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The Genetics and Functional Genomics of Osteoarthritis

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Abstract

Osteoarthritis is the most prevalent whole-joint degenerative disorder, and is characterized by the degradation of articular cartilage and the underlying bone structures. Almost 600 million people are affected by osteoarthritis worldwide. No curative treatments are available, and management strategies focus mostly on pain relief. Here, we provide a comprehensive overview of the available human genetic and functional genomics studies for osteoarthritis to date and delineate how these studies have helped shed light on disease etiopathology. We highlight genetic discoveries from genome-wide association studies and provide a detailed overview of molecular-level investigations in osteoarthritis tissues, including methylation-, transcriptomics-, and proteomics-level analyses. We review how functional genomics data from different molecular levels have helped to prioritize effector genes that can be used as drug targets or drug-repurposing opportunities. Finally, we discuss future directions with the potential to drive a step change in osteoarthritis research.

INTRODUCTION

Osteoarthritis is the most prevalent form of arthritis in adults. It is a common complex joint disease that can affect weight-bearing joints such as the knee, hip, ankle, and spine as well as non-weight-bearing joints such as the hand, thumb, and finger (44). Globally, almost 600 million people are affected by osteoarthritis, which accounts for approximately 7.6% of the world's population (36). The main symptoms of the disease include joint pain and stiffness, which places osteoarthritis among the leading causes of disability worldwide (44). The risk of developing osteoarthritis is influenced by both genetic and environmental factors, including age, female sex, obesity, and joint abnormalities. The genetic heritability of the disease has been estimated to be between 20% and 60% (4, 84). The prevalence of comorbidities in osteoarthritis patients is substantial and ranges from cardiometabolic diseases to mental health conditions (48). Considering the global rise in life expectancy and average body mass index, as well as the accompanying comorbidities, osteoarthritis represents an increasing health burden worldwide.

Osteoarthritis is a whole-joint disease that irreversibly affects articular cartilage, local bone structure, and the synovium. Cartilage degradation is the primary hallmark of osteoarthritis development. The presence of synovitis indicates the involvement of inflammatory processes in the pathophysiology of osteoarthritis, particularly in cases accompanied by pain symptoms (25). Alterations in both structural composition and cellular activity within the subchondral bone contribute significantly to the progression of osteoarthritis (40). The adipose tissue within the knee's fat pad may also contribute to a state of low-grade systemic inflammation by secreting proinflammatory cytokines, thereby potentially accelerating cartilage degradation (14).

Genome-wide association studies (GWASs) of osteoarthritis have identified multiple genetic risk loci. To further elucidate the genetic architecture of the disease, integration of the genetic findings with functional genomics studies of primary tissues such as the cartilage, synovium, and fat pad is pivotal. In this review, we provide an overview of genetic and functional genomics studies of age-related osteoarthritis and discuss how they have enhanced our understanding of the biological mechanisms underpinning disease onset and progression.

GENETIC STUDIES OF OSTEOARTHRITIS

GWASs aim to discover genetic variants contributing to the risk of developing a disease or trait. Followed by downstream functional genomics, such as fine mapping and effector gene identification, GWASs can help to unravel the mechanisms of disease etiology and aid the translation of genetic discoveries into clinical practice. Osteoarthritis is a complex polygenic disease that is characterized by low effect sizes of risk-conferring variants, and therefore large sample sizes are crucial to detect these effects.

Initial studies exploring the genetics of osteoarthritis have prioritized candidate genes associated with the disease. Miyamoto et al. (64) and Valdes et al. (100) identified a promoter variant (rs143383) in *GDF5* associated with the risk of developing both hip and knee osteoarthritis in individuals of Asian and European descent, respectively. Valdes et al. (101) were also the first to identify an intronic variant (rs12901499) in *SMAD3* associated with both hip and knee osteoarthritis. In a larger study focusing on hip osteoarthritis, Castaño Betancourt et al. (13) used hip minimum joint-space width (mJSW) as an intermediate trait for developing osteoarthritis and identified a *DOT1L* variant (rs12982744) associated with mJSW and hip osteoarthritis. Further highlights regarding weight-bearing joints include the genetic associations of *GLIS3* (rs10116772) with osteoarthritis (knee or hip replacement) (12), *CHADL* (rs532464664, frameshift) and *COMP* (c.1141G>C, rare missense variant) with high risk of hip osteoarthritis (88), and *HLA* variants

(rs7775228 and rs10947262) with the risk of knee osteoarthritis in East Asian and European ancestry populations (67).

In 2011, Arthritis Research UK Osteoarthritis Genetics (arcOGEN), the first genetics consortium for osteoarthritis, was formed as the result of a United Kingdom–wide collaborative effort (70). The arcOGEN stage 1 GWAS included 3,177 knee and hip osteoarthritis cases and 4,894 controls. The establishment of genetic consortia facilitated scientific collaborations and data sharing, leading to a significant rise in sample sizes and paving the way for large-scale GWAS meta-analyses. Evangelou et al. (26) meta-analyzed four knee osteoarthritis GWASs of individuals of European ancestry, totaling 6,709 cases and 44,439 controls. Zengini et al. (106) performed five osteoarthritis GWASs [self-reported osteoarthritis at any site ($n = 12,658$), hospital-diagnosed osteoarthritis ($n = 10,083$), hospital-diagnosed knee osteoarthritis ($n = 4,462$), hospital-diagnosed hip osteoarthritis ($n = 2,396$), and hospital-diagnosed knee and/or hip osteoarthritis ($n = 6,586$)] using the first release of genotype data of the UK Biobank (93) and evaluating for the first time the differences between self-reported and hospital-diagnosed cases. Styrkarsdottir et al. (90) performed a GWAS meta-analysis for hip ($n = 17,151$) and knee ($n = 23,877$) osteoarthritis between samples from Iceland (deCODE) and the UK Biobank (93). The authors reported 23 genome-wide significant independent associations, two of which are low-frequency missense variants for hip osteoarthritis.

