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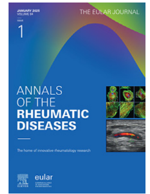

Osteoarthritis

# The epigenomic landscape of primary tissues in osteoarthritis

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ABSTRACT

*Objectives:* Osteoarthritis is a complex joint disease, affecting more than 500 million people worldwide. We investigated DNA methylation profiles and integrated these with matching genotype and transcriptomics data in primary tissues of patients with knee osteoarthritis to enhance our understanding of the regulatory framework that modulates gene expression in osteoarthritis.
*Methods:* We measured matched genotype and methylation from primary chondrocytes of macroscopically intact (low-grade) and degraded (high-grade) disease cartilage as well as synovium, infrapatellar fat pad, and blood samples, for 314 patients who underwent total knee replacement for osteoarthritis. We generated methylation quantitative trait locus (mQTL) maps in cartilage, synovium, fat pad, and blood, as well as expression quantitative trait methylation (eQTM) maps in cartilage and synovium. We integrated these results with genome-wide association studies (GWAS) to identify likely effector genes of osteoarthritis.
*Results:* We reported widespread associations between genetic variants and methylation as well as between methylation and gene expression. Through colocalisation, we find methylation sites with a potential causal role in osteoarthritis. By tissue-specific integration of colocalisation and eQTM results, we resolved GWAS signals and identified 50 likely effector genes.
*Conclusions:* We provided the largest genome-wide mQTL maps of low- and high-grade osteoarthritis cartilage and synovium. We further presented the largest cartilage eQTM map for primary cartilage and the first eQTM map in synovium. We found methylation sites with a potential mechanistically causal role in osteoarthritis and associated these with likely effector genes. Together, the presented genome-wide eQTM and mQTL maps constitute relevant resources for osteoarthritis research and beyond.

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**WHAT IS ALREADY KNOWN ON THIS TOPIC**

- Osteoarthritis is a prevalent joint disease with no curative therapy.
- Genome-wide association studies (GWAS) have identified osteoarthritis risk loci, but enhanced molecular profiles of primary osteoarthritis tissues are required to link these loci to effector genes in a tissue- and disease-specific manner.

**WHAT THIS STUDY ADDS**

- By combining whole-genome sequencing, DNA methylation, and gene expression data from 314 patients with osteoarthritis with GWAS results, we suggested 50 likely effector genes of genetic risk loci, including 19 novel ones.

**HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY**

- We prioritised novel targets for further osteoarthritis research.
- We provided publicly available, genome-wide maps of genetic effects on DNA methylation, as well as effects between DNA methylation and gene expression across relevant osteoarthritis tissue types.

## INTRODUCTION

Osteoarthritis is a complex joint disease, affecting more than 500 million people worldwide [1], but despite its high prevalence, treatment methods are limited to pain management and total joint replacement. To promote the development of novel, personalised treatment strategies, it is important to better understand the genetic and molecular basis of osteoarthritis. Large genome-wide association studies (GWAS) have generated insights into its complex genetic architecture and found 962 risk loci for osteoarthritis [2]. However, in order to reveal molecular pathways along which genetic variants exert osteoarthritis-relevant effects, it is necessary to link these genetic insights to molecular profiles of primary tissues [3−5].

A relevant regulatory molecular layer is DNA methylation, an epigenetic mark describing the covalent addition of a methyl group to the DNA that has a regulatory role on gene expression. Previous work [6,7] combined DNA methylation of joint primary tissues with genotype data, matched from the same patients with osteoarthritis, to examine genetic effects on methylation, thus providing genome-wide methylation quantitative trait locus (mQTL) maps. These maps have been generated in low-grade (n = 90 patients) [6] and high-grade osteoarthritis cartilage (n = 89 patients) [6], synovium (n = 78 patients) [6], as well as infrapatellar fat pad (n = 68 patients) [7], and constitute a valuable data resource of these tissue types to enable investigation of the causal roles of epigenetic markers in osteoarthritis. Beyond these insights into joint tissues, other osteoarthritis-relevant epigenetic studies have focused on blood methylation profiles of patients with osteoarthritis, in particular on building methylation-based classifiers to predict knee osteoarthritis progression [8].

However, genome-wide mQTL studies in osteoarthritis tissues have been limited in sample size [9] (Supplementary Fig S1). Furthermore, there is a need for genome-wide association maps between molecular layers, particularly between DNA methylation and gene expression in osteoarthritis-relevant tissues.

Here, we investigated primary osteoarthritis tissue samples of 314 patients to (1) map effects of genotype on DNA methylation and identify mQTL, (2) determine associations between gene expression and DNA methylation (expression quantitative trait methylation; eQTM), and (3) apply integrative approaches to find likely effector genes for genetic risk loci.

## METHODS

For full details of methods, see Supplementary Methods.

### *Osteoarthritis-affected individuals and study samples*

We studied data from 314 patients who underwent total knee replacement due to osteoarthritis (mean age, 70.57 years; age range, 38-93 years; 181 women and 133 men; mean body mass index [BMI], 32.23 kg/m$^2$; BMI range, 20-51 kg/m$^2$; Supplementary Table S1). Inclusion/exclusion criteria were primary osteoarthritis of the affected knee, without use of drugs known to affect bone turnover, such as steroids and bisphosphonates, within 6 months of surgery, and no current cancer diagnosis. Cartilage samples were graded according to the International Cartilage Repair Society (ICRS) macroscopic scoring system (low-grade osteoarthritis cartilage, ICRS score 0 or 1; high-grade cartilage osteoarthritis, ICRS score 3 or 4) [10]. This work was approved by the Oxford National Health Service Research Ethics Committee C (10/H0606/20, 15/SC/0132, and 20/SC/0141), and samples were collected under Human Tissue Authority licence 12182, Sheffield Musculoskeletal Biobank, University of Sheffield, UK. Before participating in the study, all osteoarthritis-affected individuals provided written, informed consent.

