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Osteoarthritis and Cartilage

Epigenomic differences between osteoarthritis grades in primary cartilage

Peter Kreitmaier # † ‡, Diane Swift §, J. Mark Wilkinson §, Eleftheria Zeggini # ‡ *

Technical University of Munich (TUM) and Klinikum Rechts der Isar, TUM School of Medicine and Health, Ismaninger Str. 22, 81675 Munich, Germany
† Graduate School of Experimental Medicine, TUM School of Medicine and Health, Technical University of Munich, 81675 Munich, Germany
‡ Institute of Translational Genomics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany
§ Division of Clinical Medicine, School of Medicine and Population Health, University of Sheffield, Sheffield S10 2RX, UK

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SUMMARY

Objective: Osteoarthritis is a common and complex joint disorder that shows higher prevalence and greater disease severity in women. Here, we investigate genome-wide methylation profiles of primary chondrocytes from osteoarthritis patients.
Design: We compare genome-wide methylation profiles of macroscopically intact (low-grade) and degraded (high-grade) osteoarthritis cartilage samples matched from osteoarthritis patients undergoing knee replacement surgery. We perform an epigenome-wide association study for cartilage degeneration across 170 patients and separately in 96 women and 74 men.
Results: We reveal widespread epigenetic differences with enrichments of nervous system and apoptosis-related processes. We further identify substantial similarities between sexes, but also sex-specific markers and pathways.
Conclusions: Together, we provide the largest genome-wide methylation profiles of primary cartilage to date with enhanced and sex-specific insights into epigenetic processes underlying osteoarthritis progression.
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Introduction

Osteoarthritis is a prevalent joint disorder, affecting more than 300 million people worldwide.[1] Existing treatment approaches are limited to pain management and replacement surgery of affected joints. Due to increasingly older populations, the impact of osteoarthritis on public health systems will increase. Together, this highlights the need for novel, personalised treatment approaches that require an enhanced understanding of the genetic and genomic basis of osteoarthritis.

To date, genome-wide association studies have identified over 150 genetic risk loci for osteoarthritis,[2] shedding insights into its complex architecture. Integration of genetic data with molecular profiles of osteoarthritis-affected tissues accessible at the point of joint replacement surgery can help identify effector genes and their mechanisms of action. DNA methylation, an epigenetic mark that describes the covalent attachment of a methyl group to the DNA, is a useful molecular tool in this regard. DNA methylation is associated with gene expression regulation, for example elevated methylation levels close to the transcription start site (particularly in promotor regions) can be associated with reduced gene expression.

DNA methylation studies have generated valuable profiles of osteoarthritis tissues,[3] such as cartilage, synovium [4] and subchondral bone.[5] In cartilage, epigenome-wide association studies (EWAS) have been conducted to compare macroscopically intact (low-grade) and degraded (high-grade) osteoarthritis cartilage samples to study epigenetic markers of cartilage degeneration.[4,6–10] However, these studies have included small numbers of patients and have thus been limited in power.

Furthermore, most methylation osteoarthritis studies combine samples of both sexes. However, osteoarthritis prevalence and incidence are higher among women [11] and female osteoarthritis patients show more osteoarthritis-related pain and disability,[12–14] suggesting potential sex-specific etiological mechanisms. A methylation study has identified a small number of sex-specific cartilage degeneration markers but was limited in sample size (52 women and 38 men).[4]

* Correspondence to: Institute of Translational Genomics, Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt, Ingolstaedter Landstraße 1, Neuherberg 85764, Germany.
E-mail address: eleftheria.zeggini@helmholtz-munich.de (E. Zeggini).

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Together, there is an urgent need for better-powered epigenetic studies of primary osteoarthritis tissues. In this study, we performed EWAS for cartilage degeneration in 170 patients (96 women and 74 men) to characterise common and sex-specific epigenetic markers of osteoarthritis.

Methods

Osteoarthritis-affected individuals and study samples

In this study, we examine cartilage samples from osteoarthritis-affected knees that were collected in 170 osteoarthritis patients (age 38–89 years, mean 70.86 years). These patients included 96 women (age 38–85 years, mean 70.68 years) and 74 men (age 50–89 years, mean 71.11 years) (Fig. S1). These individuals underwent total knee replacement due to late-stage osteoarthritis. Cartilage samples were graded agnostically to sex using the International Cartilage Repair Society (ICRS)[15] macroscopic scoring system (low-grade osteoarthritis cartilage: ICRS score 0 or 1, high-grade cartilage osteoarthritis: ICRS score 3 or 4). This work was approved by Oxford NHS REC C (10/H0606/20 and 15/SC/0132), 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.

Sample extraction

Knee chondrocytes were isolated by following a protocol reported in a previous study (Methods, section "Isolation of chondrocytes").[16]

DNA methylation preprocessing

DNA methylation was measured using the Illumina EPICv1 array. We used a R package meffil-based preprocessing pipeline (https://github.com/perishky/meffil/wiki).[17] We further tested for ethnicity outliers using the Illumina ancestry and kinship toolkit[18] (Fig. S2) and excluded samples with > 10% undetected (detection pvalue > 0.01) methylation values, sex outliers (> 5 * sd), methylated/unmethylated signal outliers (> 3* sd) and control probe signal outlier (> 5 * sd). We normalised methylation samples with the meffil function meffil.normalize.quantiles (including 16 principal components) and meffil.normalize.samples.