Leveraging data from both the UK Biobank and arcOGEN, Tachmazidou et al. (97) added a further 52 not-previously-associated variants across four osteoarthritis phenotypes [knee osteoarthritis ($n = 24,955$), hip osteoarthritis ($n = 15,704$), knee and/or hip osteoarthritis ($n = 39,427$), and any osteoarthritis ($n = 77,052$)]. The authors used for the first time a systematic approach based on independent functional-level evidence to prioritize several osteoarthritis effector genes, including *TGFB1*, *FGF18*, *CTSK*, and *IL11*, that are targets of drugs at an approved or clinical trial stage. They also reported that these prioritized genes are associated with pathways relevant to collagen formation and extracellular matrix organization as well as monogenic diseases that disrupt bone development (97).

In contrast to weight-bearing joints, the genetic component of hand osteoarthritis has been explored by only a handful of studies. The first study by Styrkarsdottir et al. (92) (623 Icelanders with severe hand osteoarthritis and 69,153 controls) identified two loci associated with severe hand osteoarthritis (overlapping *ALDH1A2* 15q22 and 1p31 loci). Following that, a study by den Hollander et al. (19) (837 cases and 77,325 controls) discovered a variant near *MGP* (rs4764133) that was associated with an increased risk of hand osteoarthritis, potentially mediated through attenuation of matrix calcification. Boer et al. (7) [8,691 cases with radiographic X-rays of the hands, part of the Rotterdam Study (45)] focused on the clustering patterns of radiographic structural characteristics of hand osteoarthritis and identified a further two novel loci on chromosomes 1 and 11, respectively, that were associated specifically with thumb osteoarthritis. The authors also highlighted *WNT9A* (rs10916199) as a putative causal gene for thumb osteoarthritis. More recently, Styrkarsdottir et al. (91) performed a meta-analysis of erosive hand osteoarthritis (a severe form of hand osteoarthritis) that included 1,484 cases and 55,680 controls of European ancestry. This study identified two previous loci (*ALDH1A2* and *MGP*) and added two new loci with effector genes associated with bone biology (*BMP6* rs11243284 and *SPP1/MEPE* rs17013495).

The largest GWAS meta-analysis to date was the first output of the Genetics of Osteoarthritis global consortium (**https://www.genetics-osteoarthritis.com**). This consortium conducted GWAS meta-analyses of 11 osteoarthritis phenotypes from both weight-bearing (knee, hip, and spine) and non-weight-bearing (hand and finger) joints (**Table 1**). Although the consortium is a global effort, most samples from their first GWAS meta-analyses are of European ancestry, with only a small proportion of East Asian ancestry (2.8%). Boer et al. (6) reported 100 independently

Table 1 Overview of sample size and ancestry proportion for each OA joint in the largest OA GWAS meta-analysis to date (6)

Joint	N_{cases}	$N_{controls}$	N_{eff}[a]	EUR %	Non-EUR %
OA at any site	177,517	649,173	557,593.5039	99.27%	0.73%
Knee OA	62,497	333,557	210,540.0963	98.92%	1.08%
Knee and/or hip OA	89,741	400,604	293,267.8303	99.12%	0.88%
Hip OA	36,445	316,943	130,745.6692	100%	0%
Total knee replacement	18,200	233,841	67,543.0775	100%	0%
Total hip replacement	23,021	296,016	85,439.4235	100%	0%
Total joint replacement	40,887	327,689	145,405.2368	100%	0%
Spine OA	28,372	305,578	103,846.1927	98.46%	1.54%
Thumb OA	10,536	236,919	40,349.6164	100%	0%
Finger OA	10,804	255,814	41,464.7842	100%	0%
Hand OA	20,901	282,881	77,851.8251	100%	0%

Abbreviations: EUR, European ancestry; GWAS, genome-wide association study; OA, osteoarthritis.

[a]The effective sample size (N_{eff}) was calculated as $4/[(1/N_{cases}) + (1/N_{controls})]$, where N_{cases} is the number of cases and $N_{controls}$ is the number of controls.

associated risk variants, 52 of which were previously unknown. Based on 12 orthogonal biological lines of evidence, including variant-level information, fine mapping, quantitative trait locus (QTL) colocalization, expression level and protein abundance in cartilage, knockout mouse phenotypes, and human rare-disease phenotypes, the authors scored the genes around the identified risk loci. Seventy-seven genes showed at least three lines of orthogonal biological evidence in support of their involvement in osteoarthritis and were defined as high-confidence effector genes for the disease. These genes were subsequently divided based on their involvement in processes of joint degradation, skeletal development, adipogenesis, muscle function, immune response, inflammation, or neuronal function and development. Of these high-confidence effector genes, 20 were tier 1 drug target candidates, representing interesting targets for drug-repurposing opportunities that can accelerate translation. The authors provided insights into biological pathways common to both weight- and non-weight-bearing joints, such as bone and cartilage development. Polygenic risk score analysis indicated that hip osteoarthritis is the most heritable osteoarthritis phenotype that was included in the study.