### *Sample extraction*

Knee chondrocytes and synoviocytes were isolated by following previously published protocols (Methods, ‘Isolation of chondrocytes’ and ‘Isolation of synoviocytes’ sections) [5]. Peripheral blood samples were stored at −80 °C prior to DNA extraction using the QIAamp DNA Blood Maxi Kit [7].

### *Whole-genome sequencing preprocessing*

Whole-genome sequencing (WGS) data preprocessing and variant, as well as sample-level quality control, were described previously [7] (Supplementary Methods).

### *DNA methylation data*

Genome-wide DNA methylation data were obtained from primary chondrocytes of macroscopically intact (low-grade) and degraded (high-grade) cartilage as well as synovium, infrapatellar fat pad, and blood samples. DNA methylation was measured using the Illumina Infinium MethylationEPIC array technology. We preprocessed methylation data from all tissues together using an R package, meffil (https://github.com/perishky/meffil/wiki) [11] (Supplementary Methods). Preprocessing fat pad methylation data and follow-up methylation cis-mQTL analysis have also been described previously [7].

### *Gene expression data*

Gene expression of the same patient cohort was collected and preprocessed as described previously [12]. Of note, tissue-specific RNA sequencing count matrices were normalised using the Trimmed Mean of M-values method. We then retained gene expression data for patients (low-grade cartilage, 227 patients and 16,991 genes; high-grade cartilage, 127 patients and 16,991 genes; synovium, 226 patients and 15,628 genes; fat pad, 45 patients and

15,726 genes) with matching DNA methylation data and performed inverse normal transformation per tissue. We then tissue-specifically estimated PEER factors using the R package peer [13], which we accounted for in the eQTM analysis.

### *Cis-mQTL analysis*

We performed cis-mQTL analysis (cis distance, 1 Mb on either side of the tested methylation site) using FastQTL (https://github.com/francois-a/fastqtl/) [14] in low- (289 patients) and high-grade osteoarthritis cartilage (172 patients), synovium (249 patients), and blood (237 patients) (Supplementary Table S1). We further compared these results with the previously published cis-mQTL map in the infrapatellar fat pad of this patient's cohort (68 patients [7]) to find signals that are tissue-specific and shared between joint tissues (Supplementary Methods).

### *Trans-mQTL analysis*

We performed trans-mQTL analysis using the R package MatrixEQTL [15] in low- and high-grade osteoarthritis cartilage, synovium, and infrapatellar fat pad, as well as blood, using the same methylation and WGS data matrices, as well as including the same covariates in the models as in the cis-mQTL analysis ('Cis-mQTL analysis' section). We considered methylation site-gene pairs of methylation sites and gene transcription starting sites on different chromosomes or with a commonly used [16,17] distance of >5 Mb. To correct for multiple testing, we chose genetic variants with the lowest nominal *P* value per methylation site. Next, we multiplied these nominal *P* values by $10^6$ to correct for the number of independent tests when studying common (minor allele frequencies (MAF) >5%) genetic variants on a genome-wide scale. We then performed false discovery rate (FDR) correction on these corrected *P* values to account for tests across methylation sites, regarding methylation sites with q value <5% Storey-Tibshirani FDR as trans-mQTL-targeted [18]. Finally, we considered the largest nominal *P* value of significant mQTL (q value < 0.05) and the lowest nominal *P* value of not significant ones (q value ≥ 0.05) and calculated the average of these 2 nominal *P* values to define a nominal *P* value threshold for trans-mQTLs.

### *Colocalisation analysis*

We performed colocalisation analysis to identify methylation sites that mediate genetic risk for osteoarthritis by integrating mQTL (for low- and high-grade cartilage, synovium, infrapatellar fat pad, and blood) and GWAS summary statistics [2] for knee osteoarthritis (172,256 cases and 1,144,244 controls), total knee replacement (48,161 cases and 958,463 controls), and osteoarthritis at any joint site (489,975 cases and 1,472,094 controls), all aligned to GRCh38. Using the *coloc* R package [19], we ran coloc.abf() to estimate the probability that the same causal variant affects both DNA methylation and osteoarthritis risk. More specifically, we considered a 100 kb ($1 \times 10^5$ bp) window around the GWAS lead variant. We performed colocalisation for mQTL-targeted methylation sites (q value ≤ 0.05) if the methylation site-specific mQTL index variant was located within this 100 kb window. For the actual analysis, we considered variants within this 100 kb window for which GWAS and cis-mQTL summary statistics were available. A signal was considered significant when the posterior probability that the methylation site and the GWAS signal share the same causal variant exceeded 80% (PP4 >80%).

### *eQTM analysis*

We further integrated methylation and gene expression data matched from the same patients to perform eQTM analysis, thus finding associations between gene expression and methylation levels of close methylation sites (<1 Mb between transcription start site and methylation site). We performed eQTM analysis tissue-specifically in low- (227 patients) and high-grade (127 patients) osteoarthritis cartilage, synovium (226 patients), and infrapatellar fat pad (45 patients) using the R package MatrixEQTL [15] (Supplementary Methods; Supplementary Table S1).