We excluded methylation probes with more than 10% of samples low bead number (< 3) or undetected methylation values (detection $p < 0.01$), probes of non-autosomal methylation sites, cross-reactive probes and probes of methylation sites that are close (within 10 base pairs) to common single nucleotide polymorphisms (minor allele frequency > 0.05) in European population.[19–21]

For downstream analysis, generated beta values were converted to Mvalues (negative M-value: more unmethylated DNA at a particular DNA methylation site; M-value is 0: equal amount of methylated and unmethylated DNA at a particular DNA methylation site; positive M-value: more methylated DNA at a particular DNA methylation site) using the beta2m function of R package lumi.[22] The resulting methylation data comprised 780,181 methylation sites for 170 patients, including 96 women and 74 men. For all 170 patients, matched low-grade and high-grade osteoarthritis samples were available.

We extracted the genomic location (hg38) and annotated genes from publicly available annotation files https://github.com/zhou-lab/InfiniumAnnotationV1/raw/main/Anno/EPIC/EPIC.hg38.manifest.tsv.gz and https://github.com/zhou-lab/InfiniumAnnotationV1/raw/main/Anno/EPIC/EPIC.hg38.manifest.gencode.v36.tsv.gz.

Differential methylation analysis

To compare epigenetic profiles between low- and high-grade osteoarthritis cartilage, we conducted principal component analysis (PCA) using the prcomp function. We then quantified association significances between cartilage types and principal components 1 and 2 by performing ANOVA (R function aov).

Next, we performed three EWAS for cartilage degeneration: One combined (170 patients) as well as in two sex-specific analyses (96 women, 74 men) to identify methylation sites associated with osteoarthritis-related cartilage degeneration. More specifically, we compared high- with low-grade osteoarthritis cartilage samples matched from the same patient. We applied functions from the R package limma (lmFit and eBayes function) to generate paired linear models to enable matched comparisons between low- and high-grade osteoarthritis cartilage samples. We further added surrogate variables (SVs) to account for technical confounders (combined analysis: 31 SV, women: 23, men: 19; these numbers were estimated using the num.sv function with the 'be' procedure).[23] These SVs also capture sequencing batches (Supplementary Note 1). This resulted in the following model:

M-values ~ cartilage_type + patient_id + SVs

Here, *patient ID* refers to the patient identifier (ensures paired modelling) and *cartilage_type* denotes the cartilage degradation status (low- vs high-grade osteoarthritis). We applied Bonferroni correction per EWAS to correct for multiple testing (threshold: 0.05/780,181 methylation sites = 6.41 × 10^(−08)). Methylation sites achieving significance below this threshold were regarded as differentially methylated sites (DMS). To identify differentially methylated regions (DMRs), we applied the R package dmrff using default parameter settings (maxgap = 500, p.cutoff = 0.05).[24] Regions are DMRs when consisting of more than one methylation site and achieving a Bonferroni-adjusted $p < 0.05$.

Replication analysis

To replicate our DMS results, we compared these findings with a previous EWAS (n = 90 patients) for cartilage degeneration.[4] We regarded DMS as replicated when showing the same direction of effect at nominal significance ($p < 0.05$) in the replication set.

Gene Ontology analysis

We performed Gene Ontology (GO) analysis to biologically characterise DMS of the combined as well as sex-specific EWAS. We applied the gometh function from the missMethyl package (version 1.24.0; we used R package GO.db 3.12.1 to load GO information).[25,26] We used gene annotations from the file EPIC.hg38.manifest.gencode.v36.txt.gz (column "genesUniq"). We included 780,181 methylation sites that passed the preprocessing procedure as background set ("all.cpg") and lists of DMS as query ("sig.cpg"). We only considered GO terms composed of between 20 and 200 genes and applied a Benjamini-Hochberg correction to account for multiple testing.

Comparing combined and sex-specific EWAS

To estimate sex-specific epigenetic markers of cartilage degeneration, we compared the results of sex-specific EWAS on a summary statistics level. Sex-specific DMS were methylation sites that (1) exceed genome-wide significance ($p < 6.41 \times 10^{-08}$) in one sex, but (2) not nominal significance in the other ($p < 0.05$). Furthermore, we compared GO analysis results of DMS identified in women and men on summary statistics level. Here, we defined sex-

specific cartilage degeneration-related GO terms as being significantly (false discovery rate (FDR) < 0.05) enriched in DMS in one sex, but not achieving nominal significance ($p < 0.05$) in DMS of the other.

Results

Widespread epigenetic markers for cartilage degeneration

We performed principal component analysis for macroscopically intact (low-grade) and degraded (high-grade) osteoarthritis cartilage samples. We identified significant differences along the first (ANOVA p = 1.04 × 10^−13) and second principal component (ANOVA $p < 2 \times 10^{-16}$) (Fig. 1A), indicating pronounced global differences in the epigenetic profiles.