Previous efforts in the genetics of osteoarthritis, including the study by Boer et al. (6), have focused mostly on European ancestry. Liu et al. (60) conducted a GWAS of knee osteoarthritis in diverse populations, including 1,217 African American participants (742 cases and 475 controls). Despite the small sample size, the authors identified one genome-wide risk variant in *LINC01006*, which had not been associated with osteoarthritis in the European ancestry GWASs at the time. Previously reported osteoarthritis risk loci were not replicated. In 2022, McDonald et al. (63) performed multiancestry and ancestry-specific meta-analyses of samples from the Million Veteran Program (35) and the UK Biobank (93). The multiancestry analysis that included all samples (n = 484,374) identified 27 independent genetic risk loci, pointing to a partial genetic etiology of osteoarthritis that traverses ancestry. This is the largest multiancestry genetic study of osteoarthritis to date, totaling 14.7% individuals of non-European ancestry. Notably, more than 94% of the Million Veteran Program participants are male. The ancestry-specific analyses were performed for each of the four major ancestry groups: European (n = 413,170), Asian (n = 10,194), African American (n = 35,753), and Hispanic (n = 10,327) (63). In the European ancestry analysis, the authors found four new loci that had not been identified in the largest osteoarthritis meta-analysis

to date, from Boer et al. (6). The same four loci were significantly associated with osteoarthritis in the multiancestry meta-analysis.

More recently, several studies have focused on the genetic risk of undergoing joint replacement surgery. A meta-analysis from Henkel et al. (38) that included more than 700,000 individuals of Northern European ancestry was the first study to investigate differences in genetic associations between hip and knee osteoarthritis depending on whether patients had joint replacement surgery (surgical knee osteoarthritis $n = 22{,}525$, nonsurgical knee osteoarthritis $n = 38{,}626$, surgical hip osteoarthritis $n = 20{,}221$, and nonsurgical hip osteoarthritis $n = 17{,}847$). The authors reported 10 novel variants associated with the surgical phenotype but not with the nonsurgical phenotype and overlapping genes involved in processes like autophagy (*ATG7* rs2447606) and mechanotransduction (*PIEZO1* rs202127176). Total hip arthroplasty, reflecting end-stage disease, was also a phenotype investigated by Kulm et al. (57). In particular, the authors performed a GWAS with 15,353 patients with end-stage osteoarthritis and 374,193 controls, identifying five novel genetic loci associated with end-stage hip osteoarthritis treated with total hip replacement.

Genetic discovery studies can glean novel insights by studying disease-related endophenotypes. Osteoarthritis-related traits that have been used as surrogate measures include radiographic images and joint morphology phenotypes. Faber et al. (27) conducted an mJSW GWAS meta-analysis of X-rays, as a proxy for cartilage thickness, of 50,745 individuals and identified 39 risk loci. The clustering of these variants highlighted growth-related mechanisms in the pathogenesis of hip osteoarthritis (27). A GWAS meta-analysis of 44,214 samples studying the genetic architecture of cam morphology using hip alpha angle as a proxy measure for hip shape (28) identified eight significantly associated risk variants. Among these eight loci, a signal at the *TNFAIP8* locus showed the strongest association. The authors reported a moderate genetic correlation between alpha angle and hip osteoarthritis ($r_g = 0.26$, 95% confidence interval 0.10–0.43) and showed evidence of causality between hip osteoarthritis liability and an increase in alpha angle (28).

METHYLATION STUDIES OF OSTEOARTHRITIS

DNA methylation describes the dynamic, tissue-specific covalent addition of a methyl group to the DNA strand. In mammals, DNA methylation mostly targets cytosines that are followed by a guanine base (CpG sites) (9). Its transfer and maintenance are catalyzed by specialized DNA methyltransferases (39). DNA methylation around the transcription start site tends to repress gene expression, whereas its role in other functional regions in the genome remains less predictable. Modern technologies, such as the Infinium microarrays (5, 66), enable genome-wide studies of methylation profiles. Large consortia, such as the Roadmap Epigenomics (58), Genotype–Tissue Expression (GTEx) (69), and Encyclopedia of DNA Elements (ENCODE) (24) projects, have produced methylation datasets of human tissues but did not include joint tissues. Therefore, additional epigenetic studies of osteoarthritis joint tissues are needed.

The majority of methylation studies of osteoarthritis joint tissues have focused on the cartilage—for example, conducting epigenome-wide association studies of cartilage degeneration by examining differences between macroscopically intact (low-grade) and degraded (high-grade) cartilage samples (8, 20, 54, 55, 65, 85, 107) of osteoarthritis-affected joints. The largest of these studies compared matched low-grade and high-grade knee osteoarthritis cartilage samples across 90 patients (55). Kreitmaier et al. (55) identified widespread and robust differences and reported 15,328 epigenetic markers, of which 7,192 (46.9%) were replicated in a smaller (17 patients) validation set (20), suggesting robust epigenetic markers of cartilage degeneration. Pathway analysis of these signals validated established osteoarthritis-related pathways, such as terms linked to skeletal development or the extracellular matrix, but also revealed an epithelium-related term

(positive regulation of epithelial cell migration), which suggests the involvement of methylation in mediating the formation of blood vessels in cartilage during knee osteoarthritis progression.

