Genome-wide analyses using UK Biobank data provide insights into the genetic architecture of osteoarthritis

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Osteoarthritis is a common complex disease imposing a large public-health burden. Here, we performed a genome-wide association study for osteoarthritis, using data across 16.5 million variants from the UK Biobank resource. After performing replication and meta-analysis in up to 30,727 cases and 297,191 controls, we identified nine new osteoarthritis loci, in all of which the most likely causal variant was noncoding. For three loci, we detected association with biologically relevant radiographic endophenotypes, and in five signals we identified genes that were differentially expressed in degraded compared with intact articular cartilage from patients with osteoarthritis. We established causal effects on osteoarthritis for higher body mass index but not for triglyceride levels or genetic predisposition to type 2 diabetes.

Osteoarthritis is the most prevalent musculoskeletal disease and the most common form of arthritis1. The hallmarks of osteoarthritis are degeneration of articular cartilage, remodeling of the underlying bone and synovitis2. A leading cause of disability worldwide, osteoarthritis affects 40% of individuals over the age of 70 and is associated with an elevated risk of comorbidity and death3. The rising health economic burden of osteoarthritis is commensurate with rising longevity and obesity rates, and there is currently no curative therapy. The heritability of osteoarthritis is ~50%, and previous genetic studies have identified 21 loci in total, traversing hip, knee and hand osteoarthritis with limited overlap4. Here, we conducted a large osteoarthritis genome-wide association study (GWAS), using genotype data across 16.5 million variants from UK Biobank. We defined osteoarthritis on the basis of both self-reported status and linkage to Hospital Episode Statistics data, as well as the joint specificity of the disease (knee and/or hip) (Supplementary Fig. 1).

Results
Disease definition and power to detect genetic associations. We compared and contrasted the hospital-diagnosed (n = 10,083 cases) and self-reported (n = 12,658 cases) osteoarthritis GWAS drawn from the same UK Biobank dataset (with selection of approximately four times more nonosteoarthritis controls than cases to preserve power for common alleles while avoiding case–control imbalance that might cause association tests to misbehave for low-frequency variants5) (Supplementary Tables 1–3, Supplementary Figs. 2–4 and Methods). We found power advantages with the self-reported dataset, thus indicating that the higher sample size overcame the limitations associated with phenotype uncertainty. When evaluating the accuracy of disease definition, we found that self-reported osteoarthritis had a modest positive predictive value (PPV, 30%) and sensitivity (37%), but high negative predictive value (95%) and specificity, correctly identifying 93% of individuals who did not have osteoarthritis (Supplementary Table 4). In terms of power to detect genetic associations, the self-reported-osteoarthritis dataset had clear advantages commensurate with its larger sample size (Fig. 1). For example, for a representative complex-disease-associated variant with a minor allele frequency (MAF) of 30% and an allelic odds ratio (OR) of 1.10, the self-reported and hospital-diagnosed osteoarthritis analyses had 80% and 56% power, respectively.
Identification of novel osteoarthritis loci. We used 173 variants with \( P < 10^{-5} \) and MAF > 0.01 for replication in an Icelandic cohort of up to 18,069 cases and 246,293 controls (Supplementary Fig. 1, Supplementary Tables 11–15 and Methods). Given the number of variants, the replication significance threshold was \( P < 2.9 \times 10^{-4} \). After meta-analysis in up to 30,727 cases and 297,191 controls, we identified six genome-wide-significant associations at novel loci and three further replicating signals just below the corrected genome-wide-significance threshold (Table 1 and Fig. 2).

We identified association between rs2521349 and hip osteoarthritis (OR 1.13 (95% confidence interval (CI) 1.09–1.17), \( P = 9.95 \times 10^{-10} \), effect-allele frequency (EAF) 0.37). rs2521349 resides in an intron of MAP2K6 on chromosome 17. MAP2K6 encodes an essential component of the p38 MAP kinase–mediated signal-transduction pathway, which is involved in various cellular processes in bone, muscle, fat-tissue homeostasis and differentiation. The MAPK signaling pathway is closely associated with osteoblast differentiation, chondrocyte apoptosis and necrosis, and has been reported to be differentially expressed in osteoarthritis synovial-tissue samples. In animal-model studies, p38 MAP kinase activity has been found to be important in maintaining cartilage health, and it has been proposed as a potential osteoarthritis diagnosis and treatment target.

rs11780978 on chromosome 8 is also associated with hip osteoarthritis with a similar effect size (OR 1.13 (95% CI 1.08–1.17), \( P = 1.98 \times 10^{-4} \), EAF 0.39). This variant is located in the intronic region of PLEC (plectin gene). We found rs11780978 to be nominally associated with the radiographically derived endophenotype of minimal joint-space width (\( \beta = 0.0291 \), s.e.m. 0.0129, \( P = 0.024 \)) (Table 2 and Methods). The direction of the effect was consistent with the established clinical association between joint-space narrowing and osteoarthritis. PLEC encodes plectin, a structural protein that interlinks components of the cytoskeleton. Functional studies in mice have shown an effect on skeletal-muscle tissue correlated with low body weight, small size and slow postnatal growth.

rs2820436, an intergenic variant located 24 kb upstream of the long-non-coding RNA gene RP11-392017.1 and 142 kb downstream of ZC3H11B (zinc-finger CCHC-type containing 11B pseudogene), is associated with osteoarthritis across any joint site (OR 0.93 (95% CI 0.91–0.95), \( P = 2.01 \times 10^{-4} \), EAF 0.65). It also resides within a region with multiple metabolic- and anthropometric-trait-associated variants, with which it was found to correlate (\( r^2 = 0.18–0.88 \)).

rs375573359 resides in an intron of ZNF345 (zinc-finger-protein 345 gene) on chromosome 19. It was prioritized on the basis of osteoarthritis at any joint site and was more strongly associated with knee osteoarthritis in the replication dataset (OR 1.21 (95% CI 1.14–1.30), \( P = 7.54 \times 10^{-5} \), EAF 0.04). Similarly, rs11335718 on chromosome 4 was associated with osteoarthritis in the discovery stage and with knee osteoarthritis in the replication stage (OR 1.11 (95% CI 1.07–1.16), \( P = 4.26 \times 10^{-6} \), EAF 0.10). We note that Bonferroni correction for the effective number of traits tested resulted in rs11335718 to no longer reach genome-wide significance, with a meta-analysis \( P = 4.26 \times 10^{-6} \) and rs11335718 is an intronic variant in ANXA3, the annexin A3 gene. Through meta-analysis of the any-site-osteoarthritis phenotype across the discovery and replication datasets, we determined \( P = 2.6 \times 10^{-3} \) and \( P = 1.32 \times 10^{-7} \) for rs375573359 and rs11335718, respectively (Supplementary Table 11). A recent mouse-model study supports the involvement of expression of a similar motif zinc-finger-protein (ZFP36L1) with osteoblastic differentiation.