Next, we performed an EWAS for cartilage degeneration by comparing paired low-grade and high-grade cartilage samples from 170 patients. Of 780,181 tested methylation sites, 146,777(18.8%) were differentially methylated (Bonferroni correction, $p < 6.41 \times 10^{-08}$) (Fig. 1B, exemplified by the most significantly DMS cg20482832 in Fig. 1C and Fig. S3, Table S1). Of these, 56,726 and 90,051 showed hyper- and hypomethylation in high-grade cartilage, respectively. We further found 4644 DMS with large methylation differences (Supplementary Note 2, Table S2 and S3). On the region level, we identified 18,661 regions to be differentially methylated between low- and high-grade osteoarthritis cartilage (Supplementary Note 3, Table S4).

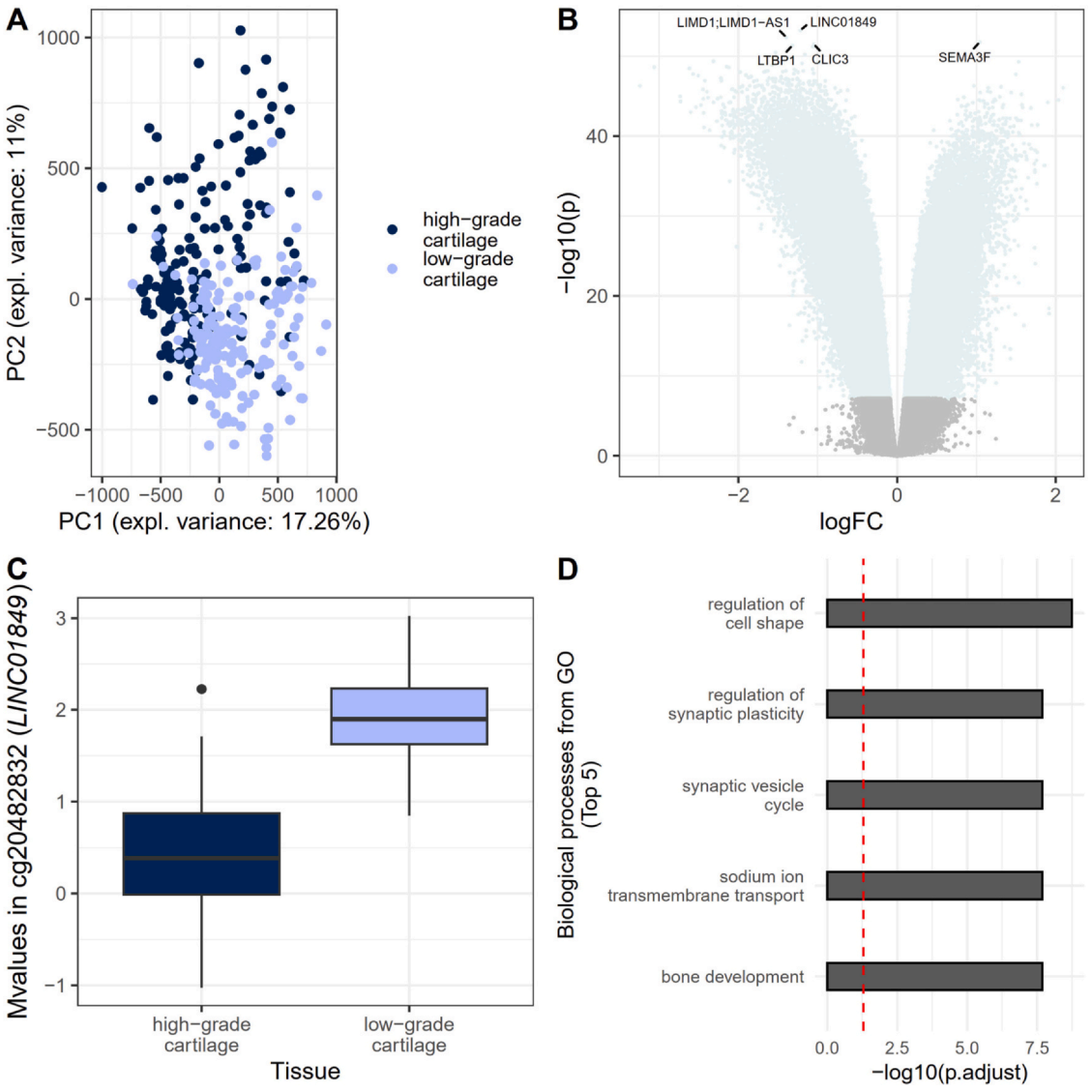

Fig. 1

Osteoarthritis and Cartilage

Methylation differences between low-grade and high-grade osteoarthritis cartilage. (A) Principal component analysis reveals global differences between low-grade and high-grade osteoarthritis cartilage samples. (B) A volcano plot visualises 146,777 DMS ($p < 6.41 \times 10^{-08}$), of which 56,726 and 90,051 are hyper- and hypomethylated, respectively. (C) The most significant DMS is cg20482832 (logfc: −1.22, $p = 5.8 \times 10^{-54}$, SE = 0.049). Whiskers extend to 1.5 times the interquartile range. (D) The five most significantly GO terms from the ontology *biological processes*. The red line indicates statistical significance (FDR < 0.05).

Comparing the DMS with a previous EWAS (n = 90 patients)[4] showed that 92% of comparable sites showed the same direction of effect at nominal significance $p < 0.05$, suggesting that the identified markers are robust (Supplementary Note 4, Table S5).