Den Hollander et al. (21) examined cartilage methylation profiles across joints by investigating 31 matched pairs of low-grade and high-grade osteoarthritis cartilage samples. Of these, 14 and 17 osteoarthritis tissue samples were collected from affected knees and hips, respectively. The authors identified different methylation-level clusters by joint type, irrespective of osteoarthritis grade, highlighting the joint specificity of the epigenetic profile.

Other genome-wide DNA methylation studies have extended to further human joint tissues, such as subchondral bone (46, 107), synovium (55), and the infrapatellar fat pad (54). Zhang et al. (108) investigated subchondral bone samples of three regions of the tibial plateau in knees from 12 patients. In these regions, the subchondral bone underlay macroscopically intact [outer region of the lateral tibial plateau (oLT)], intermediately degraded [inner region of the lateral tibial plateau (iLT)], and highly degraded [inner region of the medial tibial plateau (iMT)] cartilage. Comparative analyses revealed differentially methylated sites between these regions (iLT versus oLT, 72 sites; iMT versus oLT, 397 sites; iMT versus iLT, 257 sites), suggesting epigenetic changes during osteoarthritis progression in the subchondral bone. Some of these differentially methylated sites overlapped with the ones identified in cartilage samples (107) of the same knee regions in the same patient cohort (iMT versus oLT, 111 differentially methylated sites in subchondral bone and cartilage), suggesting similar epigenetic changes during osteoarthritis between cartilage and subchondral bone.

Kreitmaier et al. (55) compared synovium ($n = 78$ osteoarthritis patients) with low-grade ($n = 98$) and high-grade ($n = 90$) cartilage samples and identified global epigenetic differences, also suggesting tissue-specific methylation profiles. Together, these DNA methylation studies of osteoarthritis tissues have generated insights into the joint, tissue, and disease-stage specificity of epigenetic profiles.

TRANSCRIPTOMIC STUDIES OF OSTEOARTHRITIS

Transcriptomics refers to the study of RNA molecules expressed in a given organism, tissue, or cell under a specific developmental stage or condition. This enables the interpretation of the functional components of the genome (i.e., mRNAs, noncoding RNAs, and small RNAs) and the quantification of changes in their expression or structure (splicing and start/end sites) during health and disease (102). To this end, the study of transcriptional changes in osteoarthritis-affected primary tissues (i.e., cartilage, synovium, subchondral bone and infrapatellar fat pad, and joint ligaments) is essential to pinpoint molecular pathways affected in osteoarthritis (49).

Several studies have explored cartilage gene expression changes during osteoarthritis using bulk RNA sequencing from patient primary tissue. These studies mainly point to osteoarthritis being characterized by extensive remodeling of the extracellular matrix in an inflammatory environment (49). The largest study of this kind compared macroscopically intact and degraded paired cartilage tissue from 124 patients undergoing total knee replacement surgery. Katsoula et al. (50) explored transcript-level and splicing events in osteoarthritis, in addition to both coding and noncoding (long noncoding RNA) gene expression changes, identifying differential gene expression for 365 genes (larger than twofold change), differential transcript usage for 82 genes, and differential splicing events for 209 genes. The latter affect multiple extracellular matrix protein genes, including *ABI3BP* and the collagen-coding genes *COL11A1*, *COL1A1*, *COL1A2*, and *COL2A1*.

To shed light on gene regulation, Coutinho de Almeida et al. (16) examined microRNA and mRNA changes in paired preserved and lesioned cartilage from 15 osteoarthritis patients. This

study created a network of 62 microRNAs targeting 238 mRNAs, mainly involved in nervous system development. The authors also prioritized the *NTF3–miR-502-3p* interaction as being involved in osteoarthritis pathogenesis.

To capture changes in early-stage disease, Fisch et al. (31) compared osteoarthritis and non-osteoarthritis cartilage (20 patients versus 18 controls). This study identified 1,332 differentially expressed genes involved in the extracellular matrix, PI3K-Akt, HIF-1, FoxO, and circadian rhythm pathways and prioritized transcription factor networks deregulated in osteoarthritis. Circadian rhythm pathways have been also implicated in osteoarthritis by Soul et al. (83), who compared gene expression levels between osteoarthritis and non-osteoarthritis cartilage (44 patients versus 10 controls). The same study also stratified osteoarthritis patients into two subgroups that reflected chondrogenic and non-chondrogenic changes of the extracellular matrix, respectively.

Osteoarthritis endophenotypes were further refined by Steinberg et al. (86), who stratified 113 osteoarthritis patients using both cartilage and synovium gene expression patterns. This study identified two patient subgroups based on cartilage transcriptomes, one associated with extracellular matrix degradation, the other with inflammation, female sex, and prescription of proton pump inhibitors. This study further constructed a seven-gene classifier that can place knee osteoarthritis patients on the inflammation endotype axis, providing evidence for a continuous rather than discrete classification for patient stratification.

A limited number of studies have focused on other joint tissues and their transcriptomic changes in osteoarthritis or during its progression. Subchondral bone tissue has been recently studied by Tuerlings et al. (99) using RNA sequencing. This study reported 1,569 differentially expressed genes between 24 paired samples from preserved and lesioned subchondral bone. Of these differentially expressed genes, 305 had the same direction of effect in cartilage tissue. The authors prioritized *IL11* and *CHADL* as potential drug targets in both tissues.

