Evaluation of shared genetic aetiology between osteoarthritis and bone mineral density identifies SMAD3 as a novel osteoarthritis risk locus

Sophie Hackinger1, Katerina Trajanoska2, Unnur Styrkarsdottir3, Eleni Zengini4,5, Julia Steinberg1, Graham R.S. Ritchie1, Konstantinos Hatzikotoulas1, Arthur Gilly1, Evangelos Evangelou6,7, John P. Kemp8,9, arcOGEN Consortium, GEFOS Consortium, David Evans8,9, Thorvaldur Ingvarsson10,11,12, Helgi Jonsson11,13, Unnur Thorsteinsdottir3,11, Kari Stefansson3,11, Andrew W. McCaskie14, Roger A. Brooks14, Jeremy M. Wilkinson4, Fernando Rivadeneira2 and Eleftheria Zeggini1,*

1Human Genetics, Wellcome Trust Sanger Institute, Hinxton CB10 1HH, UK, 2Departments of Internal Medicine and Epidemiology, Erasmus University Medical Center, Rotterdam 3000 CA, The Netherlands, 3deCODE Genetics, Sturlugata 8, IS-101 Reykjavik, Iceland, 4Department of Oncology and Metabolism, University of Sheffield, Sheffield S10 2RX, UK, 55th Department, Dromokaiteio Psychiatric Hospital, Athens 124 61, Greece, 6Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina 45110, Greece, 7Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK, 8MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK, 9University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, QLD, Australia, 10Department of Orthopedic Surgery, Akureyri Hospital, 600 Akureyri, Iceland, 11Faculty of Medicine, University of Iceland, 101 Reykjavik, Iceland, 12Institution of Health Science, University of Akureyri, 600 Akureyri, Iceland, 13Department of Medicine, Landspitali, The National University Hospital of Iceland, 101 Reykjavik, Iceland and 14Division of Trauma & Orthopaedic Surgery, University of Cambridge, Box 180, Addenbrooke’s Hospital, Cambridge CB2 0QQ, UK

*To whom correspondence should be addressed at: Analytical Genomics of Complex Traits, Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1HH, UK. Tel: +44 1223496868; Fax: +44 1223496826; Email: eleftheria@sanger.ac.uk

Abstract

Osteoarthritis (OA) is a common complex disease with high public health burden and no curative therapy. High bone mineral density (BMD) is associated with an increased risk of developing OA, suggesting a shared underlying biology. Here, we performed the first systematic overlap analysis of OA and BMD on a genome wide scale. We used summary statistics from the GEFOS consortium for lumbar spine (n = 31,800) and femoral neck (n = 32,961) BMD, and from the arcOGEN consortium for...
three OA phenotypes (hip, n_cases=3,498; knee, n_cases=3,266; hip and/or knee, n_cases=7,410; n_controls=11,009). Performing LD score regression we found a significant genetic correlation between the combined OA phenotype (hip and/or knee) and lumbar spine BMD (r_g=0.18, P = 2.23 × 10^{-5}), which may be driven by the presence of spinal osteophytes. We identified 143 variants with evidence for cross-phenotype association which we took forward for replication in independent large-scale OA datasets, and subsequent meta-analysis with arcOGEN for a total sample size of up to 23,425 cases and 236,814 controls. We found robustly replicating evidence for association with OA at rs12901071 (OR 1.08 95% CI 1.05–1.11, P_meta=3.12 × 10^{-15}), an intronic variant in the SMAD3 gene, which is known to play a role in bone remodeling and cartilage maintenance. We were able to confirm expression of SMAD3 in intact and degraded cartilage of the knee and hip. Our findings provide the first systematic evaluation of pleiotropy between OA and BMD, highlight genes with biological relevance to both traits, and establish a robust new OA genetic risk locus at SMAD3.

Introduction

Osteoarthritis (OA) is a degenerative disease of the joints affecting over 40% of people over 70 years (1). Hallmarks of OA include cartilage degradation, joint-space narrowing, formation of osteophytes within the joint and subchondral bone remodeling (2,3). Due to a lack of therapeutic options, the main treatment strategy consists of pain management and, in severe cases, joint replacement surgery (2). OA is a complex disorder with the 18 currently known risk loci accounting for approximately 11% of disease heritability (4). The biggest OA genome-wide association study (GWAS) published to date was conducted by the arcOGEN consortium in a two-stage design, culminating in a total discovery sample of 7,410 cases and 11,009 controls (5,6). Cases in the arcOGEN study had radiographic hip or knee OA (defined as a Kellgren Lawrence score ≥ 2), and approximately 80% had undergone total joint replacement surgery, indicating disease progression to a severe degree (6).

In addition to establishing bigger OA sample collections, studying OA in the context of related phenotypes may add to the currently short list of established loci. While most GWAS to date have investigated a single phenotype, it is estimated that around 5% of risk variants and 16% of genes identified through these studies have pleiotropic effects on multiple phenotypes (7). In recent years, the accumulation of large-scale genomic datasets has made it possible to study genetic pleiotropy by incorporating data from multiple epidemiologically linked traits. Leveraging available summary data of correlated traits is expected to uncover common genetic risk factors that can help pinpoint causal pathways and refine our understanding of disease mechanisms. OA in itself is a heterogeneous disorder, with heritability varying depending on the affected joint. Of the 18 published risk loci to date, 6 and 7 are associated with knee OA only and hip OA only, respectively, while 5 are associated with both hip and knee OA (4). This further highlights how phenotypic variation is reflected by genetics, and demonstrates the need for strict phenotype definitions.

The link between bone mineral density (BMD) and OA was first reported in 1972 by Foss and Byers, who observed higher BMD in femoral heads excised during OA-related hip replacement surgery (8). Since then, a number of cross-sectional and longitudinal studies have found higher femoral neck (FN) and lumbar spine (LS) BMD, as well as total body BMD to be associated with incident OA at the hip, knee, and other joint sites (9–12).