rs3771501 (OR 0.94 (95% CI 0.92–0.96), \( P = 1.66 \times 10^{-4} \), EAF 0.53) is associated with osteoarthritis at any site and resides in an intron of TGFA (transforming growth factor alpha gene). TGFA encodes an epidermal-growth-factor-receptor ligand and is an important integrator of cellular signaling and function. We detected association of rs3771501 with minimal joint-space width (\( \beta = 0.0699 \), s.e.m. 0.0127, \( P = 3.45 \times 10^{-4} \)) (Table 2 and Methods); i.e., the osteoarthritis-risk-increasing allele was also associated with lower joint-cartilage thickness in humans. A perfectly correlated variant in this gene has previously been associated with cartilage thickness and with hip osteoarthritis; moreover, this variant has been found to be differentially expressed in osteoarthritis cartilage lesions compared with nonlesioned cartilage.
Table 1 | Association summary statistics for the nine signals

| rsID     | EA   | Discovery phenotype | Discovery EAF | Discovery OR (95% CI) | Discovery P value* | Discovery replication phenotype | Replication EAF | Replication OR (95% CI) | Replication P value* | Replication n (cases/controls) | Overall OR (95% CI) | Overall P value* | Heterogeneity P value* | Overall n (cases/controls) |
|----------|------|---------------------|---------------|-----------------------|--------------------|-------------------------------|----------------|-------------------------|-------------------|----------------------------|-------------------|----------------|---------------------|------------------------|
| rs2820436 | C    | Hospital-diagnosed osteoarthritis | 0.66 | 0.92 | 0.96 | 6.4 × 10⁻⁸ | 10,083/40,425 | Directly typed | Osteoarthritis at any site | 0.64 | 0.94 | 0.91 | 0.97 | 8.71 × 10⁻⁸ | 18,069/24,6293 | 0.99972 | 0.93 | 0.91 | 0.96 | 2.0 × 10⁻⁸ | 0.5739 | 28,152/286,718 |
| rs3771001 | G    | Self-reported osteoarthritis | 0.53 | 0.94 | 0.91 | 0.96 | 3.81 × 10⁻⁸ | 12,658/50,898 | Directly typed | Osteoarthritis at any site | 0.54 | 0.95 | 0.92 | 0.98 | 0.001069 | 18,069/24,6293 | 0.999808 | 0.94 | 0.92 | 0.96 | 1.66 × 10⁻⁸ | 0.4825 | 30,722/297,911 |
| rs1135718 | A    | Self-reported osteoarthritis | 0.11 | 1.12 | 1.07 | 1.12 × 10⁻⁸ | 0.9968932 | Knee osteoarthritis | 0.11 | 1.10 | 1.02 | 1.2 | 0.014675 | 4,672/172,791 | 0.9988999 | 1.10 | 1.07 | 1.16 | 4.26 × 10⁻⁸ | 0.792 | 14,330/223,689 |
| rs1135718 | A    | Self-reported osteoarthritis | 0.11 | 1.12 | 1.07 | 1.12 × 10⁻⁸ | 0.9968932 | Osteoarthritis at any site | 0.11 | 1.06 | 1.01 | 1.1 | 0.033023 | 18,069/24,6293 | 0.9988999 | 1.09 | 1.06 | 1.13 | 1.32 × 10⁻⁸ | 0.1254 | 30,722/297,911 |
| rs1780978 | A    | Hospital-diagnosed hip osteoarthritis | 0.4 | 1.16 | 1.08 | 1.23 | 6.24 × 10⁻⁸ | 2.396/9,593 | Hip osteoarthritis | 0.39 | 1.11 | 1.05 | 1.16 | 4.55 × 10⁻⁸ | 5,714/199,421 | 0.999673 | 1.13 | 1.08 | 1.17 | 1.98 × 10⁻⁸ | 0.2424 | 8,110/20,914 |
| rs16682138 A | Hospital-diagnosed hip and/or knee osteoarthritis | 0.02 | 1.4 | 1.22 | 1.6 | 2.96 × 10⁻⁸ | 6,586/26,384 | Directly typed | Knee osteoarthritis | 0.02 | 1.27 | 1.07 | 1.15 | 0.006552 | 4,672/172,791 | 0.998087 | 1.34 | 1.21 | 1.49 | 5.09 × 10⁻⁸ | 0.3988 | 11,258/199,175 |
| rs16682138 A | Hospital-diagnosed hip and/or knee osteoarthritis | 0.02 | 1.4 | 1.22 | 1.6 | 2.96 × 10⁻⁸ | 6,586/26,384 | Directly typed | Hip and/or knee osteoarthritis | 0.02 | 1.13 | 0.99 | 1.12 | 0.060018 | 9,429/199,421 | 0.998087 | 1.25 | 1.14 | 1.38 | 2.39 × 10⁻⁸ | 0.03456 | 16,015/222,805 |
| rs2521349 | A    | Hospital-diagnosed hip osteoarthritis | 0.38 | 1.18 | 1.11 | 1.26 | 6.85 × 10⁻⁸ | 2.396/9,593 | Hip osteoarthritis | 0.37 | 1.10 | 1.05 | 1.16 | 0.000103 | 5,714/199,421 | 0.999925 | 1.13 | 1.09 | 1.18 | 9.95 × 10⁻⁸ | 0.151 | 8,710/20,904 |
| rs864839 | T    | Self-reported osteoarthritis | 0.72 | 1.08 | 1.05 | 1.12 | 6.21 × 10⁻⁸ | 12,658/50,898 | Hip osteoarthritis | 0.7 | 1.07 | 1.02 | 1.13 | 0.008275 | 5,714/199,421 | 0.997756 | 1.08 | 1.05 | 1.11 | 2.01 × 10⁻⁸ | 0.7886 | 18,170/250,319 |
| rs864839 | T    | Self-reported osteoarthritis | 0.72 | 1.08 | 1.05 | 1.12 | 6.21 × 10⁻⁸ | 12,658/50,898 | Hip osteoarthritis | 0.7 | 1.02 | 0.99 | 1.06 | 0.18218 | 18,069/24,6293 | 0.997756 | 1.05 | 1.03 | 1.08 | 7.02 × 10⁻⁸ | 0.0121 | 30,722/297,911 |
| rs7557359 | C    | Self-reported osteoarthritis | 0.03 | 1.2 | 1.12 | 1.29 | 9.96 × 10⁻⁸ | 12,658/50,898 | Directly typed | Osteoarthritis at any site | 0.05 | 1.05 | 1.02 | 1.02 | 0.025177 | 4,672/172,791 | 0.992996 | 1.21 | 1.14 | 1.13 | 7.54 × 10⁻⁸ | 0.25 | 17,330/223,689 |
| rs7557359 | C    | Self-reported osteoarthritis | 0.03 | 1.2 | 1.12 | 1.29 | 9.96 × 10⁻⁸ | 12,658/50,898 | Directly typed | Osteoarthritis at any site | 0.05 | 1.03 | 0.96 | 1.1 | 0.47234 | 18,069/24,6293 | 0.992996 | 1.12 | 1.06 | 1.18 | 2.6 × 10⁻⁸ | 0.00403 | 30,722/297,911 |
| rs6516886 | T    | Hospital-diagnosed hip and/or knee osteoarthritis | 0.75 | 1.13 | 1.08 | 1.19 | 5.36 × 10⁻⁸ | 6,586/26,384 | Directly typed | Hip and/or knee osteoarthritis | 0.76 | 1.06 | 1.00 | 1.12 | 0.053185 | 5,714/199,421 | 0.999981 | 1.10 | 1.06 | 1.14 | 5.84 × 10⁻⁸ | 0.06276 | 12,304/225,805 |
| rs6516886 | T    | Hospital-diagnosed hip and/or knee osteoarthritis | 0.75 | 1.13 | 1.08 | 1.19 | 5.36 × 10⁻⁸ | 6,586/26,384 | Directly typed | Hip and/or knee osteoarthritis | 0.76 | 1.05 | 1.00 | 1.11 | 0.043467 | 9,429/199,421 | 0.999981 | 1.09 | 1.06 | 1.13 | 1.42 × 10⁻⁸ | 0.01914 | 16,015/222,805 |