Changes in synovial tissue during the disease have also been explored by Nanus et al. (68) using RNA sequencing in paired painful and nonpainful sites from early-stage ($n = 6$) and end-stage ($n = 6$) osteoarthritis patients. The authors reported that the gene expression of both early- and end-stage osteoarthritis painful sites is reflective of neuronal growth and nociceptive signaling, pointing to a differential synovium phenotype participating in mediating pain perception. These findings were further elaborated using single-cell RNA sequencing in synovial fibroblasts, pointing to early-stage osteoarthritis painful synovial sites containing fibroblast subsets with high expression of proinflammatory markers (*CXCL1*, *CXCL2*, *IL-6*, and *LIF*). Gandhi et al. (34) used microarrays to investigate fat pad tissue expression changes between early- and late-stage osteoarthritis in 5 early-stage and 29 end-stage osteoarthritis patients. They reported higher expression of genes for fat metabolism and energy homeostasis, including *ADIPOQ*, *LEP*, and *PPARG*.

Several bulk RNA-sequencing studies have included cartilage (2, 16, 85, 87) and subchondral bone (99) from the hip joints of a limited number of patients. These studies pointed to distinct joint-specific gene expression changes in both cartilage (2, 16, 85, 87) and subchondral bone (99) compared with knee osteoarthritis. They included up to 15 hip samples, and a well-powered study of hip osteoarthritis is still missing.

A number of single-cell studies have been conducted to characterize cell-type-level changes in osteoarthritis. In cartilage, Ji et al. (47) performed the first single-cell RNA-sequencing study comparing chondrocytes ($n = 1{,}464$) across osteoarthritis stages from 10 patients. The authors reported seven distinct chondrocyte populations, providing marker genes for four previously described populations (proliferative, pre-hypertrophic, hypertrophic, and fibrocartilage chondrocytes) and three novel populations (effector, regulatory, and homeostatic chondrocytes). This study

also described a potential transition among proliferative, pre-hypertrophic, and hypertrophic chondrocytes in osteoarthritis.

Following that study, Chou et al. (15) characterized 26,192 chondrocytes from three osteoarthritis patients, replicating on a pathway basis five of the seven chondrocyte types described by Ji et al. (47) and adding another two (reparative chondrocytes and pre-fibrochondrocytes). Both of these studies pointed to pre-hypertrophic and fibrotic chondrocytes being associated with increased cartilage degradation. Lv et al. (61) performed single-cell RNA sequencing for three pairs of intact and damaged osteoarthritis cartilage samples, profiling 17,638 chondrocytes. This study focused mainly on ferroptosis occurring in osteoarthritis cartilage and identified four chondrocyte populations, two of which had been identified by Ji et al. (47) (homeostatic and regulatory chondrocytes) and one of which was identified by both Ji et al. (47) and Chou et al. (15) (homeostatic chondrocytes). The additional two chondrocyte clusters included degenerative chondrocytes and stressed chondrocytes. The latter were further divided into subclusters, among which the authors highlighted ferroptotic chondrocytes as a potential therapeutic target for osteoarthritis.

More recently, a study from Swahn et al. (95) performed single-cell RNA sequencing of healthy ($n = 6$) and osteoarthritis ($n = 6$) cartilage, profiling a total of 70,972 cells. This study identified a total of 10 chondrocyte types in healthy cartilage, including six chondrocyte populations described by Ji et al. (47) (effector, pre-hypertrophic, hypertrophic, regulatory, homeostatic, and fibrocartilage chondrocytes) and three from Chou et al. (15) (reparative chondrocytes, pre-fibrochondrocytes, and fibrocartilage chondrocytes), and further divided the fibrocartilage and regulatory chondrocytes into two separate clusters. Integrating healthy with osteoarthritis cartilage led to the identification of 13 distinct chondrocyte populations, including the 10 chondrocyte populations of healthy cartilage with the addition of proliferative chondrocytes, a pathogenic chondrocyte population, and a chondrocyte population with high expression of metallothioneins. The authors pointed to this specific pathogenic chondrocyte population being expanded in osteoarthritis cartilage and reflecting fibrotic (TGFβ signaling), mechanotransduction (tenascin signaling), and cell senescence.

All of the aforementioned studies were performed in the knee joint. There has been one single-cell RNA-sequencing study that explored chondrocyte changes in osteoarthritis of the hand. Li et al. (59) profiled 105,142 cells from five osteoarthritis donors and reported 13 cell populations. Among those, the authors described inflammatory and fibrocartilage chondrocytes being enriched in osteoarthritis compared with non-osteoarthritis cartilage. Further comparison with the knee joint revealed that the inflammatory chondrocyte population was specific to the hand joint, along with the ferroptosis process as a key mediator of osteoarthritis pathology in the hand.

In synovium, Chou et al. (15) further profiled 10,640 synoviocytes from three osteoarthritis patients. This is the first study to explore intertissue communication between synovium and cartilage. The authors identified 12 distinct synovial cell populations, pointing to a specific group of HLA-DRA$^+$ synoviocytes as the main mediators of crucial proinflammatory osteoarthritis-related cytokines (*IL1B*, *IL1A*, *IL6*, *TNF*, *CCL2*, and *CCL3*) in articular cartilage. Cell types in synovium were further explored by Huang et al. (43), who profiled 93,208 synovial cells from 14 osteoarthritis patients. This study identified seven more general cell populations in osteoarthritis synovium: fibroblasts, antigen-presenting cells, T cells, endothelial cells, mural cells, B cells, and mast cells. Further subtyping of those clusters demonstrated that a greater variety of T cell populations (e.g., Th17) were involved in osteoarthritis pathology compared with the results of Chou et al. (15).

