the dataset containing only those variants overlapping between the 3 datasets.

The UK Biobank samples were genotyped was performed using a custom-made Affymetrix chip, UK BiLEVE Axiom33, in the first 50,000 participants, and with Affymetrix UK Biobank Axiom array in the remaining participants34.95% of the sample was genotyped at both. We performed LD-based and GWAS association analysis by using a combination of 1000 Genomes phase 3, UK10K, and HRC reference panel reference panels in two steps; The HRC panel was used as first choice option, but for SNPs not in that reference panel the UK10K + 1000 Genomes panel was used35. We only used markers imputed based on the HRC panel due to problems in the UK10K + 1000 Genomes panel imputation for this study.

Meta-analyses. Results from the Icelandic discovery results and replication datasets were combined using an inverse-variance weighted meta-analysis, where different datasets allowed to have different populations frequency for alleles and genotypes but assumed to have a common effect (fixed effect). Heterogeneity in the effect estimates was tested using a likelihood ratio test by comparing the null hypothesis of the effect being the same in both populations to the alternative hypothesis of either population having a different effect.

Genetic correlations. We used the cross-trait LD score regression method44 and the summary statistics from the Icelandic and UK Biobank datasets to perform a genome-wide estimation of genetic correlation between pairs of traits. We used results for about 1.2 million variants, well imputed in both datasets, in this analysis, and for LD information, we used pre-computed LD scores for European populations (downloaded from https://data.broadinstitute.org/alkesgroup/LDSC/t3n/eu1kchr.breed.bim). We calculated the genetic correlation (METAL) between each of the 8 DXA bone trait summary statistics for each trait and the UK GWAS summary statistic for the other traits, and the vice versa, and then meta-analyzed those results to avoid bias due to overlapping samples. Area measures for the hip were not available for the UK Biobank dataset.

Mendelian randomization. Causal relationships between DXA bone area and height and BMD were estimated with a two sample Mendelian Randomization, using GIANT and GEOFOS effect estimates for height and BMD, respectively, as instrumental variables. The Mendelian randomization was performed with the MendelianRandomization package in R46.

Functional annotation of DXA bone area associated variants. For each lead variant we identified variants in linkage disequilibrium (LD) on the basis of in-house genotype data to the nearest 100,000 variants (r2 > 0.8) to define an LD class. We then annotated these variants by intersection with chromatin immunoprecipitation (ChIP) signal data and DNA hypersensitivity data for osteoblast primary cells. The ChIP-seq data was derived from the ENCODE project56 and downloaded from the UCSC table browser (hg19)57. We carried out ChIP-seq analysis for osteoblast cells, based on H3K27ac data derived from the Encode project (GSM373379), as described by Hnisz et al.58.

To identify enhancer targets genes, we used the Joint effect of multiple enhancers (JEME) resource. Additionally, we used Genhancer elite predictions which is based on multiple data types (eQTLs, C-Hic, TF co-expression and eRNA co-expression, and distance) where the presence of at least two different types of data point to enhancer-gene targets. We perform all predictions in the elite version other than those where distance underlies the prediction, i.e., where distance is one of only two evidence levels. DEPICT analysis59 was carried out using association variants with nominal P-value < 1e−5, separately for each of the five phenotypes.

MIR196 experiments. The full length mir196A2 cDNA (RefSeq: M10000279) in pCMV-MIR vector (product ID: pCMVMIR). Transformed TOP10 cells. The ChIP-seq data was derived from the ENCODE project67 and downloaded from the UCSC table browser (hg19)57. We carried out ChIP-seq analysis for osteoblast cells, based on H3K27ac data derived from the Encode project (GSM373379), as described by Hnisz et al.58.
transcript expression was then quantified in each sample using the following primers for 5′-CAAGAAGACTGCTGAGTACATC-3′ and 3′-TGCGGAGTTCAAAACC-3′. Sanger sequencing confirmed the sequence of Mir196A2-Mut.

Transfection experiments were performed in six replicates for each condition (n = 18). One day prior to transfection, 50,000 HEK293T cells (ATCC® CRL-3216) were seeded into each well of a 6-well plate in 2 ml of Dulbecco’s Modified Eagle Medium (ThermoFisher 31966021) supplemented with 10% fetal calf serum (ThermoFisher 10082417) and 50 units/ml penicillin and 50 µg/ml streptomycin (ThermoFisher 15070063). On the day of transfection, medium was replaced with 3 ml of the identical media as before without antibiotics. For each transfected well, 3.3 µg of Mir_Empty, Mir196A2_WT or Mir196A_Mut plasmids were diluted in 155 µl Opti-Mem (ThermoFisher 31980047) and 9.9 µl Fugene HD (Promega E2312). Incubated at room temperature for 10 min before 125 µl of transfection mix was added to the appropriate well.

Forty-eight hours after transfection, cells were harvested for cell sorting. Cells were released from the plate surface with TrypLE Express (ThermoFisher 12605010) and then washed with 2% FBS (ThermoFisher 10500064) in PBS. TrypLE Express solution was added to release the cells from the plate surface, and the cells were then pelleted by centrifugation. Live cells stained with DAPI were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to exclude by gating at FSC Height/FSC Area. Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11).

First gated by size with FSC Area/SSC Area and doublets were excluded by gating at FSC Height/FSC Area. Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11). Unstained cells were then used to define the live/dead gate (Supplementary Fig. 11).

For the arcOGEN Study, see http://www.arcogen.org.uk/; for the UK Biobank, see http://www.ukbiobank.ac.uk/; for the United Kingdom Household Longitudinal Study, see https://www.understandingsoociety.ac.uk; for HRC pre-imitation checking tool, see http://www.well.ox.ac.uk/~wrayer/tools/; for re-computed LD scores for European populations, see https://data.broadinstitute.org/alkesgroup/LDSCORE/eur_w_lid_chr.tar.gz; for HapMap data, see https://www.hapmap.org/hapmap-products/breast-skeletal/horizon-dx-system, and for LUNA DX machines, see https://www.gehealthcare.com/en/products/bone-health-and-metabolic-health.

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Data availability

The Icelandic population WGS data has been deposited at the European Variant Archive under accession code PRJEB15197, and the RNA sequencing results from the Mir196A2 experiments to Gene Expression Omnibus, accession code GSE128641. The GWAS summary statistics are available at https://www.decode.com/summarydata. The authors declare that the data supporting the findings of this study are available within the article, its Supplementary Data files and upon request. A reporting summary for this Article is available as a Supplementary Information file. The source data underlying Figs. 1–4 in the main text, and for Supplementary Figs. 2, 4, 5, 6, 8, 9, 10, and Table 5, are provided as Source Data files.

Code availability

All custom codes used in this study are freely available online, in-house codes not applicable.

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