Findings with regards to the relationship between BMD and OA progression are less clear (13). Elevated bone turnover – usually a marker for decreased BMD – was reported in patients with progressive knee OA compared to patients with stable OA (14). Decreased baseline femoral neck BMD (FNBM) has also been associated with knee OA progression (15,16). Conversely, data from the Rotterdam study showed a non-significant trend of higher odds of knee OA progression with increased lumbar spine BMD (LSBMD) (17), while another study found no link between knee OA progression and total body- or FNBM (9).

The largest genetic studies on BMD to date have been carried out by the Genetic Factors for Osteoporosis (GEFOS) consortium: a large-scale GWAS in 2012 found 56 loci associated with BMD (18); more recently, a rare variant of large effect was identified combining whole-genome sequencing and GWAS imputation (19). Several biological mechanisms are implicated in both OA and BMD, such as bone remodeling, mesenchymal stem cell differentiation and inflammation (2,6,18,20). RUNX2, a key transcription factor regulating endochondral ossification and osteoblast differentiation (21,22), has been associated with both OA and BMD based on its proximity to genome-wide significant variants (6,18). The other locus with known GWAS hits for both traits is KLHL42 (or KLHDC5), although its biological relevance remains unclear (6,18).

In addition, Yerges-Armstrong and colleagues have previously shown nominal association of four BMD-linked single nucleotide polymorphisms (SNPs) with knee OA (23). However, despite the long-established epidemiologic link and shared biology, the genetic overlap of OA and BMD has not yet been assessed on a genome-wide level. Here, we present results from the first genome-wide analysis establishing shared genetic etiology between OA and BMD.

Results

Known OA risk loci in BMD

Six loci associated with OA were nominally significant for FN- or LSBMD, including the SUPP3/CDCSL locus, which is close to RUNX2, and KLHL42/PTHLH (Supplementary Material, Table S1).

Genome-wide correlation

We used linkage disequilibrium (LD) score regression to get an estimate of the genome-wide genetic correlation between OA and BMD. There was a significant correlation between combined OA and LSBMD (correlation = 0.18, P = 0.022), but not hip or knee OA and LSBMD or between FNBM and any OA phenotype (Fig. 1).

Extent of shared association signals

We found evidence for significant overlap of association signals at different P-value thresholds (P) between all three OA categories and LSBMD (permutation adjusted P-value (P_perm)<0.05) (Table 1).
Figure 1. Genetic correlation between osteoarthris (OA) and bone mineral density (BMD) as estimated by LD score regression. Rectangles show the correlation estimates (middle horizontal line) and standard errors (upper and lower bounds) of each comparison. Rectangles are colored according to the strength of correlation. Significant correlation estimates are marked by an asterisk. LSBMD = lumbar spine BMD; FNBMD = femoral neck BMD.

Table 1. SNP-based overlap analysis of OA and BMD. For each comparison, the total number of SNPs present in both datasets after LD-pruning is given, as well as the number of SNPs falling below each P-value threshold (P). $P_{\text{perm}}$ = empirical overlap p-value obtained through permutation analysis.

|                   | Combined OA FNBMD | Hip OA FNBMD | Knee OA LSBMD FNBMD |
|-------------------|-------------------|--------------|---------------------|
| **Total number of SNPs** | 75,015 | 75,161 | 74,999 | 75,147 | 75,125 | 75,270 |
| $P_t$             | SNPs | $P_{\text{perm}}$ | SNPs | $P_{\text{perm}}$ | SNPs | $P_{\text{perm}}$ | SNPs | $P_{\text{perm}}$ | SNPs | $P_{\text{perm}}$ | SNPs | $P_{\text{perm}}$ |
| 0.5               | 28,940 | 8.53 x 10^{-2} | 27,599 | 2.74 x 10^{-1} | 28,563 | 1.64 x 10^{-1} | 27,065 | 7.33 x 10^{-1} | 28,910 | 7.95 x 10^{-1} | 27,534 | 5.84 x 10^{-2} |
| 0.1               | 26,33 | 7.92 x 10^{-2} | 2,119 | 5.41 x 10^{-1} | 2,513 | 1.46 x 10^{-1} | 2,057 | 1.74 x 10^{-1} | 2,620 | 3.67 x 10^{-2} | 2,131 | 2.33 x 10^{-3} |
| 0.05              | 931 | 1.25 x 10^{-1} | 698 | 3.35 x 10^{-1} | 868 | 1.42 x 10^{-2} | 647 | 2.47 x 10^{-1} | 939 | 8.98 x 10^{-4} | 700 | 3.10 x 10^{-3} |
| 0.04              | 662 | 2.41 x 10^{-3} | 486 | 2.33 x 10^{-1} | 641 | 1.60 x 10^{-4} | 434 | 4.99 x 10^{-1} | 680 | 4.74 x 10^{-4} | 474 | 1.27 x 10^{-3} |
| 0.03              | 451 | 1.20 x 10^{-3} | 314 | 6.57 x 10^{-2} | 426 | 1.02 x 10^{-3} | 272 | 4.20 x 10^{-1} | 437 | 2.87 x 10^{-3} | 291 | 3.27 x 10^{-3} |
| 0.02              | 251 | 1.95 x 10^{-3} | 160 | 1.07 x 10^{-1} | 239 | 6.10 x 10^{-5} | 150 | 6.31 x 10^{-2} | 244 | 1.59 x 10^{-3} | 152 | 3.03 x 10^{-1} |
| 0.01              | 87 | 2.79 x 10^{-2} | 60 | 5.84 x 10^{-2} | 87 | 2.97 x 10^{-4} | 52 | 4.94 x 10^{-1} | 88 | 3.01 x 10^{-3} | 53 | 2.18 x 10^{-1} |
| 0.005             | 37 | 6.89 x 10^{-3} | 24 | 2.43 x 10^{-2} | 38 | 3.04 x 10^{-4} | 26 | 6.39 x 10^{-4} | 29 | 1.97 x 10^{-1} | 16 | 6.94 x 10^{-1} |
| 0.001             | 10 | 3.90 x 10^{-5} | 3 | 4.52 x 10^{-2} | 7 | 1.68 x 10^{-3} | 1 | 1.00 |
| $5 \times 10^{-4}$ | 4 | 6.81 x 10^{-1} | 0 | 1.00 | 6 | 5.70 x 10^{-5} | 1 | 5.64 x 10^{-2} | 4 | 4.21 x 10^{-3} | 2 | 7.12 x 10^{-2} |