To biologically characterise these 146,777 DMS, we performed GO analyses and identified 1660 GO terms (at FDR < 0.05) (Table S6, Fig. 1D). These included terms that were previously associated with cartilage degeneration, such as musculoskeletal tissue development (e.g. “bone development”, “cartilage development”, “muscle cell development”), cytoskeletal structure (e.g. “actomyosin structure organisation”, “actin filament bundle organisation”), extracellular matrix (e.g. “regulation of cell-matrix adhesion”) or the epithelium (e.g. “morphogenesis of a branching epithelium”, “branching morphogenesis of an epithelial tube”).

Notably, we also detected enrichment for nervous system (“dendrite morphogenesis”, “regulation of synaptic plasticity”, “neuron projection organisation”), neurotransmission (“synaptic vesicle cycle”, “neurotransmitter secretion”, “signal release from synapse”, “positive regulation of synaptic transmission”) and apoptosis-related terms (e.g. “regulation of extrinsic apoptotic signalling pathway”).

Together, these results suggest methylation sites and biological pathways that are associated with osteoarthritis progression in cartilage.

Next, we generated epigenetic profiles of cartilage degeneration stratified by sex. We performed EWAS separately in women (n = 96) and men (n = 74), again by comparing matched low-grade and high-grade cartilage samples from the same patient.

In women, we identified 62,313 DMS (Bonferroni correction, $p < 6.41 \times 10^{-8}$) (8.41% of tested methylation sites) (Fig. 2A, most significant DMS cg01931614 in Fig. 2B and Fig. S4, Table S7). Of these, 20,345 and 41,968 showed hyper- and hypomethylation in high-grade cartilage, respectively. These differential methylated sites were overrepresented in 361 GO terms (Table S8). On the region level, we identified 19,734 DMR in women (Supplementary Note 3, Table S9).

In men, we detected 61,513 DMS (Bonferroni correction, $p < 6.41 \times 10^{-8}$) (7.88% of tested methylation sites) (Fig. 2C, most significant DMS cg20482832 in Fig. 2D and Fig. S5, Table S10). Of these, 20,295 and 41,218 showed hyper- and hypomethylation in high-grade cartilage, respectively. These signals were enriched in 480 GO terms (Table S11). On the region level, we found 24,064 DMR in men (Supplementary Note 3, Table S12).

Together, the sex-stratified analyses also reveal widespread methylation differences between low-grade and high-grade osteoarthritis cartilage.

Sex-specific markers of cartilage degeneration

Next, we tested whether epigenetic markers for osteoarthritis are common across sexes or sex-specific (Fig. 3A). Of 62,313 and 61,513 that were identified in women and men, respectively, 43,152 overlapped (women: 69.2%, men: 70.1%). Effects of these were in concordant direction and highly correlating (Pearson $r = 0.98$, $p < 2.2 \times 10^{-16}$). Together, this suggests that a substantial part of epigenetic osteoarthritis markers in cartilage are shared between men and women.

We further detected sex-specific DMS, which are methylation sites associated with cartilage degeneration in one sex but not in the other (Method). We identified 413 (142 hyper- and 271 hypomethylated in high-grade osteoarthritis cartilage, Fig. 3B and Fig. S6, Table S13) and 539 (259 hyper- and 280 hypomethylated in high-grade osteoarthritis cartilage, Fig. 3C and Fig. S7, Table S14) DMS that are specific for women and men, respectively. Furthermore, we found DMS with larger effect sizes (> = 1.5 x |logfc|) in men (n = 2224 methylation sites) and women (n = 74 methylation sites) when compared to the respective other sex, suggesting effect size magnitude differences of DMS between sexes (Table S15).

A subset of these sex-specific methylation markers (167 of 413 women specific DMS, 215 of 539 men specific DMS) do not achieve nominal significance in the combined analysis, suggesting that some markers are unidentifiable when samples of both sexes are analysed together.

On the biological pathway level, we compared 361 and 480 GO terms enriched (FDR < 0.05) among 62,313 and 61,513 DMS in women and men, respectively, and found 19 (of 361, 5.26%, Table S16) and 51 (of 480, 10.62%, Table S17) women- and men-specific GO terms which are enriched among DMS in one sex, but not in the other (Method). These sex-specific GO terms included terms related to the immune system (for example in men: ”positive regulation of lymphocyte differentiation“; women: ”phagocytic cup”), the nervous system (men: ”regulation of synaptic vesicle cycle”, “regulation of postsynaptic membrane neurotransmitter”, “neuron migration”; women: ”regulation of axon guidance”) and hormone regulation (men: “negative regulation of hormone secretion”), suggesting sex-specific methylation changes in these pathways during osteoarthritis degeneration in cartilage.

We found 258 overlapping GO terms (71.47% and 53.75% of identified GO terms in women and men, respectively). Furthermore, these terms also overlapped with the 1660 GO terms of the combined analysis (women: 354 of 361 GO terms, men: 447 of 480), indicating substantial overlap between sex-specific and combined analyses on the biological pathway level.

Discussion

Here, we have generated the largest genome-wide methylation profile of low-grade and high-grade osteoarthritis cartilage to date. We estimate common and sex-specific epigenome-wide profiles of cartilage degeneration and identify DNA methylation markers for osteoarthritis progression across sexes and in a sex-specific manner.