*Imputation accuracy was assessed with IMPUTE2 infoscore. Heterogeneity P values were derived from Cochran’s Q test. Two-sided P value, likelihood ratio test. EA, effect allele; n, sample size.
studies have shown that TGFA regulates the conversion of cartilage to bone during the process of endochondral bone growth, and that it is a dysregulated cytokine present in degrading cartilage in osteoarthritis and a strong stimulator of cartilage degradation upregulated by articular chondrocytes in experimentally induced and human osteoarthritis\(^{-18-21}\). The function of TGFA has also been associated with craniofacial development, palate closure and small body size\(^{-22}\).

rs864839 resides in the intronic region of *JPH3* (junctophilin 3 gene) on chromosome 16 and was discovered in the any-joint-site osteoarthritis analysis. It was more strongly associated with hip osteoarthritis in the replication dataset (OR 1.08 (95% CI 1.05–1.11), \(P = 2.1 \times 10^{-6}\), EAF 0.71). Through meta-analysis of the any-site-osteoarthritis phenotype across the discovery and replication datasets, we determined \(P = 7.02 \times 10^{-6}\) (Supplementary Table 11). JPH3 is involved in the formation of junctional membrane structure, and it regulates neuronal calcium flux and has been reported to be expressed in pancreatic beta cells and in the regulation of insulin secretion.

rs116882138 was most strongly associated with hip and/or knee osteoarthritis-discovery analysis and was more strongly associated in the hip-osteoarthritis replication dataset (OR 1.10 (95% CI 1.06–1.14), \(P = 5.84 \times 10^{-4}\), EAF 0.75). rs6516886 was situated 1 kb upstream of *RWDD2B* (RWD-domain-containing 2B gene) on chromosome 21. *LTN1* (listerin E3 ubiquitin protein ligase 1 gene), which is located 28 kb from the variant, has been reported to affect musculoskeletal development in a mouse model\(^{23}\).

**Functional analysis.** Using molecular phenotyping through quantitative proteomics and RNA sequencing, we tested whether coding genes within 1 Mb of the novel osteoarthritis-associated variants were differentially expressed at 1% false discovery rate (FDR) in chondrocytes extracted from intact compared with degraded cartilage from patients with osteoarthritis undergoing total-joint-replacement surgery (Table 3 and Methods).

**PCYOX1**, located 209 kb downstream of *rs3771501*, showed significant evidence of differential expression (1.21-fold higher post-normalization in degraded cartilage at the RNA level, \(q = 0.0047\); and 1.17-fold lower abundance at the protein level, \(q = 0.0042\)). This discrepancy may indicate potential clinical relevance, because the gene product is a candidate biomarker for osteoarthritis progression. Premethylxamine oxidase 1, the protein product of this gene, is a secreted protein that catalyzes the degradation of prenylated proteins\(^{24}\) and has been identified in urinary exosomes\(^{25}\). Further investigation into the chondrocyte and peripheral secretome is warranted to assess the potential of this molecule as a biomarker for osteoarthritis.

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**Fig. 2** | Regional association plots for the nine novel osteoarthritis loci. The y axis represents the negative logarithm (base 10) of the variant \(P\) value (likelihood ratio test), and the x axis represents the position on the chromosome (chr), with the names and location of genes and nearest genes shown at the bottom. The variant with the lowest \(P\) value in the region after combined discovery and replication is marked by a purple diamond. The same variant is marked by a purple dot showing the discovery \(P\) value. The colors of the other variants indicate their \(r^2\) with the lead variant.
osteoarthritis progression. PCYOX1 has been reported to be over-expressed in human dental-pulp-derived osteoblasts compared with osteosarcoma cells. FAM136A, located 188 kb upstream of the same variant (rs3771501), showed 1.13-fold-lower transcriptional levels in chondrocytes from degraded articular cartilage ($q = 0.0066$).

BACH1 and MAP3K7, located in the vicinity of rs6516886, showed evidence of differential transcription (1.26-fold higher, $q = 0.0019$, and 1.37-fold higher, $q = 0.0021$, respectively, in degraded tissue). BACH1 is a transcriptional repressor of heme oxygenase-1. Studies in BACH1-deficient mice have independently suggested inactivation of Bach1 as a novel target for the prevention and treatment of meniscal degeneration and of osteoarthritis.

Finally, PLAA and ZNF382, located proximal to rs116882138 and rs375573559, respectively, showed higher transcription levels in degraded cartilage compared with intact cartilage (1.15-fold, $q = 0.0027$, and 1.31-fold, $q = 0.0031$, respectively). BOP1, located $451$ kb downstream of rs11780978, showed 1.17-fold lower levels of transcription in degraded tissue ($q = 0.003$).

We examined evidence for expression quantitative trait loci (eQTLs) in the Genotype-Tissue Expression GTEx tissues and found that none of the eQTLs identified at $5\%$ FDR overlapped with the genes identified as differentially expressed between osteoarthritis intact and degraded cartilage (Supplementary Note and Supplementary Table 16).

Fine mapping indicates noncoding variants at all loci. For five of the new loci, the sum of probabilities of causality of all variants in the fine-mapped region was $>0.95$ ($>0.99$ for two signals) and was $>0.93$ for two further loci (Supplementary Table 17 and Methods). Most variants within each credible set had marginal posterior probabilities, whereas only a small number of variants had a posterior probability of association (PPA) $>0.1$; these accounted for 25–92% of PPA across the different regions. The credible set of four signals was narrowed down to three variants, one signal to two variants, and one signal to one variant, with a probability of causality $>0.1$. For all nine regions, the variant identified as most likely to be causal was noncoding (Supplementary Table 18, Supplementary Note and Supplementary Fig. 5).

Gene-based analyses. Gene-set analysis identified UQCC1 and GDF5, located close to each other on chromosome 20, as key genes with consistent evidence of significant association with osteoarthritis across phenotype definitions (Supplementary Table 19 and Supplementary Note). UQCC1 and GDF5 were significantly associated with four and three of the five osteoarthritis definitions, respectively. GDF5 encodes growth differentiation factor 5, a member of the TGFβ superfamily, and accruing evidence indicates that it plays a central role in skeletal health and development. Pathway analyses identified significant associations between self-reported osteoarthritis and anatomical-structure morphogenesis ($P = 4.76 \times 10^{-5}$) or ion-channel transport ($P = 8.98 \times 10^{-5}$); hospital-diagnosed hip osteoarthritis and activation of MAPK activity ($P = 1.61 \times 10^{-3}$); hospital-diagnosed knee osteoarthritis and histidine metabolism ($P = 1.02 \times 10^{-3}$); and hospital-diagnosed hip and/or knee osteoarthritis and recruitment of mitotic centrosome proteins and complexes ($P = 8.88 \times 10^{-5}$) (Supplementary Table 20 and Supplementary Fig. 6).

Genetic links between osteoarthritis and other traits. Established clinical risk factors for osteoarthritis include old age, female sex, obesity, occupational exposure to high levels of joint loading activity, previous injury, smoking status and family history of osteoarthritis. We estimated the genome-wide genetic correlation between osteoarthritis and 219 other traits and diseases and identified 35 phenotypes with significant (5% FDR) genetic correlation with osteoarthritis across definitions, with large overlap between the identified phenotypes (Supplementary Fig. 7, Fig. 3, Supplementary Table 21 and Methods).