Analysis of the combined OA and LSBMD data resulted in significant overlap P-values at $P_t=0.001$ and 0.005, as well as at less stringent $P_t$. Four SNPs overlap at $P_t=5\times10^{-4}$ ($P_{\text{perm}}=5.7\times10^{-5}$). The highest overlap was observed between hip OA and LSBMD, with six SNPs overlapping at $P_t=5\times10^{-4}$ ($P_{\text{perm}}=5.7\times10^{-5}$). Two of these SNPs are genome-wide significantly associated with BMD in GEFOSS (rs1524928, $P=5.29\times10^{-9}$ and rs716255, $P=2.07\times10^{-4}$). A significant overlap was also observed for $P_t$ of 0.001, 0.005 and 0.01 in the hip OA-LSBMD comparison.

Compared to the hip OA and LSBMD analysis, overlap P-values for the knee OA and LSBMD comparison were at least one order of magnitude smaller. Four SNPs (rs7104420, rs9466056, rs881803 and rs4536164) overlapped at $P_t=5\times10^{-4}$ in this analysis ($P_{\text{perm}}=4.21\times10^{-3}$). Two of these, rs4536164 and rs9466056, fall within known BMD risk loci (18).

Overlap signal was much weaker for the OA and FNBMD comparisons, with only five $P_t$ reaching statistical significance ($P_t=0.001$ for knee OA, $P_t=0.005$ and 0.01 for hip OA, and $P_t=0.001$ and $P_t=0.005$ for combined OA). The SNP overlapping at $P_t=5\times10^{-4}$ for hip OA and FNBMD (rs1524928) was also among the six SNPs identified in the hip OA and LSBMD analysis. Two SNPs overlapped at this $P_t$ for knee OA, one being rs9466056 and the other rs1283614, which maps to an intron of the BMD locus MEF2C (24).

**Evidence for colocalising regions**

We employed a regional Bayesian colocalisation test that measures the posterior probabilities (PP) for each of four alternative hypotheses compared to one global null hypothesis (i.e. no associations in that region). We identified four independent genomic regions with a high posterior probability (PP) of harbouring one causal variant common to both traits analysed (PP for hypothesis 3 $>0.9$) (Table 2). The region containing the RPS6KAS gene was identified by three comparisons (combined OA and LSBMD, hip OA and LSBMD, and hip OA and FNBMD). The most strongly associated SNPs in this region have genome-wide significant associations with increased BMD at both the LS and FN (rs1286147 and rs1286063, $P<5\times10^{-8}$) and nominally significant associations with increased risk of combined and hip OA (rs1286077, $P<0.05$); the three SNPs are in perfect LD ($r^2=1.00$ for each pairwise combination).

Two further regions were identified in the hip OA and LSBMD analysis; one of these spans a known BMD locus close to the KCNMA1 gene, while the other does not contain genome-wide significant variants for either OA or BMD.
The region identified in the knee OA and FNBMD analysis contains one lead SNP for both traits, rs9466056, which is a known variant associated with high FN and LSBMD mapping to an intergenic region between CDKAL1 and SOX4.

We also identified three regions (Table 2) with a high PP of harbouring two distinct causal variants (PP for hypothesis $4 \geq 0.9$). All three of these contain a known BMD locus, with the top SNPs for LSBMD mapping to introns of IDUA, CDC170 and PDXDC1. The top SNPs for knee and hip OA are nominally associated ($P < 0.05$) with these respective phenotypes in arcOGEN.

**Gene and pathway analysis**

Of the individual genes significantly associated ($Q < 0.05$) with at least one OA or BMD phenotype, SUPH3, COL11A1, and APCDD1 overlapped between OA and BMD (Supplementary Material, Table S2). All three include variants that were identified in the SNP-wise overlap analysis and taken forward for replication.

There were no pathways significantly associated with any OA phenotype in any of the analyses. One of the CP pathways was associated with FNBMD ("basal cell carcinoma", $Q = 0.02$) when allowing a 20 kilobase (kb) window around genes. Using GO annotations, a total of 33 unique pathways were associated with either phenotype using strict or lenient gene definitions (Supplementary Material, Tables S3–S5), including several with direct biological relevance, such as "regulation of ossification" or "osteoblast development".

**Cross-phenotype meta-analysis**

To search for potential novel associations not identified by single-trait GWAS, we performed a cross-phenotype meta-analysis between each pairwise combination of OA and BMD datasets. Using the CPASSOC method (25), we computed two statistics, $S_{num}$ and $S_{nat}$, which assume homogeneous and heterogeneous effects across studies, respectively. The quantile–quantile and Manhattan plots of these analyses are shown in Supplementary Material, Figures S1–S4. We identified 13 independent associations not previously reported for BMD or OA, which we followed up in the UK Biobank combined OA dataset (Supplementary Material, Table S10). One SNP, rs11164649, was nominally significant ($P < 0.05$). This SNP lies in an intron of the COL11A1 gene and is in strong LD ($r^2 = 0.92$) with a variant (rs1903787) identified in the SNP-wise overlap analysis which was taken forward for replication.