Meniscus has been recently explored by Sun et al. (94) ($n = 3$ healthy individuals and $n = 4$ osteoarthritis patients), leading to the identification of seven cell populations: four found in cartilage (regulatory, pre-hypertrophic, hypertrophic, and fibrocartilage chondrocytes) along with cartilage progenitor cells, fibrochondrocyte progenitors, and endothelial cells. For degenerated meniscus,

the authors reported three specific cell populations: monocyte-derived dendritic cells, degenerated meniscus progenitors, and hypertrophic chondrocytes. They pointed to the differentiation trajectory of fibrochondrocyte progenitors to meniscus progenitors as a key element for meniscus degeneration. A complementary study by Fu et al. (32) profiled 45,744 cells from healthy and degenerated meniscus samples of four osteoarthritis patients and four healthy controls. The authors identified and localized (inner and outer meniscus) five chondral and two pericyte-like cell clusters along with endothelial, Schwann, and infiltrated immune cells. The pericyte-like cells were reported to be decreased in degenerated meniscus, along with two chondrocyte populations characterized by the expression of extracellular matrix decomposers (*MMP/ADAM/ADAMTS* family molecules). Notably, this study did not identify the trajectory of fibrochondrocyte progenitors to meniscus progenitors and reported that the pericyte-like cell populations were similar to fibrochondrocyte progenitors. Swahn et al. (95) also profiled 78,017 meniscus cells from seven healthy controls and six osteoarthritis patients and evaluated shared mechanisms in osteoarthritis. They identified 19 chondrocyte clusters, including the populations described by Sun et al. (94), Ji et al. (47), and Chou et al. (15), pointing to reparative chondrocytes, chondrocytes with high expression of metallothioneins, proliferative chondrocytes, and homeostatic chondrocytes as being depleted in osteoarthritis meniscus. Notably, they also found that the same pathogenic chondrocyte subset (characterized by extracellular matrix deregulation, tenascin, and TGFβ signaling) was expanded in both osteoarthritis meniscus and cartilage.

Lastly, cellular changes in subchondral bone during osteoarthritis have been profiled by Hu et al. (41) using lateral (control) and medial (osteoarthritis-affected) tibial plateaus of two osteoarthritis patients (26,379 cells). They identified 10 cell types, including several infiltrated immune cells (T, B, natural killer, natural killer T, dendritic, monocyte, and macrophage cells) and bone-related cells (endothelial cells, osteoblasts, and mesenchymal stem cells). The authors pointed to increased osteoblasts and endothelial cells in osteoarthritis-affected subchondral bone compared with control tissue. They highlighted the aberrant vascularization patterns mediated by endothelial cells during osteoarthritis and specific osteoblast subpopulations (endothelial osteoblasts, stromal osteoblasts, and mineralized osteoblasts) associated with the vascularization, matrix manufacturing, and mineralization that take place during the disease.

In the age of single-cell sequencing, the aforementioned studies have uncovered specific cell populations that play a role in the pathophysiology of osteoarthritis within a wide range of affected tissues, such as cartilage, synovium, and meniscus (**Table 2**). However, the majority of studies to date have been restricted to a single tissue and focus mainly on the knee joint. A welcome step forward would be to analyze matched joint tissues to interrogate separate and shared mechanisms of osteoarthritis pathogenesis.

PROTEOMIC STUDIES OF OSTEOARTHRITIS

The number of osteoarthritis studies focusing on proteomics has increased in recent years (**Supplemental Table 1**). Access to technologies that allow researchers to conduct high-throughput untargeted proteomics analyses, such as liquid chromatography tandem mass spectrometry, has improved. Targeted approaches, such as different array-based methods, can provide important information on potential novel biomarkers for the early detection and progression of osteoarthritis.

Supplemental Material >

Cartilage remains the most studied solid tissue in osteoarthritis and has been analyzed in seven recent papers (including one studying meniscus). However, some studies have examined bone (two studies) and synovium tissues (four studies), which helps put together a more complete picture of the proteome changes in joints during osteoarthritis progression (**Supplemental Table 1**). There

Table 2 Overview of single-cell studies in OA primary tissues

Study	Joint	Tissue	Number of cells	Number of patients
Cartilage				
Ji et al. (47)	Knee	OA cartilage	1,464	10
Chou et al. (15)	Knee	OA intact and degraded cartilage	26,192	3
Lv et al. (61)	Knee	OA intact and degraded cartilage	17,638	3
Swahn et al. (95)	Knee	Healthy and OA degraded cartilage	70,972	6 controls, 6 OA
Li et al. (59)	Hand	OA intact and degraded cartilage	105,142	5
Synovium				
Nanus et al. (68)	Knee	Synovial fibroblasts (nonpainful early OA and painful late-stage OA)	4,180	4
Chou et al. (15)	Knee	Synovium	10,640	3
Huang et al. (43)	Knee	Synovium	93,208	14
Meniscus				
Sun et al. (94)	Knee	Healthy and OA degraded meniscus	3,639	3 controls, 4 OA
Fu et al. (32)	Knee	Healthy and degenerated meniscus	45,744	4 controls, 4 OA
Swahn et al. (95)	Knee	Healthy and OA degraded meniscus	78,017	7 controls, 6 OA
Bone				
Hu et al. (41)	Knee	Subchondral bone	26,379	2