The phenotypes with significant genetic correlations (rg) fell into the following broad categories: obesity, body mass index (BMI) and related anthropometric traits (rg $>0$); type 2 diabetes (rg $>0$); educational achievement (rg $<0$); neuroticism, depressive symptoms (rg $>0$) and sleep duration (rg $<0$); mother’s, father’s or parents’ age at death (rg $<0$); reproductive phenotypes, including age at first birth (rg $<0$) and number of children born (rg $>0$); smoking, including age of smoking initiation (rg $<0$) and having ever smoked (rg $<0$), and lung cancer (rg $>0$) (Fig. 3, Supplementary Table 21). The four phenotypes with significant genetic correlation in all analyses were number of years of schooling, waist circumference, hip circumference and BMI.

We found a nominally significant positive genetic correlation with rheumatoid arthritis, which did not pass multiple-testing correction for self-reported and hospital-diagnosed osteoarthritis (rg $= 0.14–0.19$, FDR 10–12%). Among musculoskeletal phenotypes, lumbar-spine bone mineral density showed a positive genetic correlation with hospital-diagnosed hip and/or knee osteoarthritis (rg $= 0.2$, FDR $= 3\%$) but did not reach significance in other analyses.

Disentangling causality. We undertook Mendelian randomization (MR) analyses to strengthen causal inference regarding modifiable exposures that might influence osteoarthritis risk (Supplementary Tables 22–25 and Methods). Each kg/m$^2$ increment in body mass

**Table 2 | Association of the nine osteoarthritis loci with radiographically derived osteoarthritis endophenotypes**

| rsID   | EA     | EAF  | $\beta$ | s.e. | $P$ value | EA     | EAF  | $\beta$ | s.e. | $P$ value | EA     | EAF  | $\beta$ | s.e. | $P$ value |
|-------|--------|------|---------|------|-----------|--------|------|---------|------|-----------|--------|------|---------|------|-----------|
| rs2820436 | A     | 0.317 | -0.0146 | 0.0135 | 0.2817 | A     | 0.317 | -0.0104 | 0.1301 | 0.9363 | A     | 0.318 | 0.0165 | 0.0675 | 0.8073 |
| rs3771501 | A     | 0.484 | -0.0699 | 0.0127 | 3.454 x 10^{-8} | A     | 0.474 | 0.1943 | 0.199 | 0.0105 | A     | 0.4779 | -0.0144 | 0.0626 | 0.8176 |
| rs1335718 | N/A   | N/A   | N/A     | N/A   | N/A     | N/A   | N/A   | N/A     | N/A   | N/A     | N/A   | N/A   | N/A     | N/A   | N/A     |
| rs377180978 | A     | 0.389 | -0.0291 | 0.0129 | 0.02419 | A     | 0.386 | 0.078 | 0.1239 | 0.5291 | A     | 0.3866 | 0.0035 | 0.0644 | 0.9564 |
| rs2521349 | A     | 0.398 | 0.0229 | 0.0128 | 0.07404 | A     | 0.391 | 0.0998 | 0.1236 | 0.4192 | A     | 0.3921 | -0.0262 | 0.0644 | 0.6835 |
| rs684839 | N/A   | N/A   | N/A     | N/A   | T       | 0.702 | -0.0206 | 0.325 | 0.8766 | T     | 0.7026 | -0.0081 | 0.0691 | 0.907 |
| rs375573599 | N/A   | N/A   | N/A     | N/A   | N/A     | N/A   | N/A   | N/A     | N/A   | N/A     | N/A   | N/A   | N/A     | N/A   | N/A     |
| rs16882138 | A     | 0.0137 | -1.1388 | 0.5276 | 0.0309 | A     | 0.0135 | 0.1814 | 0.2607 | 0.4865 |
| rs6516886* | T     | 0.272 | -0.0222 | 0.0143 | 0.1206 | A     | 0.265 | -0.1491 | 0.1373 | 0.2773 | A     | 0.263 | 0.0544 | 0.0713 | 0.4458 |

*For minimal joint-space width, proxy variant rs2150403 ($r^2 = 0.99$ with rs6516886) was used.

Sample size = 13,013; Sample size = 6,880; Sample size, cases = 639; Sample size, controls = 4,339; Two-sided P value following inverse variance-based meta-analysis. EA, effect allele; s.e., standard error; N/A, not available.
logical relationships. To assess causality and disentangle complex cross-trait epidemiologies between osteoarthritis and multiple molecular, physiological primary statistics across complex traits to identify genetic correlation with clinically relevant radiographic endophenotypes and dataset, coupled with independent replication, independent association with other obesity-related measures, such as waist circumference (OR 1.03 per cm increment; 95% CI 1.02–1.05, P = 5 × 10⁻⁴) and hip circumference (OR 1.03 per cm increment; 95% CI 1.01–1.06, P = 0.021). The OR values for type 2 diabetes liability and triglycerides were consistently small across estimators and osteoarthritis definitions; given that the analyses involving those traits were well powered (Supplementary Table 26), these results are compatible with either a weak or no causal effect. The results for years of schooling were not consistent across estimators, and there was evidence of directional horizontal pleiotropy, thus hampering any causal interpretation (Fig. 4). For lumbar-spine bone mineral density, there was evidence of a causal effect with OR per s.d. increment of 1.28 (95% CI 1.11–1.47, P = 0.002) for hip and/or knee osteoarthritis. This effect appeared to be site specific, with OR of 1.29 (95% CI 1.06–1.57, P = 0.014) for knee osteoarthritis, whereas the OR for hip osteoarthritis ranged from 0.71 to 1.57. There was also some evidence of a site-specific causal effect of height on knee osteoarthritis (OR 1.13 per s.d. increment; 95% CI 1.02–1.25, P = 0.023), which was consistent across estimators. One-sample MR analyses corroborated these findings, and obesity-related phenotypes presented strong statistical evidence after multiple-testing correction (Supplementary Table 27). These analyses did not detect reliable effects of smoking or reproductive traits on osteoarthritis (Supplementary Tables 28 and 29).

Discussion
To improve understanding of the genetic etiology of osteoarthritis, we conducted a study combining genotype data in up to 327,918 individuals. We identified six novel, robustly replicating loci associated with osteoarthritis, three of which fell just under the corrected genome-wide-sigificance threshold. These loci provide a substantial increase in the number of known osteoarthritis loci. Together, all established osteoarthritis loci accounted for 26.3% of trait variance (Supplementary Fig. 8). The key attributes of this study were the large sample size and the homogeneity of the UK Biobank dataset, coupled with independent replication, independent association with clinically relevant radiographic endophenotypes and functional genomics follow-up in primary osteoarthritis tissue. We further capitalized on the wealth of available genome-wide summary statistics across complex traits to identify genetic correlations between osteoarthritis and multiple molecular, physiological and behavioral phenotypes, and we performed formal MR analyses to assess causality and disentangle complex cross-trait epidemiological relationships.