**Replication and meta-analysis for OA**

We meta-analysed 143 SNPs identified in the colocalisation and/or $P$-value based overlap analysis in arcOGEN, UK Biobank and deCODE (Supplementary Material, Table S6). None of the SNPs taken forward are genome-wide significantly associated with arcOGEN. We found a significant excess of independent SNPs with the same direction of effect in all three meta-analysis cohorts among variants with $P_{meta} < 0.05$ (binomial sign test $P = 7.75 \times 10^{-14}$), as well as all independent SNPs included in the meta-analysis (binomial sign test $P = 0.03$).

Variants within several genes linked to bone, cartilage and extracellular matrix biology, including APCDD1, SUPH3, COL11A1, NOTCH4, SEMA3A, LGR4, PTC1 and RS6KAS, were associated at $P_{meta} < 0.05$ (Supplementary Material, Table S6).

Two variants reached genome-wide significance in the meta-analysis across arcOGEN, deCODE and UK Biobank: rs12901071 (OR 1.08 95% CI 1.05–1.11, $P_{meta} = 3.12 \times 10^{-14}$) and rs10518707 (OR 1.07, 96% CI 1.03–1.09, $P_{meta} = 2.15 \times 10^{-8}$). Both are intronic variants in the SMAD3 gene ($r^2 = 0.645$) and were identified in the SNP-wise overlap analysis of combined OA vs. LSBMD and hip OA vs. LSBMD, respectively.

Both new genome-wide significant SNPs for OA were imputed in the arcOGEN data (imputation info score > 0.95) and are nominally associated with combined OA (Supplementary Material, Table S6; Fig. S2). They are also nominally associated with increased LSBMD in GEFOS (rs12901071, $P = 1.58 \times 10^{-3}$ and rs10518707, $P = 3.47 \times 10^{-3}$) (Fig. 2), but not FNBMD (rs12901071, $P = 3.72 \times 10^{-1}$ and rs10518707, $P = 2.46 \times 10^{-1}$). SMAD3 is associated ($P < 0.05$) with LS and FNBMD, hip and combined OA in the gene analysis (Supplementary Material, Table S7), although this association only holds for LSBMD after using false discovery rate (FDR) correction ($Q = 6.92 \times 10^{-5}$).

In the colocalisation analysis, the region in which both top SNPs reside (chr15:67,095,629-69,017,421) has a PP of containing a single pleiotropic variant associated with hip OA and LSBMD (hypothesis 3) of 0.88.

**Functional follow-up of SMAD3**

Using RNA sequencing data, we confirmed the expression of SMAD3 in low-grade degenerate articular cartilage of 12 knee and 9 hip OA patients undergoing total joint replacement (26) (Supplementary Material, Fig. S5). SMAD3 is among the 30% most expressed genes in the knee articular cartilage samples, and among the 15% most expressed genes in the hip articular cartilage samples.

### Table 2. Regions with strong evidence of pleiotropy

For each region the number of SNPs, start and stop position in basepairs (bp) and most strongly associated SNPs for OA and BMD are given. Chromosome coordinates are in hg19. Hypothesis 3 = one causal variant; hypothesis 4 = two distinct causal variants; PP = posterior probability.

| Analysis                  | SNPs | Chr   | Start (bp) | Stop (bp) | Top SNP BMD | Top SNP OA | PP   |
|---------------------------|------|-------|------------|-----------|-------------|-----------|------|
| Hypothesis 3 combinedOA and LSBMD | 817  | chr14 | 91297823   | 93129850  | rs1286147   | rs1286077  | 0.95 |
| hipOA and FNBMD           | 817  | chr14 | 91297823   | 93129850  | rs1286147   | rs1286077  | 0.98 |
| hipOA and LSBMD           | 817  | chr14 | 91297823   | 93129850  | rs1286147   | rs1286077  | 0.99 |
| hipOA and LSBMD           | 1242 | chr10 | 78708452   | 80875213  | rs7071206   | rs716255   | 0.92 |
| hipOA and LSBMD           | 531  | chr1  | 44974119   | 46897698  | rs7551423   | rs7546984  | 0.91 |
| kneeOA and FNBMD          | 1235 | chr6  | 19208477   | 21677746  | rs9466056   | rs9466056  | 0.99 |
| kneeOA and FNBMD          | 268  | chr4  | 6968648    | 1415698   | rs3755995   | rs3755920  | 0.97 |
| kneeOA and LSBMD          | 382  | chr16 | 14464538   | 16152940  | rs4958155   | rs9955327  | 0.95 |
| kneeOA and LSBMD          | 1070 | chr6  | 150255029   | 151910904 | rs4869742   | rs9384514  | 0.90 |
and the production of bone matrix (31,32). It also represses the activity and BMI more strongly associated with the latter (45).

Recent efforts in the development of statistical methods to de- monstrate linkage between OA and LSBMD measurements obtained via dual X-ray absorptiometry (42). While this may have contributed to the observed association of FN- and LSBMD with OA remain unclear. One possible explanation could be the correlation between LSBMD and OA, characterised by spinal osteophytes, which could lead to increased LSBMD measurements. However, in one study, the presence of osteophytes at the lumbar spine did not change the strength of association between OA and LSBMD (10). Damage to the spine accumulates over time and can lead to changes such as breakdown of the invertebral discs, scoliosis and osteochondrosis. This process is also referred to as degenerative disc disease (DDD). Although the association between DDD and LSBMD remains inconclusive (38-41), it is known that the presence of degenerative features can increase LSBMD measurements obtained via dual X-ray absorptiometry (42). While this may have contributed to the observed association between LSBMD and OA, we found genetic correlations of a similar magnitude between OA and skull, as well as total body BMD measurements in a paediatric cohort (43) (Supplementary Material, Table S11; Supplementary Methods). As DDD and related features such as osteophytes are unlikely to be present in young individuals, these results suggest that the correlation between OA and LSBMD is not purely artefactual.