We conducted the largest sex-combined (170 patients) and sex-specific (96 women, 74 men) EWAS for cartilage degeneration which almost doubles the sample size of the next largest study.[4] These analyses identify widespread epigenetic markers of cartilage degeneration, highlighting the distinctness of the methylation profile between early and late cartilage degeneration grades.

We compared sex-specific EWAS results and found osteoarthritis-related epigenetic markers and pathways to be largely overlapping between sexes. This suggests that the molecular processes contributing to osteoarthritis in cartilage are largely the same. We further identified a small number of epigenetic markers solely identified in men (n = 539 DMS) and women (n = 413 DMS), which suggests a few sex-specific epigenetic mechanisms.

GO analysis of combined EWAS results revealed biological processes previously associated with cartilage degeneration in genome-wide methylation studies,[4,6–9] including musculoskeletal tissue development, cytoskeletal structure, or extracellular matrix.

Notably, we identified apoptosis-related GO terms (such as “regulation of extrinsic apoptotic signalling pathway”) which may point to chondrocyte apoptosis that has been associated with cartilage matrix breakdown.[27]

The nervous system (e.g. “dendrite morphogenesis”, “regulation of synaptic plasticity”, “neuron projection organisation”) and neurotransmission-related (e.g. “synaptic vesicle cycle”, “neurotransmitter secretion”, “signal release from synapse”, “positive regulation of synaptic transmission”) terms were strongly represented, thus confirming a small number of nervous system-related signals in smaller osteoarthritis cartilage EWAS.[7–9] These signals may point to the innervation in the diseased cartilage,