Table 3 | Genes in the osteoarthritis-associated signals with significantly different gene expression and/or protein abundance in intact versus degraded articular cartilage

| Index variant | Gene | Position (chromosome: start-end) | Distance from index variant (kb) | Proteomics logFC | Proteomics FDR q value | RNA-seq logFC | RNA-seq FDR q value |
|---------------|------|---------------------------------|---------------------------------|-----------------|------------------------|---------------|---------------------|
| rs3771501     | PCYOX1 | 2: 70484518–70508323            | 209.3                          | -0.27           | 0.0042                 | 0.27          | 0.0047              |
| rs3771501     | FAM136A | 2: 70523707–70529222            | 188.4                          | N/A             | N/A                    | -0.20         | 0.0066              |
| rs6516886     | BACHI | 21: 30566392–31003071           | 172.7                          | N/A             | N/A                    | 0.32          | 0.0019              |
| rs6516886     | MAP3K7CL | 21: 30449792–30548210         | 56.1                           | N/A             | N/A                    | 0.41          | 0.0021              |
| rs11780978    | BOPI | 8: 145486055–145515082          | 451.2                          | N/A             | N/A                    | -0.26         | 0.0030              |
| rs16682138    | PLAA | 9: 26904081–26947461            | 366                            | -0.07           | 0.601                  | 0.20          | 0.0027              |
| rs375757359   | ZNF382 | 19: 37095719–37119499          | 233.8                          | N/A             | N/A                    | 0.39          | 0.0031              |

logFC, log, fold change based on normalized values (increase indicates higher value in degraded cartilage); FDR, Benjamini-Hochberg FDR; N/A, proteomics data not available.

index was predicted to increase the risk of self-reported osteoarthritis by 1.11 (95% CI 1.07–1.15, P = 8.3 × 10⁻⁷). This result was consistent across MR methods (OR 1.52–1.66) and disease definition (OR 1.66–2.01). Consistent results were also observed for other obesity-related measures, such as waist circumference (OR 1.03 per cm increment; 95% CI 1.02–1.05, P = 5 × 10⁻⁴) and hip circumference (OR 1.03 per cm increment; 95% CI 1.01–1.06, P = 0.021). The OR values for type 2 diabetes liability and triglycerides were consistently small across estimators and osteoarthritis definitions; given that the analyses involving those traits were well powered (Supplementary Table 26), these results are compatible with either a weak or no causal effect. The results for years of schooling were not consistent across estimators, and there was evidence of directional horizontal pleiotropy, thus hampering any causal interpretation (Fig. 4). For lumbar-spine bone mineral density, there was evidence of a causal effect with OR per s.d. increment of 1.28 (95% CI 1.11–1.47, P = 0.002) for hip and/or knee osteoarthritis. This effect appeared to be site specific, with OR of 1.29 (95% CI 1.06–1.57, P = 0.014) for knee osteoarthritis, whereas the OR for hip osteoarthritis ranged from 0.71 to 1.57. There was also some evidence of a site-specific causal effect of height on knee osteoarthritis (OR 1.13 per s.d. increment; 95% CI 1.02–1.25, P = 0.023), which was consistent across estimators. One-sample MR analyses corroborated these findings, and obesity-related phenotypes presented strong statistical evidence after multiple-testing correction (Supplementary Table 27). These analyses did not detect reliable effects of smoking or reproductive traits on osteoarthritis (Supplementary Tables 28 and 29).

Most novel signals were at common frequency variants and conferred small to modest effects, in line with a highly polygenic model underpinning osteoarthritis risk. We identified one low-frequency variant associated with osteoarthritis (MAF 0.02) with a modest effect size (combined OR 1.34). Even though our study was well powered to detect such variants, we found no evidence of a role of low-frequency variation of large effect in osteoarthritis susceptibility (Supplementary Table 5). The power of this study was very limited for low-frequency variants with OR <1.50 and for rare variants. We estimate a requirement of up to 40,000 osteoarthritis cases and 160,000 controls to recapitulate the effects identified in this study at genome-wide significance, on the basis of the sample-size-weighted effect-allele frequencies and replication-cohort odds-ratio estimates (Table 1 and Supplementary Table 30).

We integrated functional information with statistical evidence for association to fine map the locations of likely causal variants and genes. All the predicted most likely causal variants resided in noncoding sequence: six were intronic, and three were intergenic. We were able to refine the association signal to a single variant in one instance, and to variants residing within a single gene in three instances, although the mechanisms of action may be mediated through other genes in the vicinity.

We empirically found self-reported osteoarthritis definition to be a powerful tool for genetic association studies, as evidenced, for example, by the genome-wide significance reached for the established GDF5 osteoarthritis locus in only the self-reported disease-status analyses. Published epidemiological studies investigating osteoarthritis via self-reporting⁶,⁷ and validation of self-reported status against primary-care records has yielded similar conclusions¹⁴. We also found very high genetic correlation between self-reported and hospital-diagnosed osteoarthritis, as well as similar variant-based heritability estimates, thus corroborating the validity of self-reported osteoarthritis status for genetic studies. However, we also note that the hospital-diagnosed-ostearthritis analyses had higher heritability and yielded stronger evidence of effect-direction concordance at established loci, thus indicating that larger sample sizes would afford the power required to convincingly detect the established loci. Hospital-diagnosed-ostearthritis data may potentially capture a different patient demographic than self-reported data (Supplementary Note). From the results of this study, we deduce that there is no gold standard for osteoarthritis definition in genetics studies, and we identified advantages in using both methods of defining disease to broadly maximize discovery power.

We identified strong genome-wide correlation between hip and knee osteoarthritis, thus indicating a substantial shared genetic etiology that has been hitherto overlooked. We therefore sought to replicate signals across these highly correlated phenotypes and...
to identify multiple instances of signals detected in the larger discovery analysis of osteoarthritis and independently replicated in joint-specific definitions of disease. Indeed, when examining the replication phenotypes, we found no instances of confirmed replication in which the replication phenotype was not captured within the accompanying discovery-phenotype definition. Further analysis in larger sample sets with precise phenotyping should help distinguish signal specificity.

Two of the newly identified signals, indexed by rs11780978 and rs2820436, resided in regions with established metabolic- and anthropometric-trait associations. Osteoarthritis is epidemiologically associated with high BMI, and the association is stronger for knee osteoarthritis. In line with this finding, we observed higher genetic correlation between BMI and knee osteoarthritis (rg = 0.52, P = 2.2 × 10^-11) compared with hip osteoarthritis (rg = 0.28, P = 4 × 10^-4). BMI is also known to be genetically correlated with...
education phenotypes, depressive symptoms, and reproductive and other phenotypes; hence, some of the genetic correlations for osteoarthritis observed here may be mediated through BMI. However, for the education and personality/psychiatric phenotypes, the strength of the genetic correlations observed here for osteoarthritis was substantially higher than that observed for BMI (for example, hospital-diagnosed osteoarthritis and years of schooling had $r_g = -0.45$, $P = 5 \times 10^{-27}$, whereas BMI and years of schooling had $r_g = -0.27$, $P = 9 \times 10^{-10}$; hospital-diagnosed osteoarthritis and depressive symptoms had $r_g = 0.49$, $P = 6 \times 10^{-7}$, whereas BMI and depressive symptoms had $r_g = 0.10$, $P = 0.023$). Epidemiologically, lower educational levels are known to be particularly associated with risk of knee osteoarthritis, even with adjustment for BMI$^{39}$.

MR provided further insight into the nature of the genetic correlations that we observed. In the case of BMI and other obesity-related measures, there was evidence of a causal effect of those phenotypes on osteoarthritis. This result corroborated findings from conventional observational studies$^{39}$, which are prone to important limitations (such as reverse causation and residual confounding) regarding causal inference$^{38}$. For all other exposure phenotypes, there was no convincing evidence of a causal effect on osteoarthritis risk, thus suggesting that the genetic correlations detected by linkage disequilibrium (LD)-score regression may be mostly due to horizontal pleiotropy, although for some phenotypes the MR analyses were underpowered (Supplementary Table 26). In the case of triglycerides and liability to type 2 diabetes, the MR analyses had sufficient power to rule out nonsmall causal effects, thus suggesting that these phenotypes have at most weak effects on osteoarthritis risk.