Our analyses showed a greater degree of overlap between hip OA and both BMD measurements than between knee OA and BMD. Hip OA is estimated to have a higher heritability than knee OA (44), with environmental risk factors such as physical activity and BMI more strongly associated with the latter (45).

The analyses outlined here present the first comprehensive evaluation of genetic overlap between BMD and radiographic OA. Our results lend further support to the hypothesis of common genetic factors underlying these two traits, and establish SMAD3 as a genome-wide significant risk locus for OA with a potential pleiotropic effect on BMD.

Our work exemplifies the potential to uncover new disease risk loci by combining data of epidemiologically linked traits. Recent efforts in the development of statistical methods to detect pleiotropy, as well as the establishment of cross-disorder working groups (46) and of biobank collections (47), have made it possible to systematically assess pleiotropy in human disease.
Materials and Methods

Datasets

All analyses outlined here were conducted using summary association statistics from the arcOGEN (6) and GEFOs consortia (18). The arcOGEN data comprised three OA phenotypes: knee OA, hip OA, and knee and/or hip OA (combined OA). A detailed description of the contributing studies and phenotype definitions can be found in (18). Briefly, OA case status was determined radiographically as a Kellgren-Lawrence grade score ≥ 2. Most cases included in arcOGEN had progressed to a severe disease endpoint, as evident from the fact that 80% had undergone total joint replacement surgery.

For the P-value-based overlap analysis as well as the gene and pathway analysis, we excluded samples from London-based cohorts (TwinsUK and Chingford Study) from the arcOGEN datasets to avoid overlap with GEFOs. After exclusion of 714 samples, we carried out genome-wide association analyses on the arcOGEN dataset for each of the three phenotype groups using the “–method score” option in SNPTEST v2.5 (50). The full arcOGEN dataset was used for all other analyses described.

The BMD data consisted of meta-analysis summary statistics for FN and LSBMD (18). For replication, we used summary statistics from two OA GWAS: the UK Biobank (47) and the deCODE study. Details on case definitions in UK Biobank and deCODE can be found in the Supplementary Methods.

Sample sizes for each dataset are outlined in Supplementary Material, Table S8.

Genome-wide correlation

We performed LD score regression analysis (51) on each pairwise combination between the BMD and OA datasets, using pre-computed LD scores based on the European sample of the 1000 Genomes Project (52). We accounted for sample overlap between each pair of datasets through tetrachoric correlation of the Z-scores of SNPs present in both studies.

Assessment of shared association signals

For each pairwise combination between the two BMD and three OA phenotypes we assessed the extent of shared signals at different p-value cutoffs, following the approach described by Elliott and colleagues (53). Briefly, we filtered both datasets to a common set of SNPs on which p-value-informed LD pruning was performed. To this end, SNPs were sorted based on their association with OA, and, starting with the lowest p-value, any SNP in LD with the index SNP (r² > 0.05) was removed. The next SNP was then considered, and so on.

The extent of shared association signals between OA and BMD was assessed by counting the number of overlapping variants above and below ten different p-value thresholds (P: 0.5, 0.1, 0.05, 0.04, 0.03, 0.02, 0.01, 0.005, 0.001, 5 × 10⁻⁵). To test for significance of overlap, a chi-squared test on the resulting 2×2 contingency tables was performed at each P

Empirical overlap p-values were obtained by repeating the chi-squared test after randomly permuting the GEFOs p-values. This was done 1,000,000 times to obtain a null distribution of overlap p-values against which the original overlap p-value could be compared.

Colocalisation analysis

We employed a Bayesian colocalisation method to search for genomic regions harbouring cross-phenotype associations between OA and BMD (48). Briefly, the algorithm uses Z-scores and standard errors from two association studies to generate posterior probabilities for each of five hypotheses:

H₀: the region contains no variants associated with trait 1 or trait 2
H₁: the region contains one variant associated with trait 1
H₂: the region contains one variant associated with trait 2
H₃: the region contains one variant associated with both trait 1 and trait 2
H₄: the region contains one variant associated with trait 1 and a second variant associated with trait 2

Genomic regions were defined according to approximately independent LD-blocks (54). Analogous to LD score regression, we used the tetrachoric correlation of the Z-scores of SNPs present in datasets as an estimate of sample overlap.

Gene and pathway analysis

Gene- and pathway analyses were performed on each OA and BMD dataset using MAGMA (55). First, SNPs are assigned to genes, which are tested for their association with the phenotype. Results from this step are then combined into pathway-based association statistics.

For the gene analysis, we grouped variants into genes using SNP locations from dbSNP version 135 and NCBI 37.3 gene definitions. We performed this step twice, once annotating SNPs to a gene only if they fell within the gene’s transcription start and stop site, and once including SNPs that fell within a 20 kilobase window of the gene.

We ran two separate pathway analyses, one using the Molecular Signatures Database canonical pathways collection (56), comprising 1,329 manually curated gene-sets from nine databases (Supplementary Material, Table S9), and one using 6,166 gene-sets from the Gene Ontology pathway database (57). Significance was defined using a 5% FDR equivalent to a Q-value of 0.05 for both the gene and pathway analyses (58).

Cross-phenotype meta-analysis

We used a multi-trait extension to meta-analysis to search for novel associations in each pairwise combination of arcOGEN and
Replication and meta-analysis for OA

We took forward a total of 143 SNPs for in-silico replication. This set comprises the two most strongly associated variants (one for each trait) in each region from the Bayesian colocalisation test, as well as all variants overlapping at P<0.005 in the SNP-based overlap analyses. We used the METAL (60) software package to perform inverse variance weighted meta-analysis of these SNPs in relation to hip and/or knee OA using summary statistics from the arcOGEN combined OA dataset (including London samples), the UK Biobank (47) and the deCODE (61) study.