Abbreviation: OA, osteoarthritis.

are no recently published proteomic studies on fat pad tissues, which should be easily accessible from the surgical waste of osteoarthritis patients undergoing arthroplasty. However, the lack of such data might be due to technical reasons, as tissues with high fat content might be complicated to analyze. A very promising tissue to study is the synovial fluid, as collecting it is less invasive than collecting solid joint tissues, thus making the healthy control tissues accessible. Furthermore, as synovial fluid is an ultrafiltrate of blood plasma, filtered by synovial membrane cells and in contact with all other joint tissues, it reflects the systemic and local molecular pattern at the same time. Six recent studies have analyzed synovial fluid to explain osteoarthritis's pathogenesis, and nine recent studies on osteoarthritis have analyzed different contents of blood, such as plasma, serum, and platelets.

The vast majority of proteomic studies also focus on knee osteoarthritis (21 out of 27 studies analyzed knee osteoarthritis). Hip, hand, and foot joints are included in 4 studies, 3 studies, and 1 study, respectively. There are also 7 studies where the affected joints were not reported, and these might include patients with different joints affected by osteoarthritis. Several studies have demonstrated differences in molecular patterns among different joints affected by osteoarthritis.

The proteomic studies on different local and systemic tissues cover different aspects that can be analyzed to further understand the triggering and progression mechanisms of osteoarthritis. In the case of cartilage from osteoarthritis-affected joints, these studies have discovered novel osteoarthritis-specific hypertrophic chondrocytes (22); detected differences in patterns of posttranslational modifications, such as phosphorylation and N-glycosylation (23, 62); described changes in the calcified cartilage zone (29); further explained the impact of subchondral bone necrosis on cartilage degeneration (82); and described the proteome profile of the meniscus, focusing more on extracellular matrix proteins (71). In subchondral bone from osteoarthritis-affected joints, altered N-glycosylation levels were detected (30), and type 2 diabetes effects on osteoarthritis progression have been described (110). Synovial tissue proteome analyses have been applied to define osteoarthritis molecular endotypes in weight-bearing and non-weight-bearing joints in

obese and nonobese patients (103), to characterize thymus cell antigen 1 fibroblast-like synoviocytes and their role in synovitis (17), and to demonstrate differences among osteoarthritis, chronic pyrophosphate arthropathy, and rheumatoid arthritis (18, 74). Proteome analyses from synovial fluid have revealed changes in synovial fluid composition after joint injury depending on sex, injury pattern, and location of bone bruises (10), as well as changes that may lead to posttraumatic osteoarthritis (73); mapped the proteome pattern without osteoarthritis and during osteoarthritis progression (3, 56, 105); and demonstrated the differences in the proteomes of exosomes from synovial fluid collected from osteoarthritis, rheumatoid arthritis, gout, and axial spondylarthritis patients (42).

At the systemic level, the proteomes of serum, plasma, and platelets collected from osteoarthritis patients, rheumatoid arthritis patients, and control individuals have been analyzed. Differences between the serum samples of osteoarthritis and rheumatoid arthritis patients and those of controls have been demonstrated (37, 109), and contrasts between obese and nonobese osteoarthritis patients' serum protein profiles have been found (98). In the case of plasma samples, differences in proteome patterns between osteoarthritis patient and control samples (80, 89) and among knee, hip, and hand osteoarthritis patient samples (96) have also been described. Blood platelet proteome analyses demonstrated dysregulated pathways in osteoarthritis patients compared with controls (52).

Several potential biomarkers for evaluating osteoarthritis stage and progression, such as S100 calcium-binding protein A7 (S100A7) and heat shock cognate 71 kDa protein (HSPA8) (29) in cartilage and serpin family A member 5 (serpinA5) (111) in blood and cartilage, have been suggested. Synovial fluid proteins reflecting catabolic inflammatory pathways (CRIP1, S100A11, PLS3, POSTN, and VIM) and anabolic chondroprotection [CHI3L2 (YKL-39), TNFAIP6/TSG6, DEFA1, SPP1, and CILP] have been put forward as early detection markers for osteoarthritis (73). GITRL, CEACAM-1, FSH, EG-VEGF, FGF-4, PIGF, cystatin EM, and NT-4 have been reported as potential diagnostic markers and treatment targets for osteoarthritis (105). Catalase has been suggested as a pathogenic indicator (109), and CRTAC1, FBN1, VDBP, and SERPINF1 have been suggested as potential diagnostic biomarkers in serum samples (98). An osteoarthritis prediction model containing 12 serum proteins (SPARC, PLTP, SPARCL1, IGFALS, NCF2, COL11A2, SLC11AA, HTRA, HRG, COMP, IGFALS, and CSPG4) has been developed (33).