### *Effector gene analysis*

To identify potential effector genes of GWAS risk signals, we overlaid colocalisation results with eQTM maps of the same tissues. More specifically, we tested whether methylation sites with a potential causal role in osteoarthritis (colocalisation posterior probability 'PP4' > .8) are significantly associated with a gene (eQTM q value < 0.05) in the same tissue.

To assess novelty, we tested whether identified likely effector genes have been found in a previous large meta-GWAS for osteoarthritis [2], which integrates 24 orthogonal lines of evidence and reports 700 effector genes (≥3 lines of evidence). If likely effector genes are within these 700, we regard them as confirmed; otherwise, as novel. We further used this effector gene analysis resource [2] to investigate whether mQTL signals have been colocalised with osteoarthritis GWAS risk signals before.

To assess the respective druggability of identified likely effector genes, we overlaid these with the OpenTarget (version 25.09) [20] database using the R package otargen (function: knownDrugsGeneQuery) [21] and filtered for drugs that completed clinical trial phase 2.

### *Data visualisation*

We used the R packages ggplot2 (version 3.4.3) and ggpubr (version 0.4.0) to generate Figures 1, 2, and 3 (Supplementary Methods).

### *Patient and public involvement statement*

There was no involvement of patients and the public in the design, conduct, reporting, or dissemination plans of this research.

## RESULTS

### *Genome-wide mQTL maps in tissues from patients with primary osteoarthritis*

We performed mQTL analysis to study the effect of common variants (MAF > 0.05) on methylation in low- (n = 289 patients) and high-grade osteoarthritis cartilage (n = 172), as well as in synovium (n = 249) and blood (n = 237). We found widespread associations (q value < 0.05) between genetic variants and close (<1 Mb; 'cis-mQTL') methylation sites in all tested tissues: low- (207,422 mQTL-targeted methylation sites; Fig 1A; most significant mQTL in Fig 1B) and high-grade cartilage (128,876; Fig 1C; most significant mQTL in Fig 1D), synovium (160,257; Fig 1E; most significant mQTL in Fig 1F) and blood (127,233; Fig 1G; most significant mQTL in Fig 1H).