Importantly, structural changes in joints usually precede the onset of osteoarthritis symptoms. Articular cartilage is an avascular, aneurual tissue. It provides tensile strength, compressive resilience and a low-friction articulating surface. Chondrocytes are the only cell type in cartilage. The mode of function of noncoding DNA is linked to context-dependent regulation of gene expression, and identification of the causal variants and the genes that they affect requires experimental analysis of genome regulation in the proper cell type. Our functional analysis of genes in osteoarthritis-associated
regions and pathways identified differentially expressed molecules in chondrocytes extracted from degraded compared with intact articular cartilage. Cartilage degeneration is a key hallmark of osteoarthritis pathogenesis, and regulation of these genes may be implicated in disease development and progression.

Osteoarthritis is a leading cause of disability worldwide, and it imposes a substantial public-health and health economic burden. Here, we gleaned novel insights into the genetic etiology of osteoarthritis and implicated genes with translational potential. The cohorts contributing to this study were composed of European-descent populations. In the future, large-scale whole-genome-sequencing studies of well-pheno-typed individuals across diverse populations should capture the full allele frequency and variation spectrum, and afford further insights into the causes of this debilitating disease.

**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at [https://doi.org/10.1038/s41588-018-0079-y](https://doi.org/10.1038/s41588-018-0079-y).

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Association analyses: E. Zengini, K.H., I.T., L.S., J.S., S.H. and A.G. Mendelian randomization: F.P.H. and G.D.S. Functional genomics sample collection: A.McC., J.M.W. and E. Zeggini. Functional genomics analyses: J.S. and L.S. Endophenotype analyses: C.G.B., A.G.U. and J.B.J.v.M. Replication analyses: U.S., T.I., H.J., U.T. and K.S. Bioinformatics: A.G., D.S. and B.K. Student supervision: K.H., G.C.B., G.D.S., J.M.W. and E. Zeggini. Manuscript writing: E. Zengini, K.H., I.T., J.S., F.P.H., L.S., C.G.B., U.S., D.S., J.B.J.v.M., G.D.S., J.M.W. and E. Zeggini.

Competing interests

U.T., U.S. and K.S. are employees of deCODE genetics/Amgen.

Additional information

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Methods

Accuracy of self-reported data. We evaluated the classification accuracy of self-reported disease status by estimating the sensitivity, specificity, PPV and negative predictive values (NPV) in the self-reported and hospital-diagnosed disease-definition datasets. We performed a sensitivity analysis by varying the threshold for assigning self-reported disease status. For this purpose, we evaluated the true-negative rate by calculating the proportion of individuals diagnosed with osteoarthritis that were correctly identified as such in the self-reported analysis, then performed a specificity test to evaluate the true-negative rate by calculating the proportion of individuals not diagnosed with osteoarthritis that were correctly identified as such in the control set. The number of individuals overlapping between the self-reported ($n_s$ = 12,658) and hospital-diagnosed ($n_{hc}$ = 10,083) datasets was $n_{overlap}$ = 3,748. The total number of individuals was $n_{total}$ = 138,997. Sensitivity = $\frac{n_{overlap}}{n_{hc}}$, specificity = $\frac{n_{overlap}}{n_{s}}$, PPV = $\frac{n_{overlap}}{n_{s}}$, NPV = $\frac{n_{overlap}}{n_{hc}}$.

Discovery GWAS. UK Biobank’s scientific protocol and operational procedures were reviewed and approved by the North West Research Ethics Committee (REC reference no. 08/MRE08/65). The first UK Biobank release of genotype data included ~150,000 volunteers between 40 and 69 years old from the UK, genotyped at approximately 820,967 SNPs. 50,000 samples were genotyped with the UKBiLEVE array, and the remaining samples were genotyped with the UK Biobank Axiom array (Affymetrix; URLs). The UK Biobank Axiom SNP update of UKBiLEVE, and the two arrays share 95% content. In total, after sample and SNP quality control (QC), which was carried out centrally, 152,763 individuals and 806,466 directly typed SNPs remained. Phasing, imputation and derivation of principal components were also carried out centrally. Briefly, the combined UK10K/1000 Genomes Project haplotype reference panel was used to impute untyped variants for the UK Biobank Discovery GWAS. After imputation, the number of variants reached 73,355,667 in 152,249 individuals. We performed additional QC checks and excluded samples with call rate ≤ 97%. We checked samples for sex discrepancies, excess heterozygosity, relatedness and ancestry, and removed possibly contaminated and withdrawn samples. After QC, the number of individuals was 138,997. We excluded 528 SNPs that had been centrally flagged as being subject to exclusion due to failure in one or more additional quality metrics.

To define osteoarthritis cases, we used the self-reported status questionnaire and the Hospital Episode Statistics data (Supplementary Table 3 and Supplementary Note). We conducted five osteoarthritis-discovery GWAS and one sensitivity analysis. The case strata were as follows: self-reported osteoarthritis at any site, $n_{sas} = 12,658$; sensitivity analysis (a random subset of the self-reported cohort equal to the sample size of the hospital-diagnosed cohort), $n_{sas} = 10,083$; hospital-diagnosed osteoarthritis at any site, on the basis of IC10 and/or ICD9 hospital-record codes, $n_{hc} = 10,083$; hospital-diagnosed hip osteoarthritis, $n = 2,396$; hospital-diagnosed knee osteoarthritis, $n = 4,462$; and hospital-diagnosed hip and/or knee osteoarthritis, $n = 6,586$. We applied exclusion criteria to minimize misclassification in the control datasets to the extent possible (using approximately four times the number of cases for each definition) (Supplementary Table 2 and Supplementary Fig. 1). We restricted the number of controls used and did not use the full set of available genotyped control samples from UK Biobank to guard against association with the statistical behavior of conservatively estimating the presence of stark case–control imbalance for alleles with minor allele count (MAC) < 400 (ref. 41) (analogous to MAF of < 0.02 in the self-reported and hospital-diagnosed osteoarthritis datasets). For the control set, we excluded all participants participated all participants with any musculoskeletal disorder or having relevant symptoms or signs, such as pain and arthritis, and we selected older participants to ensure that we minimize the number of controls that were diagnosed with osteoarthritis in the future, while keeping the number of males and females balanced (Supplementary Table 1).

At the SNP level, we further filtered for Hardy–Weinberg equilibrium $P < 10^{-8}$, MAF ≤ 0.001 and info score < 0.4 (Supplementary Fig. 1). We tested for association by using the frequency-matched ratio test (LM and method.ml in SNPTEST v2.5.2 (ref. 42)) with adjustment for the first ten principal components to control for population structure. Power calculations were carried out in Quanto v2.5.2 (URLs).