Functional follow-up of SMAD3

Samples were obtained and processed as in Ref. 25. Briefly, articular cartilage was obtained from 12 patients undergoing total joint replacement for knee OA, and 9 patients for hip OA. Cartilage was graded using the OARSI cartilage classification system (62,63).

Supplementary Material

Supplementary Material is available at HMG online.

Acknowledgements

This study utilised genotype data from arcOGEN (http://www.arcogen.org.uk/) funded by a special purpose grant from Arthritis Research UK (grant 18030).

This study makes use of data generated by the Wellcome Trust Case-Control Consortium (the 1958 British Birth Cohort collection and the UK Blood Services Collection). A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk.

We would like to acknowledge the Cambridge Biomedical Research Center Human Research Tissue Bank for storing the hip gene expression and methylation replication samples.

This research has been conducted using the UK Biobank Resource under Application Number 9979.

Conflict of Interest statement. U.S., U.T. and K.S. are employed by deCODE Genetics/Amgen. All other authors declare no competing interests.

Funding

This work was funded by the Welcome Trust (WT098051). Funding to pay the Open Access publication charges for this article was provided by the Welcome Trust.

References

1. Zhang, Y. and Jordan, J.M. (2010) Epidemiology of osteoarthritis. Clin. Geriatr. Med., 26, 355–369.
2. Glyn-Jones, S., Palmer, A.J.R., Agricola, R., Price, A.J., Vincent, T.I., Weinans, H. and Carr, A.J. (2015) Osteoarthritis. Lancet, 386, 376–387.
3. Hochberg, M.C., Yerges-Armstrong, L., Yau, M. and Mitchell, B.D. (2013) Genetic epidemiology of osteoarthritis: recent developments and future directions. Curr. Opin. Rheumatol., 25, 192–197.
4. Zengini, E., Finan, C. and Wilkinson, J.M. (2016) The genetic epidemiological landscape of hip and knee osteoarthritis: Where are we now and where are we going? J. Rheumatol., 43, 260–266.
5. Panoutsopoulou, K., Southam, L., Elliott, K.S., Wrayner, N., Zhai, G., Beazley, C., Thorleifsson, G., Arden, N.K., Carr, A., Chapman, K. et al. (2011) Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. Ann. Rheum. Dis., 70, 864–867.
6. Zeggini, E., Panoutsopoulou, K., Southam, L., Rayner, N.W., Day-Williams, A.G., Lopes, M.C., Boraska, V., Esco, T., Evangelou, E., Hofman, A. et al. (2012) Identification of new susceptibility loci for osteoarthritis (arcOGEN): A genome-wide association study. Lancet, 380, 815–823.
7. Sivakumaran, S., Agakov, F., Theodoratou, E., Prendergast, J.G., Zgaga, L., Manolio, T., Rudan, I., McKeigue, P., Wilson, J.F. and Campbell, H. (2011) Abundant pleiotropy in human complex diseases and traits. Am. J. Hum. Genet., 89, 607–618.
8. Foss, M.V. and Byers, P.D. (1972) Bone density, osteoarthritis of the hip, and fracture of the upper end of the femur. Ann. Rheum. Dis., 31, 259–264.
9. Nevitt, M.C., Zhang, Y., Javaid, M.K., Neogi, T., Curtis, J.R., Niu, J., McCulloch, C.E., Segal, N.A. and Felson, D.T. (2010) High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study. Ann. Rheum. Dis., 69, 163–168.
10. Hart, D.J., Mootosamy, I., Doyle, D.V. and Spector, T.D. (1994) The relationship between osteoarthritis and osteoporosis in the general population: the Chingford Study. Ann. Rheum. Dis., 53, 158–162.
11. Hannan, M.T., Anderson, J.J., Zhang, Y., Levy, D. and Felson, D.T. (1993) Bone mineral density and knee osteoarthritis in...
elderly men and women. the framingham study. Arthritis Rheum., 36, 1671–1680.

12. Hardcastle, S. a., Dieppe, P., Gregson, C.L., Smith, G.D. and Tobias, J.H. (2015) Osteoarthritis and bone mineral density: are strong bones bad for joints?. Bonekey Rep., 4, 1–8.

13. Hardcastle, S. a., Dieppe, P., Gregson, C.L., Arden, N.K., Spector, T.D., Hart, D.J., Edwards, M.H., Dennison, E.M., Cooper, C., Sayers, A. et al. (2015) Individuals with high bone mass have an increased prevalence of radiographic knee osteoarthritis. Bone, 71, 171–179.

14. Bettica, P., Cline, G., Hart, D.J., Meyer, J. and Spector, T.D. (2002) Evidence for increased bone resorption in patients with progressive knee osteoarthritis: Longitudinal results from the Chingford study. Arthritis Rheum., 46, 3178–3184.

15. Hart, D.J., Cronin, C. and Daniels, M. (2002) The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee. Arthritis Rheum., 46, 92–99.

16. Zhang, Y., Hannan, M.T., Chaixson, C.E., McAlindon, T.E., Evans, S.R., Aliabadi, P., Levy, D. and Felson, D.T. (2000) Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the Framingham Study. J. Rheumatol., 27, 1032–1037.

17. Bergink, A.P., Uitterlinden, A.G., Van Leeuwen, J.P.T.M., Hofman, A., Verhaar, J.A.N. and Pols, H.A.P. (2005) Bone mineral density and vertebral fracture history are associated with incident and progressive radiographic knee osteoarthritis in elderly men and women: the Rotterdam Study. Bone, 37, 446–456.

18. Estrada, K., Styrkardottir, U., Evangelou, E., Hsu, Y.H., Duncan, E.L., Mzani, E.E., Oei, L., Albargha, O.M.E., Amin, N., Kemp, J.P. et al. (2012) Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat. Genet., 44, 494–501.