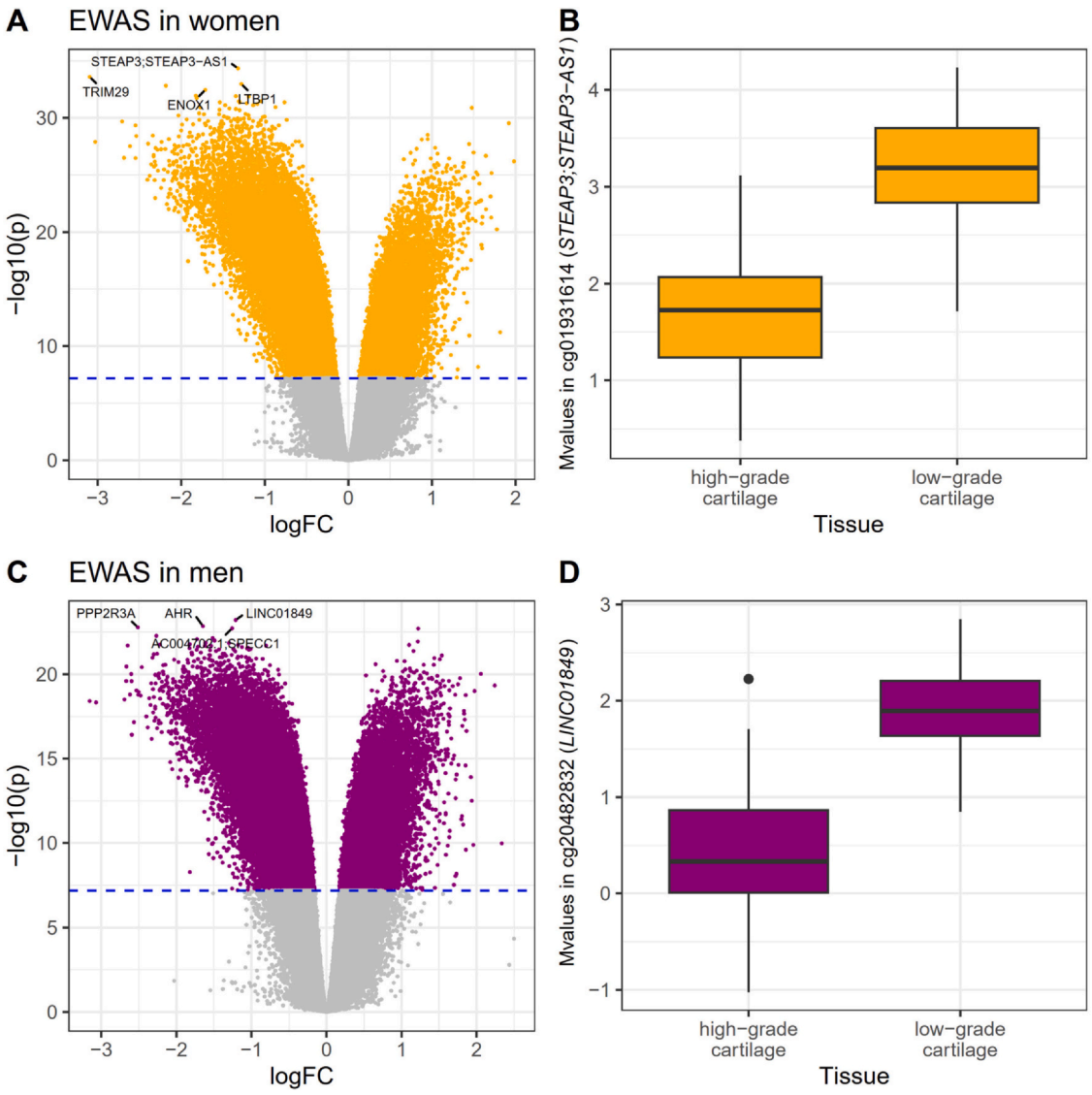

Fig. 2

Osteoarthritis and Cartilage

Methylation profiles of cartilage degeneration in women and men. (A) A volcano plot visualises 62,313 DMS (20,345 and 41,968 are hyper- and hypomethylated, respectively) in women. (B) Cg01931614 is the most significant DMS in women (logfc: −1.32, p = 4.63 × 10^(−35), SE = 0.06). (C) In Men, 61,513 DMS (20,295 and 41,218 are hyper- and hypomethylated, respectively) are detected. (D) Cg20482832 is the most significant DMS in men (logfc: −1.21, p = 6.17 × 10^(−24), SE = 0.073). Blue lines indicate genome-wide significance (p < 6.41 × 10^−08).

potentially contributing to pain sensation in affected joints.[28] Notably, some nervous system-related terms were identified in only one sex, suggesting sex differences in the innervation and neurotransmission during osteoarthritis progression. This may be related with women being more likely to develop pain in osteoarthritis joints.[13]

Other sex-specific GO terms are related to the immune system, which may be linked with higher pro-inflammatory factor levels in chondrocyte cell cultures (*IL1A*, *IL6*, and *IL8* expression levels in cultured chondrocytes of low-grade osteoarthritis cartilage are higher in women[29]) or an overrepresentation of female patients in a high inflammation cluster.[30] Our results suggest a sex-specific regulative role of cartilage DNA methylation on parts of the immune system during osteoarthritis.

A hormone-related term ("negative regulation of hormone secretion") was only enriched in osteoarthritis markers in men, indicating sex differences in hormone regulation. Previous studies have observed associations between sex hormones and osteoarthritis.[31,32] Furthermore, cultured chondrogenic progenitor cells of osteoarthritis knees have been shown to demonstrate sex-dependent effects of sex hormones on gene expression.[33] Together, this suggests a sex-dependent role of some hormones in osteoarthritis.

Altogether, we compare low-grade (early degeneration state) and high-grade (late degeneration state) osteoarthritis cartilage samples matched from the same patients. By using the largest cohort of its kind, we generate insights at unprecedented power, in turn enabling enhanced insights into the osteoarthritis-related epigenetic signature in cartilage.

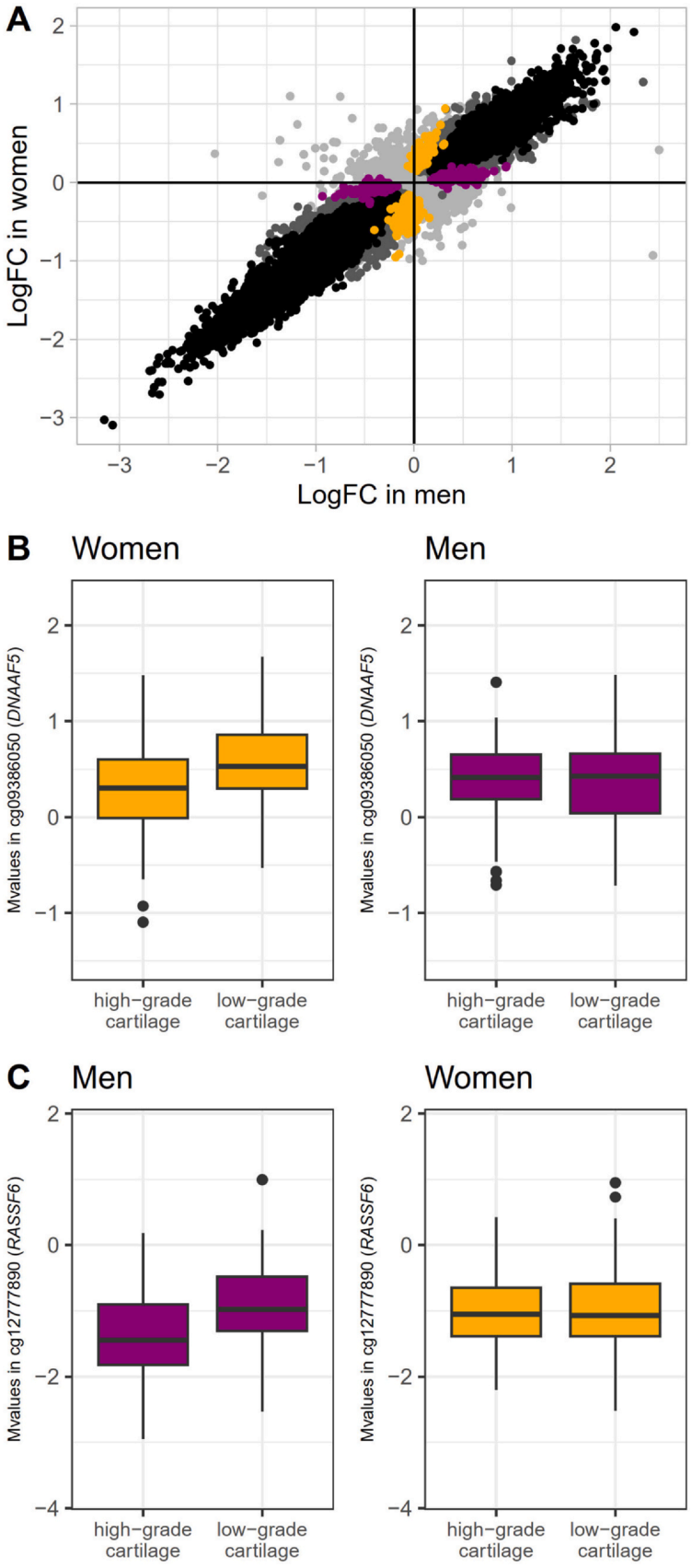

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Fig. 3 Osteoarthritis and Cartilage