Replication. Two hundred independent and novel variants with $P < 1.0 \times 10^{-6}$ in the discovery analyses were used for in silico replication in an independent cohort from Iceland (deCODE) through fixed-effects inverse-variance-weighted meta-analysis in METAL. One hundred seventy-three variants were present in the replication cohort. The number of variants had ambiguous alleles (i.e., those incompatible because of alignment issues) and were not included in further analyses. The significance threshold for association in the replication stage was hence $0.05/173 = 2.9 \times 10^{-5}$. The deCODE dataset comprised four osteoarthritis phenotypes: any osteoarthritis site (18,069 cases and 246,293 controls), hip osteoarthritis (5,714 cases and 199,421 controls), knee osteoarthritis (4,672 cases and 172,791 controls) and hip and/or knee osteoarthritis (9,429 cases and 199,421 controls). We performed meta-analyses (association tests or osteoarthritis definitions), using summary statistics from the UK Biobank osteoarthritis analyses and deCODE. We used $P < 2.8 \times 10^{-5}$ as the threshold corrected for the effective number of traits to report genome-wide significance.

Association with osteoarthritis-related endophenotypes. The nine replicating genetic loci were examined for association in radiographic osteoarthritis endophenotypes. This examination was done for three endophenotypic joint-space width (mJSW) and two measures of hip-shape deformities known to be strong predictors of osteoarthritis: acetabular dysplasia (measured with center-edge (CE) angle) and cam deformity (measured with alpha angle). For mJSW, association statistics for the variants were looked up in a previously published GWAS, which performed joint analysis of data from Rotterdam Study I (RS-I), Rotterdam Study II (RS-II), TwinsUK, SOF and MRos by using standardized age-, sex- and population stratification (four principal components)-adjusted residuals from linear regression. For the two hip-shape phenotypes, CE angle and alpha angle were measured as previously described. CE angle was analyzed as a continuous trait in the GWAS and the most strongly associated variants were reviewed and approved by the North West Research Ethics Committee and the National Bioethics Committee of Iceland. Informed consent was obtained from all participants.

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mapping of gene name and Ensembl ID, we used 1,705 genes with significantly different abundance between intact and degraded cartilage at 1% FDR.

Fine mapping. We constructed regions for fine mapping, by taking a window of at least 0.1 cM to either side of each index variant. The region was extended to the furthest variant with \( r^2 > 0.1 \) with the index variant within a 1-Mb window. LD calculations for extending the region were based on whole-genome-sequenced EUR samples from the combined reference panel from UK10K and the 1000 Genomes Project. For each region we implemented the Bayesian mode-averaging method CAVIARBF\(^{41}\), which uses association summary statistics and correlations among variants to calculate Bayes’ factors and the posterior probability of each variant being causal. We assumed a single causal variant in each region and calculated 95% credible intervals, which contained the minimum set of variants jointly having at least 95% probability of including the causal variant. We also applied the extended CAVIARBF method, which uses functional annotation scores to upweight variants according to their predicted functional scores. To this end, we downloaded precalculated CADD\(^{53}\) and Eigen\(^{54}\) scores from their equivalent websites. We observed better separation of severe-consequence genic variants with the CADD score and better separation of regulatory variants with the Eigen score, and we therefore created a combined score in which splice-acceptor, splice-donor, stop-loss, stop-gain, missense and splice-region variants were assigned their CADD-PheRed scores, and the rest were assigned their Eigen-PheRed scores.

Functional enrichment analysis. We used genome-wide summary statistics to test for enrichment with functional annotations. We used GARFIELD\(^{21}\), with the customized functional annotations, making use of the functional genomics data that we generated in primary articular chondrocytes by using RNA sequencing and quantitative proteomics. We defined differentially expressed genes separately at the RNA (transcriptional) level and at the protein level when comparing intact and degraded cartilage (1% FDR). We extended each differentially regulated gene by 5kb on each side. Using GARFIELD’s approach, we calculated the effective number of independent annotations to be 1,995, which led to an adjusted \( P \)-value significance level of 0.025. We tested for enrichment by using variants with \( P < 1.0 \times 10^{-5} \), and no analysis surpassed the corrected significance threshold.

LD regression. We used LDHub\(^{40} \) (accessed 23–27 January 2017) to estimate the genome-wide genetic correlation between each of the osteoarthritis definitions and 219 other human traits and diseases. In each analysis, we extracted variants with r2s (range 11999963–15561966) and uploaded the corresponding association summary statistics to LDHub; the analysis yielded 896,076–1,172,130 variants overlapping with LDHub. We corrected for multiple testing by defining significance at 5% Benjamini–Hochberg FDR for each of the five osteoarthritis analyses.

Mendelian randomization analysis. We used MR to assess the potential causal role of the phenotypes identified in the LD-score regression analysis on osteoarthritis. We also included birth weight and height (Supplementary Table 22). In all analyses, the primary outcome variable was self-reported osteoarthritis. We used data from hospital records (which were available for a much smaller number of individuals) as sensitivity analyses and to identify potential site-specific effects.

Data sources. Genetic instruments were identified from publicly available summary GWAS results through the TwoSampleMR R package, which allows for extraction of the data available in the MR-Base database\(^1\). Only results that combined both sexes were extracted. Preference was given to studies restricted to European populations to minimize the risk of bias due to population stratification; however, for several traits, those results were either not available or corresponded to much smaller studies (Supplementary Table 22). However, this aspect was unlikely to substantially bias the results, because all studies used correction methods, and even multiancestral studies were composed of mostly European populations. The exception was for number of children born and age of the individual at birth of the first child: because the GWAS of reproductive traits by Barban and colleagues\(^39\) was not available in MR-Base, we extracted association results from the variants that achieved genome-wide significance directly from the paper and used coefficients from each sex in sex-specific analyses. The search was performed on 19 June, 2017. For each trait, all genetic instruments achieved the conventional levels of genome-wide significance (i.e., \( P < 5.0 \times 10^{-8} \)) and were mutually independent (i.e., \( r^2 < 0.001 \) between all pairs of instruments).

Two-sample MR. For the exposure phenotypes with at least one genetic instrument available, we used two-sample MR analysis to evaluate their causal effects on osteoarthritis risk. The exceptions were smoking and reproductive traits, which were performed with one-sample MR only, because of the need to perform the analysis within specific subgroups. All summary association results used for two-sample MR are shown in Supplementary Table 23, and Supplementary Table 24 provides an overall description of each set of genetic instruments. We applied the following methods:

- Ratio method. For exposure phenotypes with only one genetic instrument available, MR was performed with the ratio method, which consists of dividing the instrument-outcome regression coefficient by the instrument-exposure regression coefficient. The standard error of the ratio estimate can be calculated by dividing the instrument-outcome standard error by the instrument-exposure regression coefficient. Confidence intervals and \( P \) values were calculated with the normal approximation.

- Inverse-variance weighting (IVW). This method allows for combination of the ratio estimates from multiple instruments into a single pooled estimate. We used a multiplicative random-effects version of the method, which incorporates between-instrument heterogeneity in the confidence intervals.

- MR–Egger regression. This method yields consistent causal-effect estimates even if all instruments are invalid, provided that horizontal pleiotropic effects are uncorrelated with instrument strength (i.e., the instrument strength independent of direct effects InSIDE assumption holds).

Weighted median. This method allows for consistent causal-effect estimation even if the InSIDE assumption is violated, provided that up to (but not including) 50% of the weights in the analysis come from invalid instruments.

Mode-based estimate (MBE). The weighted MBE relies on the zero modal pleiotropy assumption (ZEMPA), which postulates that the largest subgroup (or the subgroup carrying the largest amount of weight in the analysis) of instruments that estimate the same causal-effect estimate is composed of valid instruments. This procedure allows for consistent causal-effect estimation even if most instruments are invalid. The stringency of the method can be regulated by the \( \phi \) parameter. We tested two values of \( \psi \), \( \psi = 1 \) (i.e., the default) and \( \psi = 0.5 \) (half the default, or twice as stringent).