19. Zheng, H., Forgetta, V., Hsu, Y., Estrada, K., Rosello-Diez, A., Leo, P.J., Dahia, C.L., Park-Min, K.H., Tobias, J.H., Kooperberg, C. et al. (2015) Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. Nature, 526, 112–117.

20. Geusens, P.P. and van den Bergh, J.P. (2016) Osteoporosis and osteoarthritis: shared mechanisms and epidemiology. Curr. Opin. Rheumatol., 28, 97–103.

21. Balooch, G., Balooch, M., Nalla, R.K., Schilling, S., Filvaroff, D.J., Mitchell, B.D., Dieppe, P., Gregson, C.L., Smith, G.D. and Alliston, T. (2005) TGF-beta regulates the mechanical properties and composition of bone matrix. Proc. Natl. Acad. Sci. U.S.A., 102, 18813–18818.

22. Panoutsopoulou, K. and Zeggini, E. (2013) Advances in osteoarthritis genetics. J. Med. Genet., 50, 715–724.

23. Yerges-Armstrong, L.M., You, M.S., Liu, Y., Krishnan, S., Renner, J.B., Eaton, C.B., Kwoh, C.K., Nevitt, M.C., Duggan, D.J., Mitchell, B.D. et al. (2014) Association analysis of BMD-associated SNPs with knee osteoarthritis. J. Bone Miner. Res., 29, 1373–1379.

24. Rivadeneira, F., Styrkardottir, U., Estrada, K., Hallóðórsson, B.V., Hsu, Y.-H., Richards, J.B., Zillikens, M.C., Kavvoura, F.K., Amin, N., Aulchenko, Y.S. et al. (2009) Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat. Genet., 41, 1199–1206.

25. Zhu, X., Feng, T., Tayo, B.O., Liang, J., Young, J.H., Franceschini, N., Smith, J. a., Yanek, L.R., Sun, Y.V., Edwards, T.L. et al. (2015) Meta-analysis of correlated traits via summary statistics from GWASs with an application in hypertension. Am. J. Hum. Genet., 96, 21–36.

26. Steinberg, J., Ritchie, G.R.S., Roumellotis, T.I., Jayasuriya, R.L., Brooks, R.A., Binch, A.L.A., Shah, K.M., Coyle, R., Pardo, M., Le Maitre, C.L. et al. (2016) Integrative epigenomics, transcriptomics and proteomics of patient chondrocytes reveal genes and pathways involved in osteoarthritis. bioRxiv, https://doi.org/10.1101/038067.

27. Shimomura, Y., Agalió, D., Venica, A., Luria, V., Wajid, M., Baumer, A., Bélli, S., Petukhova, L., Schinzel, A., Brivanlou, A.H. et al. (2010) APCDD1 is a novel Wnt inhibitor mutated in hereditary hypotrichosis simplex. Nature, 464, 1043–1047.

28. Rodriguez-Fontenla, C., Calaza, M., Evangelou, E., Valdes, A.M., Arden, N., Blanco, F.J., Carr, A., Chapman, K., Deloukas, P., Doherty, M. et al. (2014) Assessment of osteoarthritis candidate genes in a meta-analysis of nine genome-wide association studies. Arthritis Rheumatol., 66, 940–949.

29. Styrkardottir, U., Thorleifsson, G., Sulem, P., Gudbjartsson, D.F., Sigurdsson, A., Jonassott, A., Jonassott, A., Oddsson, A., Helgason, A., Magnusson, O.T. et al. (2013) Nonsense mutation in the LGR4 gene is associated with several human diseases and other traits. Nature, 497, 517–520.

30. Fukuda, T., Takeda, S., Xu, R., Ochi, H., Sunamuro, S., Sato, T., Shibata, S., Yoshiya, Y., Gu, Z., Kimura, A. et al. (2013) Sema3A regulates bone-mass accrual through sensory innervations. Nature, 497, 490–493.

31. Alliston, T., Choy, L., Ducy, P., Karsenty, G., Derynck, R., Akiyoshi, S., Isoue, H., Hanai, J., Kusaganagi, K., Nemoto, N. et al. (2001) TGF-beta-induced repression of CBFA1 by Smad3 decreases cbfa1 and osteocalcin expression and inhibits osteoblast differentiation. emboj., 20, 2254–2272.

32. Chen, C.G., Thuillier, D., Chin, E.N. and Alliston, T. (2012) Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. Arthritis Rheum., 64, 3278–3289.

33. van de Laar, I.M.B.H., Oldenburg, R.A., Pals, G., Roos-Hesselink, J.W., de Graaf, B.M., Verhagen, J.M.A., Hoedemaekers, Y.M., Willemsen, R., Severijn, L.-A., Venselaar, H. et al. (2011) Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat. Genet., 43, 121–126.

34. Valdes, A.M., Spector, T.D., Tamm, A., Kisdian, K., Doherty, S.A., Dennison, E.M., Mangino, M., Tamm, A., Kerna, I., Hart, D.J. et al. (2010) Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. Arthritis Rheum., 62, 2347–2352.

35. Raine, E.V.A., Reynard, L.N., van de Laar, I.M.B.H., Bertoli-Avella, A.M. and Loughlin, J. (2014) Identification and analysis of a SMAD3 cis-acting eQTL operating in primary osteoarthritis and in the aseumetics and osteoarthritis syndrome. Osteoarthritis Cartil., 22, 698–705.

36. Aref-Eshghi, E., Liu, M., Razavi-Lopez, S.B., Hirassawa, K., Harper, P.E., Martin, G., Furey, A., Green, R., Sun, G., Rahman, P. et al. (2016) SMAD3 is upregulated in human osteoarthritisc cartilage independent of the promoter DNA methylation. J. Rheumatol., 43, 388–394.