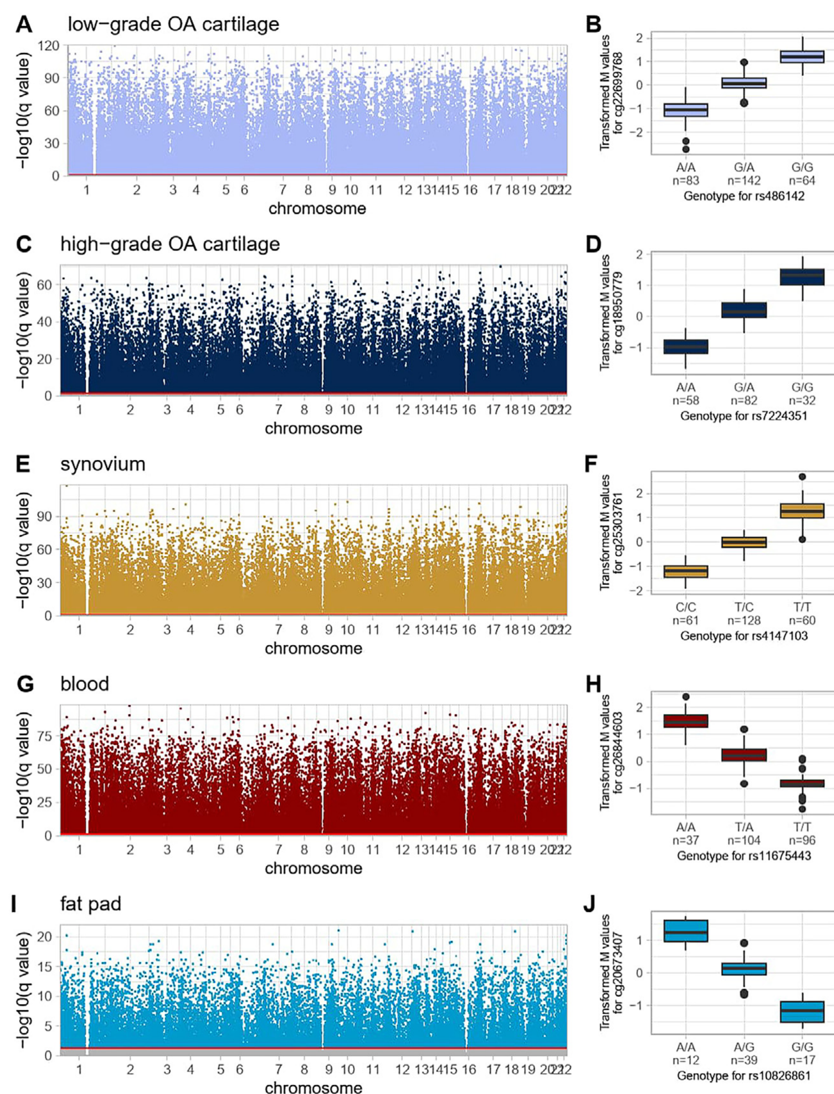


**Figure 1.** Methylation quantitative trait locus (mQTL) landscapes in cartilage, synovium, blood and fat pad in patients with osteoarthritis. The cis-mQTL maps in (A) low- and (C) high-grade osteoarthritis cartilage, as well as in (E) synovium, (G) blood and (I) fat pad in patients with osteoarthritis. Manhattan plots showing the negative log of the *q* value of the most significant association per methylation site across genetic variants in cis (<1 Mb distance to the tested methylation site). Quantitative trait locus (QTL)-targeted methylation sites (q value < 0.05) are coloured light blue (A), dark blue (C), yellow (E), red (G), and blue (I), otherwise dark grey. Red lines indicate genome-wide significance (q < 0.05). The boxplots exemplify mQTL signals by showing the most significant effects in (B) low-grade osteoarthritis crtilage (rs486142 on cg22699768, with beta = 1.27, SE = 0.02, *P* value = $3.59 \times 10^{-142}$), (D) high-grade osteoarthritis cartilage (rs7224351 on cg18950779, with beta = 1.21, SE = 0.03, *P* value = $2.40 \times 10^{-89}$), (F) synovium (rs4147103 on cg25303761, with beta = 1.36, SE = 0.02, *P* value = $6.35 \times 10^{-140}$), (H) blood (rs11675443 on cg26844603, with beta = −1.22, SE = 0.02, *P* value = $1.75 \times 10^{-118}$) and (J) fat pad (rs10826861 on cg20673407, with beta = −1.40, SE = 0.05, *P* value = $4.15 \times 10^{-33}$). Of note, the cis-mQTL fat pad map has been published previously [7], and Figures (I) and (J) were included in this earlier work. The boxplots represent the 25th, 50th, and 75th percentiles, and whiskers extend to 1.5 times the IQR. OA, osteoarthritis.

Furthermore, we have generated the first cis-mQTL map in infrapatellar fat pad samples (n = 68 patients; 35,948 cis-mQTL-targeted methylation sites; Fig 1I; most significant mQTL in Fig 1J), which was published previously [7].

In low- and high-grade cartilage as well as synovium, we found cis-mQTL-targeted methylation sites that were not identified in the previous largest mQTL maps [6] in these tissues (low-grade osteoarthritis cartilage, 162,314 of 207,422 quantitative trait locus (QTL)-targeted methylation sites; high-grade osteoarthritis cartilage, 99,818 of 128,876; synovium, 136,315 of 160,257), underlining the novelty of these QTL maps and ultimately the expanded regulatory landscape uncovered by these maps.

We performed follow-up comparative analyses by overlapping cis-mQTL-targeted methylation sites (q < 0.05) in low- and high-grade osteoarthritis cartilage, synovium, and fat pad, identifying signals that are tissue-specific and shared between joint tissues (Supplementary Note S1; Supplementary Fig S2).

We further tested for novel long-range effects of genetic variants on DNA methylation. We found trans-mQTL effects in low- (2836 trans-mQTL-targeted methylation sites; 439 and 2424 DNA methylation sites being targeted by long-range intrachromosomal (>5 Mb) and interchromosomal effects, respectively; $P < 1.82 \times 10^{-10}$; Supplementary Table S2) and high-grade osteoarthritis cartilage (1488 trans-mQTL-targeted methylation sites; 216 and 1279 DNA methylation sites being targeted by long-range intrachromosomal and interchromosomal effects, respectively; $P < 9.55 \times 10^{-11}$; Supplementary Table S3), synovium (1999 trans-mQTL-targeted methylation sites; 307 and 110 DNA methylation sites being targeted by long-range intrachromosomal and interchromosomal effects, respectively; $P < 1.28 \times 10^{-10}$; Supplementary Table S4), blood (1993 trans-mQTL-targeted methylation sites; 260 and 1746 DNA methylation sites being targeted by long-range intrachromosomal and interchromosomal effects, respectively; $P < 1.27 \times 10^{-10}$; Supplementary Table S5) and infrapatellar fat pad (191 trans-mQTL-targeted methylation sites; 25 and 166 DNA methylation sites being targeted by long-range intrachromosomal and interchromosomal effects, respectively; $P < 1.29 \times 10^{-11}$; Supplementary Table S6). Together, these results constitute the largest mQTL data sets for knee osteoarthritis to date and reveal QTL effects in both the short and long-distance ranges.

### *Genome-wide eQTM maps in tissues from patients with primary osteoarthritis*

Next, we integrated DNA methylation with available gene expression data [12] from the same patient cohort and tissue types. We generated eQTM maps to associate methylation sites and expression levels of their proximal genes (<1 Mb) in low- (n = 227 patients) and high-grade osteoarthritis cartilage (n = 127 patients), as well as synovium (n = 226 patients) and infrapatellar fat pad (n = 45). In total, we found 3270 eQTM-targeted genes (q < 0.05) across joint tissues, which showed enrichments (FDR < 0.05) in 100 gene ontology (GO) terms