For exposure phenotypes with more than one but fewer than ten genetic instruments, only the IVW method was applied, because the remaining methods are typically less powered and require a relatively large number of genetic instruments to provide reliable results. The degree of weak instrument bias (which corresponds to regression-dilution bias in two-sample MR) for the IVW and MR–Egger methods was quantified with the \( I^2 \)\(^{10} \) statistic, respectively.

Both range from 0% to 100%, and \( 100(1-I^2)\% \) and \( 100(1-I^2_{\text{GXE}})\% \) can be interpreted as the amount of dilution in the corresponding causal-effect estimates. Given that only genome-wide-significant variants were selected as instruments, the \( I^2 \)\(^{10} \) statistic was necessarily high (at least ~95%). However, the \( I^2 \)\(^{10} \) statistic depends on both instrument strength and heterogeneity between instrument-exposure associations, thus suggesting that regression dilution bias in MR–Egger can exists even if instruments are individually strong. Indeed, for some traits, the \( I^2 \)\(^{10} \) statistic was very low (Supplementary Table 24). Therefore, all MR–Egger regression analyses were corrected for regression dilution with a simulation extrapolation (SIMEX) approach.

Horizontal pleiotropy tests. We additionally assessed the robustness of our findings to potential violations of the assumption of no horizontal pleiotropy by applying two tests of horizontal pleiotropy. One test was the MR–Egger intercept, which can be interpreted as the average instrument-outcome coefficient when the instrument-exposure coefficient is zero. If there is no horizontal pleiotropy, the intercept should be zero. Therefore, the intercept provides an indication of overfitting horizontal pleiotropy. The second test was Cochran’s Q test of heterogeneity, which relies on the assumption that all valid genetic instruments estimate the same causal effect.

Power calculations. We performed power calculations to estimate the power of our two-sample MR analysis to detect odds ratios of 1.2, 1.5 and 2.0 (Supplementary Note).

One-sample MR. UK Biobank data were used to perform one-sample MR with the same genetic instruments as in the two-sample MR (Supplementary Note).

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. All RNA-sequencing data have been deposited in the European Genome/Phenome Archive (cohort 1, EGAD00001001331; cohort 2, EGAD00001003354; cohort 3, EGAD00001003355).

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1. Sample size
   Describe how sample size was determined.

We used the data provided by the interim release of the UK Biobank resource, which is a large population-based study of over 152,000 genotyped subjects, to conduct the largest osteoarthritis (OA) genome-wide association study (GWAS) to date. We defined 5 OA case strata based on both self-reported status and through linkage to Hospital Episode Statistics data, and on joint-specificity of disease (knee and/or hip). We used the full set of available OA cases with directly genotyped and imputed data. Non-OA controls were drawn from the same UK Biobank dataset. We applied exclusion criteria to minimise misclassification in the control dataset which was selected to be ~4x the number of cases to preserve power for common alleles while avoiding case:control imbalance causing association tests to misbehave for low frequency variants. For each of the 5 OA definitions, we performed power calculations and found that we have >80% power to detect variants at genome-wide significance (P<5x10^-8) with modest effect size (odds ratio of 1.15 to 1.50) for common variants (minor allele frequency (MAF) 0.5 to 0.2). The power of this study is very limited for low frequency variants with OR<1.50, and for rare variants.

2. Data exclusions
   Describe any data exclusions.

Sample and variant quality control (QC) was carried out centrally. We performed additional QC checks with exclusion criteria as follows: i) call rate ≤97% ii) gender discrepancies, iii) excess heterozygosity, iv) duplicates and/or high relatedness, v) ethnicity outliers, vi) possibly contaminated and vii) withdrawn samples. We excluded 528 SNPs that had been centrally flagged as subject to exclusion due to failure in one or more additional quality metrics. At the SNP level, we further filtered for Hardy Weinberg equilibrium (HWE) P≤10^-6, MAF≤0.001 and info score<0.4. All the above are typical QC steps for quality assurance in GWAS data. We also restricted the number of controls used and did not utilise the full set of available genotyped control samples from UK Biobank in order to guard against association test statistics behaving anti-conservatively in the presence of stark case: control imbalance for alleles with minor allele count (MAC) <400.

3. Replication
   Describe whether the experimental findings were reliably reproduced.

Our findings were reliably reproduced. We took 173 variants with P<1.0x10^-5 and MAF>0.01 forward to replication in an independent cohort from Iceland (deCODE) of up to 18,069 cases and 246,293 controls. Following meta-analysis in up to 30,727 cases and 297,191 controls, we report seven genome-wide significant associations at novel loci, and two further new replicating signals just below the genome-wide significance threshold (P<6.0x10^-8).

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

Randomization of experimental groups were not required to this study. Participants were allocated into experimental groups according to their OA...
5. Blinding
Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not applicable to this study.

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 the Methods section if additional space is needed).

n/a

- [x] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- [x] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
- [x] A statement indicating how many times each experiment was replicated.
- [x] 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).
- [x] A description of any assumptions or corrections, such as an adjustment for multiple comparisons.
- [x] The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted.
- [x] A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range).
- [x] Clearly defined error bars.

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

confirmed

Software
Policy information about availability of computer code

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

We used SNPTEST v2.5.245 for association analysis. Power calculations were carried out using Quanto v1.2.4. We performed a fixed effects inverse-variance weighted meta-analysis in METAL. The results of the discovery cohorts of from RS-I, RS-II and CHECK were quality checked using EASYQC. To investigate the narrow sense heritability and the genetic correlation between the five osteoarthritis disease definitions, we ran the LDscore method. We tested proteins for differential abundance using limma in R. The cram rna-seq files were converted to bam files using samtools 1.3.157 and then to fastq files using biobambam 0.0.191. We obtained transcript-level quantification using salmon 0.8.259 and the GRCh38 cDNA assembly release 87 downloaded from Ensembl. We used tximport to convert transcript-level to gene-level count estimates. Limma-voom was used to remove heteroscedasticity from the estimated expression data. We tested genes for differential expression using limma in R. For fine-mapping we used CAVIARBF. We used GARFIELD for functional enrichment analysis. Gene-based and gene-set analyses were performed using MAGMA v1.06. We used DAPPLE for visualization of the pathways and protein-protein interaction (PPIs) relationships among the genes in each gene-set by integrating data from the InWeb database. LDHub was used to estimate the genome-wide genetic correlation between each of the OA definitions and 219 other human traits and diseases. In Mendelian randomization (MR) analysis, genetic instruments were identified from publicly-available summary GWAS results through the TwoSampleMR R package, which allows extracting the data available in the MR-Base database.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The Nature Methods guidance for providing algorithms and software for publication may be useful for any submission.
Materials and reagents

Policy information about availability of materials

8. 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.
   No unique materials used.

9. Antibodies
   Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).
   No antibodies were used.

10. Eukaryotic cell lines
    a. State the source of each eukaryotic cell line used.
    b. Describe the method of cell line authentication used.
    c. Report whether the cell lines were tested for mycoplasma contamination.
    d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
    No eukaryotic cell lines were used.

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.
    Animals were not used in this study.

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.
    This information can be found in the online methods:
    1) Discovery GWAS
    2) Replication
    3) Association with OA-related endophenotypes
    4) Functional genomics
    Consent was obtained for each individual as stated in each of the above sections.