Identification of sex-specific epigenetic markers for cartilage degeneration. (A) Scatterplot comparing effects of DMS in women and men. Orange and purple dots refer to 413 and 539 women- and men-specific DMS, respectively, which are defined by achieving genome-wide significance ($p < 6.41 \times 10^{-08}$) in one sex, but not nominal significance ($p < 0.05$) in the other. Black dots are DMS identified in both sexes. Dark grey dots refer to methylation sites that pass genome-wide significance in only one sex. Other methylation sites are light grey. (B) cg09386050 is a DMS in women (logfc: −0.30, $p = 4.24 \times 10^{-9}$, SE = 0.046), but does not exceed nominal significance in men ($p = 0.83$). (C) Similarly, cg12777890 (logfc: −0.67, $p = 3.35 \times 10^{-14}$, SE = 0.07) is a DMS in men, but not nominal significant in women ($p = 0.13$).

We identify a multitude of methylation sites across the genome that are differentially methylated between these two osteoarthritis stages. These sites are associated ('markers') with osteoarthritis-related cartilage degeneration, thus are linked with osteoarthritis progression rather than disease onset.

In this work, we have studied the methylation profile of primary cartilage from osteoarthritis patients at the point of knee replacement surgery. Therefore, the identified DMS could be a consequence, rather than a cause, of osteoarthritis development. However, access to age-matched healthy cartilage tissue can be challenging. To reveal causal links between osteoarthritis and cartilage methylation, it is necessary to generate methylation quantitative trait locus maps and integrate these with osteoarthritis GWAS results using colocalisation or causal inference analyses.

Our study highlights widespread epigenetic markers for cartilage degeneration linked to a large spectrum of biological pathways, including apoptosis and neuronal development. We reveal large similarities in the epigenetic signature of osteoarthritis across sexes, but also find a number of sex-specific markers, thus providing enhanced insights into the osteoarthritis related epigenetic signature in cartilage.

Author contributions

Study design: E.Z., J.M.W.; Clinical collection: D.S., J.M.W.; Data analysis: P.K.; Interpretation of results: P.K., E.Z.; Manuscript drafting: P.K., E.Z.; Manuscript reviewing and editing: P.K., E.Z., J.M.W.

Conflict of interest

The authors declare no competing interests.

Data Availability

All software used in this study is available from free repositories or manufacturers, as referenced in the Materials and Methods section. Full summary statistics can be obtained online (https://hmgubox2.helmholtz-muenchen.de/index.php/s/pCbEFC9oHNdpNkA).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.joca.2024.07.008.

References

1. Safiri S, Kolahi AA, Smith E, *et al.* Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017. Ann Rheum Dis 2020;79(6):819–28. https://doi.org/10.1136/annrheumdis-2019-216515
2. Boer CG, Hatzikotoulas K, Southam L, *et al.* Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. Cell 2021;184(18):4784–4818.e17. https://doi.org/10.1016/j.cell.2021.07.038
3. Katsoula G, Kreitmaier P, Zeggini E. Insights into the molecular landscape of osteoarthritis in human tissues. Curr Opin Rheumatol 2022;34(1):79–90. https://doi.org/10.1097/BOR.0000000000000853
4. Kreitmaier P, Suderman M, Southam L, *et al.* An epigenome-wide view of osteoarthritis in primary tissues. Am J Hum Genet 2022;109(7):1255–71. https://doi.org/10.1016/j.ajhg.2022.05.010
5. Zhang Y, Fukui N, Yahata M, *et al.* Identification of DNA methylation changes associated with disease progression in subchondral bone with site-matched cartilage in knee osteoarthritis. Sci Rep 2016;6,34460. https://doi.org/10.1038/srep34460
6. den Hollander W, Ramos YFM, Bomer N, *et al.* Transcriptional associations of osteoarthritis-mediated loss of epigenetic control in articular cartilage. Arthritis Rheumatol 2015;67(8):2108–16. https://doi.org/10.1002/art.39162
7. Zhang Y, Fukui N, Yahata M, *et al.* Genome-wide DNA methylation profile implicates potential cartilage regeneration at the late stage of knee osteoarthritis. Osteoarthritis Cartilage 2016;24(5):835–43. https://doi.org/10.1016/j.joca.2015.12.013
8. Moazedi-Fuerst FC, Hofner M, Gruber G, *et al.* Epigenetic differences in human cartilage between mild and severe OA. J Orthop Res 2014;32(12):1636–45. https://doi.org/10.1002/jor.22722
9. Bonin CA, Lewallen EA, Baheti S, *et al.* Identification of differentially methylated regions in new genes associated with knee osteoarthritis. Gene 2016;576(0):312–8. https://doi.org/10.1016/j.gene.2015.10.037
10. Steinberg J, Ritchie GRS, Roumeliotis TI, *et al.* Integrative epigenomics, transcriptomics and proteomics of patient chondrocytes reveal genes and pathways involved in osteoarthritis. Sci Rep 2017;7(1):8935. https://doi.org/10.1038/s41598-017-09335-6
11. Srikanth VK, Fryer JL, Zhai G, Winzenberg TM, Hosmer D, Jones G. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. Osteoarthritis Cartilage 2005;13(9):769–81. https://doi.org/10.1016/j.joca.2005.04.014
12. de Kruijf M, Stolk L, Zillikens MC, *et al.* Lower sex hormone levels are associated with more chronic musculoskeletal pain in community-dwelling elderly women. Pain 2016;157(7):1425–31. https://doi.org/10.1097/j.pain.0000000000000535
13. McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Knee pain and disability in the community. Br J Rheumatol 1992;31(3):189–92. https://doi.org/10.1093/rheumatology/31.3.189
14. Odding E, Valkenburg HA, Algra D, Vandenouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip

and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57(4):203–8. https://doi.org/10.1136/ard.57.4.203
15. Mainil-Varlet P, Aigner T, Brittberg M, *et al*. Histological assessment of cartilage repair: a report by the histology endpoint committee of the International Cartilage Repair Society (ICRS). JBJS. 2003;85(suppl_2):45.
16. Steinberg J, Southam L, Roumeliotis TI, *et al*. A molecular quantitative trait locus map for osteoarthritis. Nat Commun 2021;12(1):1309. https://doi.org/10.1038/s41467-021-21593-7
17. Min JL, Hemani G, Davey Smith G, Relton C, Suderman M. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics 2018;34(23):3983–9. https://doi.org/10.1093/bioinformatics/bty476
18. Arthur R, Schulz-Trieglaff O, Cox AJ, O'Connell J. AKT: ancestry and kinship toolkit. Bioinformatics 2017;33(1):142–4. https://doi.org/10.1093/bioinformatics/btw576
19. McCartney DL, Walker RM, Morris SW, McIntosh AM, Porteous DJ, Evans KL. Identification of polymorphic and off-target probe binding sites on the Illumina Infinium MethylationEPIC BeadChip. Genom Data 2016;9:22–4. https://doi.org/10.1016/j.gdata.2016.05.012
20. Pidsley R, Zotenko E, Peters TJ, *et al*. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol 2016;17(1):208. https://doi.org/10.1186/s13059-016-1066-1
21. Chen Y an, Lemire M, Choufani S, *et al*. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. Epigenetics 2013;8(2):203–9. https://doi.org/10.4161/epi.23470
22. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microarray. Bioinformatics 2008;24(13):1547–8. https://doi.org/10.1093/bioinformatics/btn224
23. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 2012;28(6):882–3. https://doi.org/10.1093/bioinformatics/bts034
24. Suderman M, Staley JR, French R, Arathimos R, Simpkin A, Tilling K. dmrff: identifying differentially methylated regions efficiently with power and control. bioRxiv 2018. https://doi.org/10.1101/508556. Published online December 31.
25. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. Bioinformatics 2016;32(2):286–8. https://doi.org/10.1093/bioinformatics/btv560
26. Maksimovic J, Oshlack A, Phipson B. Gene set enrichment analysis for genome-wide DNA methylation data. Genome Biol 2021;22(1):173. https://doi.org/10.1186/s13059-021-02388-x
27. Hwang HS, Kim HA. Chondrocyte apoptosis in the pathogenesis of osteoarthritis. Int J Mol Sci 2015;16(11):26035–54. https://doi.org/10.3390/ijms161125943
28. Suri S, Gill SE, Camin SM, de, McWilliams DF, Wilson D, Walsh DA. Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis. Ann Rheum Dis 2007;66(11):1423–8. https://doi.org/10.1136/ard.2006.063354
29. Pan Q, O'Connor MI, Coutts RD, *et al*. Characterization of osteoarthritic human knees indicates potential sex differences. Biol Sex Differ 2016;7:27. https://doi.org/10.1186/s13293-016-0080-z
30. Steinberg J, Southam L, Fontalis A, *et al*. Linking chondrocyte and synovial transcriptional profile to clinical phenotype in osteoarthritis. Ann Rheum Dis 2021;80(8):1070–4. https://doi.org/10.1136/annrheumdis-2020-219760
31. Freystaetter G, Fischer K, Orav EJ, *et al*. Total serum testosterone and western ontario and mcmaster universities osteoarthritis index pain and function among older men and women with severe knee osteoarthritis. Arthritis Care Res 2020;72(11):1511–8. https://doi.org/10.1002/acr.24074
32. Roman-Blas JA, Castañeda S, Largo R, Herrero-Beaumont G. Osteoarthritis associated with estrogen deficiency. Arthritis Res Ther 2009;11(5):241. https://doi.org/10.1186/ar2791
33. Koelling S, Miosge N. Sex differences of chondrogenic progenitor cells in late stages of osteoarthritis. Arthritis Rheum 2010;62(4):1077–87. https://doi.org/10.1002/art.27311