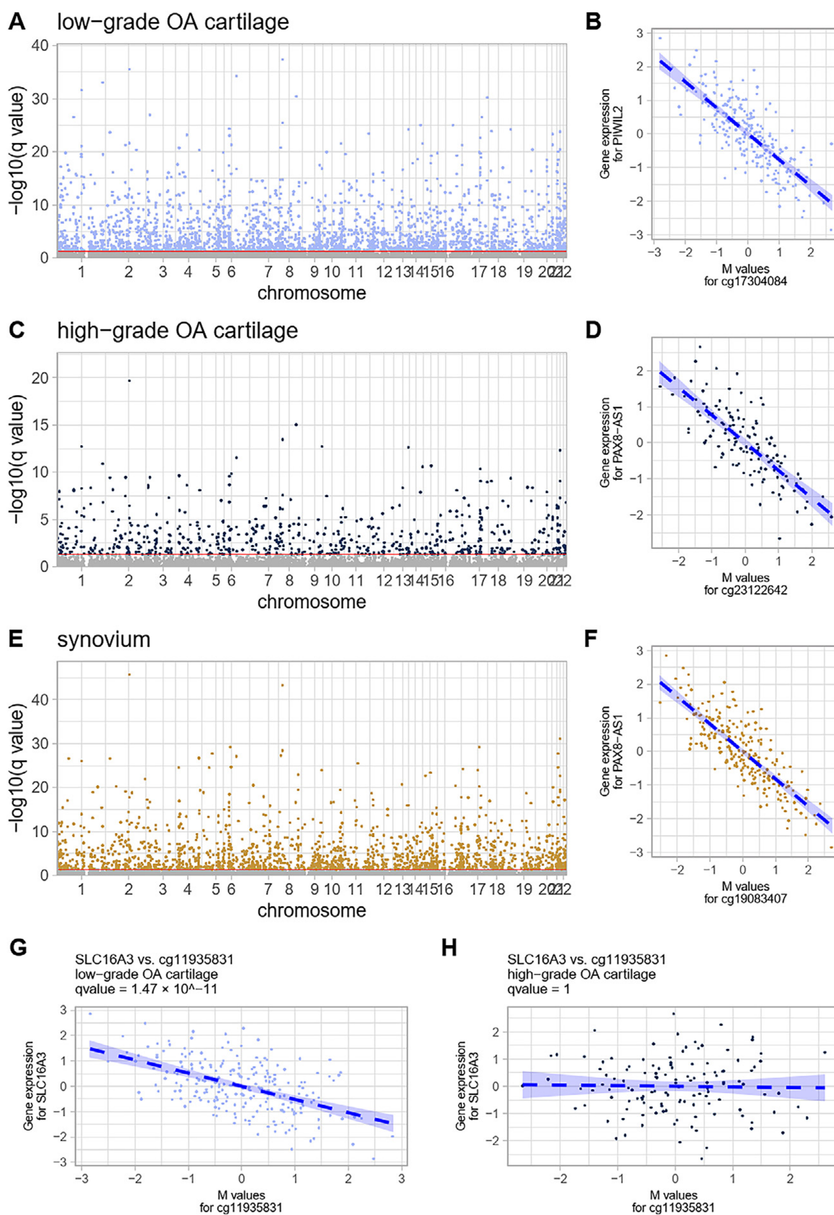


**Figure 2.** Expression quantitative trait methylation (eQTM) landscapes in cartilage and synovium. The eQTM maps and respective top signals in (A) low- and (C) high-grade osteoarthritis cartilage, as well as in (E) synovium. Manhattan plots showing the negative log of the q value of the most significant association per gene across all tested methylation sites in cis (<1 Mb distance to the tested gene transcription starting site). Methylation-associated genes are shown in light violet (A), dark violet (C), or yellow (E), otherwise dark grey. Red lines indicate genome-wide significance (q < 0.05). Scatterplots exemplify eQTM effects by showing the most significant associations between methylation and gene expression levels in (B) low-grade osteoarthritis cartilage (cg17304084 and *PIWIL2* with beta = −0.77, SE = 0.04, *P* value = $2.25 \times 10^{-45}$), (D) high-grade osteoarthritis cartilage (cg23122642 and *PAX8-AS1* with beta = −0.77, SE = 0.05, *P* value = $1.90 \times 10^{-27}$) and (F) synovium (cg19083407 and *PAX8-AS1* with beta = −0.81, SE = 0.04, *P* value = $1.83 \times 10^{-53}$). Furthermore, we show an example for a differential eQTM effect between cg11935831 and *SLC16A3* that is present in (G) low-grade osteoarthritis cartilage (beta = −0.52, SE = 0.06, *P* value = $7.26 \times 10^{-17}$, q value = $1.47 \times 10^{-11}$), but not (H) high-grade osteoarthritis cartilage (*P* value = .8, q value = 1). In Figures 2B, 2D, 2F, 2G, and 2H, the blue dashed line and shaded region visualise the regression line as well as its 95% CI, respectively. OA, osteoarthritis.

(Supplementary Note S2; Supplementary Table S7). By tissue, we identified genome-wide significant eQTM effects in low- (11,204 methylation sites associated with 2388 genes; Fig 2A; most significant eQTM in Fig 2B; Supplementary Table S8) and high-grade osteoarthritis cartilage (3116 methylation sites associated with 649 genes; Fig 2C; most significant eQTM in Fig 2D; Supplementary Table S9) as well as synovium (7620 methylation sites associated with 1661 genes; Fig 2E; most significant eQTM in Fig 2F; Supplementary Table S10). The fat pad eQTM map revealed effects at nominal significance (*P* < .05) (Supplementary Note S3; Supplementary Fig S3; Supplementary Table S11). To biologically characterise eQTM-targeted genes in a tissue-specific manner, we performed GO enrichment analysis separately in low-grade osteoarthritis cartilage (116 GO terms with FDR < 0.05; Supplementary Table S12) and high-grade osteoarthritis cartilage (5 GO terms with FDR < 0.05; Supplementary Table S13), as well as in synovium (4 GO terms with FDR < 0.05; Supplementary Table S14), and found osteoarthritis-linked terms in cartilage (including terms related to extracellular matrix or innervation) and synovium (including terms related to the cytoskeleton or cell development), suggesting effects between DNA methylation and genes contributing to disease-relevant pathways in primary joint tissues (Supplementary Note S4).