37. Hochberg, M.C., Lethbridge-Cejku, M. and Tobin, J.D. (2004) Bone mineral density and osteoarthritis: Data from the Baltimore Longitudinal Study of Aging. Osteoarthr. Cartil., 12, 45–48.

38. Harada, A., Okuizumi, H., Miyagi, N. and Genda, E. (1998) Correlation between bone mineral density and intervertebral disc degeneration. Spine (Phila. Pa. 1976), 23, 857–861.
39. Grams, A.E., Rehwald, R., Bartsch, A., Honold, S., Freyschlag, C.F., Knoflach, M., Gizewski, E.R. and Glodny, B. (2016) Correlation between degenerative spine disease and bone mineral density: a retrospective investigation. BMC Med. Imaging, 16, 17.

40. Pye, S.R., Reid, D.M., Adams, J.E., Silman, A.J. and O’Neill, T.W. (2006) Radiographic features of lumbar disc degeneration and bone mineral density in men and women. Ann. Rheum. Dis., 65, 234–238.

41. Miyakoshi, N., Itoi, E., Murai, H., Wakabayashi, I., Ito, H. and Minato, T. (2003) Inverse relation between osteoporosis and spondylosis in postmenopausal women as evaluated by bone mineral density and semiquantitative scoring of spinal degeneration. Spine (Phila. Pa. 1976), 28, 492–495.

42. Rand, T., Seidl, G., Kainberger, F., Resch, A., Hittmair, K., Schneider, B., Glider, C.C. and Imhof, H. (1997) Impact of spinal degenerative changes on the evaluation of bone mineral density with dual energy X-ray absorptiometry (DXA). Calcif. Tissue Int., 60, 430–433.

43. Kemp, J.P., Medina-Gomez, C., Estrada, K., St Pourcain, B., Hepp, D.H.M., Warrington, N.M., Oel, L., Ring, S.M., Kruithof, C.J., Timpon, N.J. et al. (2014) Phenotypic dissection of bone mineral density reveals skeletal site specificity and facilitates the identification of novel loci in the genetic regulation of bone mass attainment. PLoS Genet., 10, e1004423.

44. Valdes, A.M. and Spector, T.D. (2011) Genetic epidemiology of hip and knee osteoarthritis. Nat. Rev. Rheumatol., 7, 23–32.

45. Blagojevic, M., Jinks, C., Jeffery, A. and Jordan, K.P. (2010) Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. Osteoarthr. Cartil., 18, 24–33.

46. Smoller, J.W. (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet, 381, 1371–1379.

47. Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M. et al. (2015) UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med., 12, e1001779.

48. Pickrell, J., Berisa, T., Segurel, L., Tung, J.Y. and Hinds, D. (2015) Detection and interpretation of shared genetic influences on 42 human traits. Nat. Genet., 48, 709–717.

49. Solovieff, N., Cotsapas, C., Lee, P.H., Purcell, S.M. and Smoller, J.W. (2013) Pleiotropy in complex traits: challenges and strategies. Nat. Rev. Genet., 14, 483–495.

50. Marchini, J., Howie, B., Myers, S., McVean, G. and Donnelly, P. (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. Nat. Genet., 39, 906–913.

51. Bulik-Sullivan, B., Finucane, H.K., Anttila, V., Gusev, A., Day, F.R., Loh, P.-R., ReproGen Consortium, Psychiatric Genomics Consortium, Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case Control Consortium 3, Duncan, L. et al. (2015) An atlas of genetic correlations across human diseases and traits. Nat. Genet., 47, 1236–1241.

52. Bulik-Sullivan, B.K., Loh, P.-R., Finucane, H.K., Ripke, S., Yang, J., Schizophrenia Working Group of the Psychiatric Genomics Consortium, Patterson, N., Daly, M.J., Price, A.L. and Neale, B.M. (2015) LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat. Genet., advance on, 291–295.

53. Elliott, K.S., Chapman, K., Day-Williams, A., Panoutsopoulou, K., Southam, L., Lindgren, C.M., Arden, N., Aslam, N., Birrell, F., Carluke, I. et al. (2013) Evaluation of the genetic overlap between osteoarthritis with body mass index and height using genome-wide association scan data. Ann. Rheum. Dis., 72, 935–941.

54. Berisa, T. and Pickrell, J.K. (2016) Approximately independent linkage disequilibrium blocks in human populations. Bioinformatics, 32, 283–285.

55. de Leeuw, C.A., Mooij, J.M., Heskes, T. and Posthuma, D. (2015) MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput. Biol., 11, e1004219.

56. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S. et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci., 102, 15545–15550.

57. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T. et al. (2000) Gene ontology: tool for the unification of biology. Nat. Genet., 25, 25–29.

58. Benjamini, Y. and Hochberg, Y. (1999) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B., 57, 289–300.

59. Park, H., Li, X., Song, Y.E., He, K.Y. and Zhu, X. (2016) Multivariate analysis of anthropometric traits using summary statistics of genome-wide association studies from GIANT Consortium. PLoS One, 11, e0163912.

60. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: Fast and efficient meta-analysis of genomewide association scans. Bioinformatics, 26, 2190–2191.

61. Gudbjartsson, D.F., Helgason, H., Gudjonsson, S.A., Zink, F., Oddson, A., Gylfason, A., Besenbacher, S., Magnusson, G., Halldorsson, B.V., Hjartarson, E. et al. (2015) Large-scale whole-genome sequencing of the Icelandic population. Nat. Genet., 47, 435–444.

62. Mankin, H.J., Dorfman, H., Lippio, L. and Zarins, A. (1971) Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritis human hips: II. correlation of morphology with biochemical and metabolic data. J. Bone Jt. Surg., 53, 523–537.

63. Pearson, R.G., Kurien, T., Shu, K.S.S. and Scammell, B.E. (2011) Phenotypic dissection of osteoarthritic human hips: II. correlation of morphologic and biochemical data. J. Bone Jt. Surg., 93, 285–290.