Comparing these eQTM maps between tested osteoarthritis tissues revealed eQTM effects that are present in 1 but not the other tissue type (q < 0.05 in 1 tissue and *P* > .05 in the other). For example, we found 4166 eQTM effects (involving 1600 genes) that are present in low-grade osteoarthritis cartilage but not high-grade osteoarthritis cartilage (exemplified using the eQTM effect between *SLC16A3* and cg11935831 being present in low- but not high-grade osteoarthritis cartilage in Fig 2G,H) (Supplementary Table S15). Vice versa, we found 253 eQTM effects (involving 188 genes) in high- but not low-grade osteoarthritis cartilage (Supplementary Table S16). In total, we found 1726 genes that were targeted by a differential eQTM effect between low- and high-grade osteoarthritis cartilage, which were enriched (FDR < 0.05) in 93 GO terms (Supplementary Note S5; Supplementary Table S17). These differences between cartilage sample types suggest eQTM effects are switched on/off during cartilage degeneration. Similarly, we found differences between the synovium and low-grade (3300 eQTM effects involving 1140 genes present in synovium but not low-grade cartilage; 5593 effects for 1626 genes vice versa; 2449 genes involved in total) as well as between the synovium and high-grade osteoarthritis cartilage (4329 eQTM effects involving 1328 genes present in synovium but not high-grade cartilage;

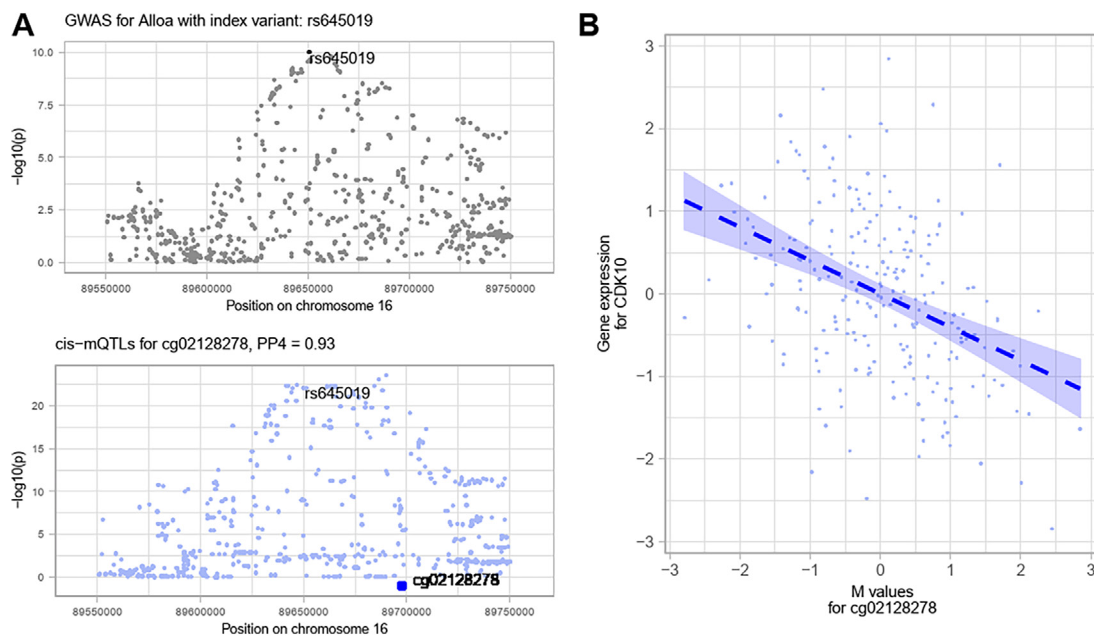


**Figure 3.** Highlights the likely effector gene *CDK10* in low-grade osteoarthritis cartilage. (A) The methylation quantitative trait locus (mQTL) signal for low-grade osteoarthritis cartilage at cg02128278 colocalises with a GWAS signal (index variant, rs645019) for osteoarthritis at any site (posterior probability, 92.8%). (B) Methylation levels of cg02128278 are negatively associated with *CDK10* expression in low-grade osteoarthritis cartilage (beta, −0.40; SE, 0.06; *P* value, $2.90^{-10}$). This exemplifies linking GWAS risk signals to a likely effector gene using methylation. In Figure 3B, the blue dashed line and shaded region visualise the regression line as well as its 95% CI, respectively. GWAS, genome-wide association studies.

964 effects for 335 genes vice versa; 1365 genes involved in total) (Supplementary Tables S18-21). Enrichment analysis found 95 (FDR < 0.05) enriched GO terms in genes targeted by a differential eQTM between cartilage and synovium (Supplementary Note S6; Supplementary Tables S22 and S23). These insights suggest tissue-specific effects between DNA methylation and gene expression in these biological processes.

Furthermore, we found a small number of eQTM effects to be significant in cartilage as well as synovium but show different directions of effect (synovium vs low-grade osteoarthritis cartilage, 50 eQTM effects involving 33 genes; synovium vs high-grade osteoarthritis cartilage, 14 eQTM effects involving 10 genes), suggesting tissue-specific effects between DNA methylation and gene expression.

Together, these results suggest widespread genome-wide effects between DNA methylation and gene expression in knee cartilage and synovium tissue, as well as disease-stage-specific effects.

### *Linking methylation sites and osteoarthritis GWAS signals*

We performed colocalisation analysis by considering GWAS risk signals [2] for osteoarthritis at any site, knee osteoarthritis, and total knee replacement, to identify methylation sites that potentially underlie risk variant effects on osteoarthritis. Applying colocalisation between mQTL and osteoarthritis GWAS risk loci detected 1455 methylation sites with a putative causal role in osteoarthritis. We found osteoarthritis-linked methylation sites in low- (n = 736 methylation sites, colocalising with 166 GWAS signals; Supplementary Table S24) and high-grade osteoarthritis cartilage (n = 473 methylation sites, colocalising with 141 GWAS signals; Supplementary Table S25), synovium (549 methylation sites, colocalising with 144 GWAS signals; Supplementary Table S26), blood (n = 347 methylation sites, colocalising with 123 GWAS signals; Supplementary Table S27) and infrapatellar fat pad (n = 104 methylation sites, colocalising with 64 GWAS signals; Supplementary Table S28). We further found that the majority of these detected methylation sites have not been linked with osteoarthritis GWAS signals in the respective tissue previously [6,7] (low-grade cartilage, mQTL signals from 728 of 736 methylation sites have not been colocalised with osteoarthritis GWAS signals previously; high-grade osteoarthritis cartilage, 469 of 473 methylation sites; synovium, 543 of 549 methylation sites; infrapatellar fat pad, 95 of 104 methylation sites), highlighting the novelty of these colocalisation results. Together, these colocalisation results suggest methylation sites that may mediate genetic osteoarthritis risk in affected tissues.

### *Resolution of GWAS signals*

Next, we tested whether methylation sites with a likely causal role in osteoarthritis (through colocalisation) show genome-wide significant associations with genes (through look-up in the eQTM map of the same tissue). Together, this would suggest molecular mechanisms through which genetic risk signals might exert their osteoarthritis-promoting effects, namely through colocalised methylation sites and methylation-associated effector genes. In total, we identified 50 likely effector genes for GWAS risk signals, of which 31 (62%) were reported in the largest osteoarthritis GWAS [2] to date, and 19 (38%) are novel.

Stratified by tissue, we identified 44 likely effectors in genes of low-grade osteoarthritis cartilage (Supplementary Table S29). Of these 44 genes, 29 were suggested as likely effector genes of genetic variants previously [2], whereas 15 genes are novel. In high-grade osteoarthritis cartilage, we found 8 likely effector genes (Supplementary Table S30). Of these 8 genes, 4 were found as effector genes of genetic variants previously [2], whereas 4 are novel. In the synovium, we detected 13 likely effector genes (Supplementary Table S31). Of these, 3 were identified previously [2], whereas 10 genes are novel. Together, by associating putative osteoarthritis-related methylation sites with genes, we can link GWAS risk signals to likely effector genes. We further compared novel (Supplementary Fig S4) and

confirmed (Supplementary Fig S5) likely effector genes across tissues, thus identifying tissue-specific and shared signals (Supplementary Note S7).

The 31 previously identified effector genes include previously osteoarthritis-associated genes, such as *ALD1A2* [6], *WWP2* [22], or *SMAD3* [23]. For 13 of 31 previously identified effector genes, we, for the first time, suggest mQTL signals that colocalise with osteoarthritis GWAS risk signals, thus providing additional mechanistic insights for these osteoarthritis-relevant genes. These genes include *CYP19A1*, a regulator of the osteoarthritis-linked hormone oestrogen [23]. More specifically, we identified 2 methylation sites in low-grade osteoarthritis cartilage for which mQTL signals colocalise with GWAS risk signals (eg, methylation site cg02325664 with a knee osteoarthritis GWAS risk signal with 95.0% posterior probability and methylation site cg00004322 colocalising with a total knee replacement GWAS risk signal with 97.8% posterior probability, both GWAS signals with lead variant rs146939415 and risk allele C) and that are associated with its *CYP19A1* gene expression levels (cg02325664 with beta, 0.45; *P*, $8.95 \times 10^{-13}$; cg00004322 with beta, −0.27; *P*, $4.51 \times 10^{-05}$). This proposes a contributing role of these methylation sites in the disease-promoting effect of genetic risk loci through *CYP19A1*.

Similarly, for 17 of 19 novel, likely effector genes (including *GDNF*, which encodes a pain-related neurotrophic factor [24]; *EXOSC6*: component of the RNA exosome complex; *CDK10*: cyclin-dependent kinase), we found for the first time mQTL signals colocalising with osteoarthritis GWAS risk signals. Thus, by linking these GWAS risk signals to DNA methylation sites (through colocalisation analysis) and DNA methylation sites to gene expression (through eQTM association analysis), we suggest, for the first time in these likely effector genes, enhanced molecular insights into how epigenetics and transcriptomics might mediate the osteoarthritis-promoting effect of genetic risk loci in primary tissues.

For 5 (*ZNF641, PDPR, PAM, EXOSC6,* and *CDK10*) of these novel likely effector genes, a previous GWAS study reported 2 lines of evidence supporting their relevance to osteoarthritis, which we now supplement with epigenetic insights.

Together, integrating GWAS, mQTL, and eQTM maps enables additional mechanistic insights into previously identified osteoarthritis effector genes and reveals novel candidates.

To estimate translational potentials, we further performed druggability analysis for the 50 likely effector genes and found links to drugs with at least 1 completed clinical trial phase 2 for 5 genes [20] (Supplementary Table S32). These 5 genes comprise *CDK10*, for which a previous GWAS [2] found a close genetic osteoarthritis risk signal. We identified *CDK10* DNA methylation in low-grade osteoarthritis cartilage for which mQTL signals (for cg02128278, 92.8% posterior probability, Fig 3A; cg08616182, 97.5% posterior probability) colocalise with a GWAS risk signal for osteoarthritis at any site (lead variant, rs645019; risk allele, T). In addition, we associated *CDK10* methylation and gene expression levels (Fig 3B). *CDK10* is involved in the cell cycle, has been associated with cell proliferation [25], and contributes to the regulation of actin cytoskeleton organisation [26]. Of note, *CDK10* is targeted by AT-7519, a drug that has completed clinical trial phase 2. Together, these results suggest translational potential for these likely effector genes.

## DISCUSSION

This study provided the largest genome-wide maps of genetic effects on DNA methylation (mQTL maps) in low- and high-grade osteoarthritis cartilage as well as synovium and, for the first time, reports long-range (trans) effects between genetic variants and methylation sites in osteoarthritis tissues. We presented the largest maps of associating DNA methylation sites and genes (eQTM maps) in cartilage and the first in synovium. Integrating osteoarthritis GWAS results, mQTL, and eQTM maps revealed a multitude of methylation sites with a potential causal role in osteoarthritis, as well as 50 likely effector genes in a tissue-specific manner.

We identified novel mQTL, extending our understanding of the genetic effects on the epigenetic profile. Colocalisation of mQTL with GWAS risk signals [2] finds novel methylation sites that putatively mediate the genetic risk of osteoarthritis risk loci, highlighting epigenetic marks that contribute to osteoarthritis risk in primary tissues. We linked these osteoarthritis-related methylation sites to 19 novel likely effector genes, which have not been reported by the largest genetic study for osteoarthritis to date [2]. These novel effector genes include *CDK10*, which encodes a part of the protein kinase CDK10/Cyclin M, which regulates actin cytoskeleton organisation [26]. *EXOSC6* encodes a subunit of the RNA exosome complex, which regulates RNA-processing and RNA-decay functions within the cell [27]. The RNA exosome complex has also been implicated in regulating cytokine production and thereby modulating innate immune signalling and inflammatory responses [28]. Furthermore, depletion of the RNA exosome complex has been linked to disrupted cellular homeostasis and cell senescence [29]. Furthermore, GDNF is a neurotrophic factor that has been linked to skeletal pain in a rat model [24]. More specifically, bone pain involves activation and sensitisation of nonpeptidergic neurones through the GDNF/GFR$\alpha$1 signalling pathway [24], suggesting a potential osteoarthritis pain-related role.

We suggest that these genes contribute to osteoarthritis pathogenesis through genetically driven changes in DNA methylation and gene expression in disease-relevant tissues.

Our integrative, methylation-focused approach further confirms 31 previously reported effector genes [2], including *ALDH1A2* (linked to the retinoic acid signalling pathway, which regulated genes involved in skeletal [30], organ and limb development [31]), *SMAD3* (mediates the signalling of the osteoarthritis-relevant transforming growth factor $\beta$ pathway that contributes to skeletal development, cartilage and bone formation, inflammation, remodelling of the extracellular matrix, and changes of the synovial tissue [2]) and *WWP2* (regulator in chondrocytes [22]), highlighting that some findings confirm previous results.

Of note, overlapping the 50 effector genes across tissues revealed tissue-shared as well as tissue-specific signals, suggesting that effector genes mediate genetic risk loci in a tissue-specific manner as well as across joint tissues.

Genome-wide methylation studies in osteoarthritis primary tissues [7,32] often apply rigid, distance-based information, annotating methylation sites to the closest genes for further interpretation, although previous eQTM maps in human tissues found associations between DNA methylation sites and genes to be partly tissue-specific [33] or across moderate distances (per tissue median distance between CpG site and gene transcription start site in Oliva et al [33]: 119-157 kb). Therefore, we generate eQTM maps of tissue (cartilage and synovium) and disease stage (low- and high-grade osteoarthritis cartilage) specifically and annotate methylation sites to genes.

Furthermore, these eQTM maps reveal enrichments of DNA methylation-associated genes in osteoarthritis-relevant GO terms in cartilage as well as in synovium, suggesting that regulatory effects between DNA methylation sites and genes play a role in osteoarthritis-relevant pathways in primary joint tissues.

This study focuses on individuals of European ancestry; it does not reflect global genetic diversity. There is a need to

extend omics studies to diverse populations, particularly including cohorts of non-European ancestry, to enhance maps of genetic and molecular variation and ensure the general applicability of findings [34].

Our study presented the largest genome-wide DNA methylation profiles across tissues and disease stages. We described the effects of genetics on DNA methylation as well as the relationship between DNA methylation and gene expression. We enabled novel insights into the important role of DNA methylation in osteoarthritis and, ultimately, into molecular mechanisms underpinning genetic risk loci. We provided molecular maps of primary tissues in osteoarthritis patients, which represent relevant resources for the musculoskeletal disease research community and beyond.

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## Contributors

EZ and JMW designed the study. JMW, KMS, and DS collected the clinical data. GK preprocessed the gene expression data. PK, OSS, GK, MT, and NB analysed the data. PK and EZ interpreted the results and drafted the manuscript. All authors reviewed, edited, and approved the final manuscript.

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## Competing interests

The authors declare no competing interests.

## Patient consent for publication

Not applicable.

## Ethics approval

This work was approved by Oxford NHS REC C (10/H0606/20, 15/SC/0132 and 20/SC/0141), and samples were collected under Human Tissue Authority license 12182, Sheffield Musculoskeletal Biobank, University of Sheffield, UK. Before participating in the study, all osteoarthritis-affected individuals provided written, informed consent.

## Provenance and peer review

Not commissioned; externally peer reviewed.

## Data availability statement

Full summary statistics can be obtained online through the MSK portal (http://mskkp.org). All software used in this study is available from free repositories or manufacturers as referenced in the Methods section.

## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.01.020.

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