In plasma samples, LRG1 has been put forward as a marker for inflammation and joint stiffness (80), and CRTAC1 was the most compelling and robust biomarker for osteoarthritis severity and progression (96) as well as for progression to joint replacement (89). Several treatment targets have been suggested, such as BRAF inhibitor in cartilage (23), components in oxidative stress and low-grade chronic inflammation pathways in subchondral bone (110), and PDK3 in synovium (17). In addition, biomarkers such as CRTAC1 and molecular endotypes for stratifying patients for clinical trials have been proposed (89, 103). Finally, general N-glycosylation levels in joint cartilage and bone have been proposed as applicable for developing both diagnostic and therapeutic methods for primary knee osteoarthritis (30).

MOLECULAR QUANTITATIVE TRAIT LOCI OF OSTEOARTHRITIS SAMPLES

Molecular QTL (molQTL) analysis integrates genetic data with molecular phenotypes, such as transcriptomics, proteomics, or epigenomics. This enables the identification of genetic variants associated with quantitative levels of a molecular trait, including gene expression (eQTLs), protein abundance (pQTLs), and methylation levels of CpG sites (methylQTLs) (1). These molQTLs

Table 3 Overview of the largest genome-wide molecular QTL maps from primary OA tissues, including cartilage, synovium, and infrapatellar fat pads

Tissue	Molecular level	Number of patients	Reference
Intact cartilage	DNA methylation	97	55
	Gene expression	87	87
	Proteomics	99	87
Degraded cartilage	DNA methylation	89	55
	Gene expression	95	87
	Proteomics	99	87
Synovium	DNA methylation	78	55
	Gene expression	77	87
Infrapatellar fat pad	DNA methylation	68	54

Abbreviations: OA, osteoarthritis; QTL, quantitative trait locus.

can help resolve GWAS signals, especially those located in noncoding regions of the genome, leading to a better understanding of the molecular mechanisms underlying disease development and progression (1). By integrating molQTL data on osteoarthritis GWAS risk loci, researchers can pinpoint causal variants and their effects on gene regulation. This helps prioritize effector genes and consequently uncover previously unknown pathways that contribute to osteoarthritis development and progression. Regulation of molecular traits is a complex process that differs among tissues and cells. To better elucidate osteoarthritis GWAS risk signals using molQTLs, it is essential to unveil the specific molecular details from primary tissues or cells relevant to this disease, such as cartilage, infrapatellar fat pads, or synovium.

Steinberg et al. (87) collected macroscopically intact and highly degraded cartilage as well as synovial tissue from 115 osteoarthritis patients undergoing joint replacement and generated genome-wide genotype data to define *cis*-eQTLs and *cis*-pQTLs in these three tissues. **Table 3** provides an overview of the sample sizes for each osteoarthritis primary tissue included in this study, which is the most extensive eQTL and pQTL study for osteoarthritis primary tissues to date. For each gene, the authors considered all genetic variants within 1 Mb of the transcription start site in order to create tissue- and disease-stage-specific eQTL and pQTL maps for osteoarthritis. Because the sample collection was from the same individuals, a comparison between disease stages characterized by degraded and intact cartilage was possible. In total, genetic variants associated with the expression of 1,891 genes in at least one tissue were identified, and variants associated with protein levels were identified for 38 genes in at least one tissue. Integration between osteoarthritis GWAS risk signals and the established cartilage molQTLs using genetic colocalization led to the prioritization of five genes (*ALDH1A2*, *NPC1*, *SMAD3*, *FAM53A*, and *SLC44A2*). All five colocalization index variants reside in noncoding regions of the genome.

For a subset of 98 patients from the osteoarthritis patient cohort from Steinberg et al. (87), Kreitmaier et al. (55) combined DNA methylation data for intact as well as degraded cartilage and synovium with matching genotype data to generate a genome-wide *cis*-methylQTL map for these three primary tissues (**Table 3**). The authors reported 73,836, 52,819, and 40,361 significant (false discovery rate < 0.05) methylation sites associated with at least one methylQTL in intact cartilage, degraded cartilage, and synovial tissue, respectively. A high effect size correlation between intact and degraded methylQTLs was identified. In addition, sex-specific methylQTLs were identified ($n = 282$ in intact cartilage, $n = 337$ in degraded cartilage, and $n = 874$ in synovium). Causal inference analysis using two-sample Mendelian randomization detected 19 methylation sites associated with cartilage degeneration with a putative causal effect on knee osteoarthritis.

COLGALT2, *MFHAS1*, and *WWP2* are examples of genes that are annotated to these methylation sites. By combining the results of genetic colocalization analysis between methylQTLs and osteoarthritis with eQTL data from Steinberg et al. (87), the authors prioritized seven genes.

Kreitmaier et al. (54) generated DNA methylation profiles of infrapatellar fat pads matched to genotype data from the blood of 70 osteoarthritis patients to establish a genome-wide *cis*-methylQTL map of this primary osteoarthritis tissue (**Table 3**). Data were collected during total knee replacement surgeries. The authors identified 35,948 methylQTL-targeted methylation sites and detected 16 colocalizing methylation sites in 11 knee osteoarthritis GWAS signals. They performed causal inference analysis using two-sample Mendelian randomization to further resolve these GWAS signals. Independent methylQTLs were used as instrumental variables to assess the causality between methylation and knee osteoarthritis. Taken together, these methods enabled the identification of 37 potential causal methylation sites for osteoarthritis. These methylation sites were linked to established osteoarthritis effector genes such as *WWP2*, *COL27A1*, and *ALDH1A2* and to novel genes such as *USP8*, *TSKU*, and *FER1L4*.

In addition to the genome-wide *cis*-molQTL maps summarized above, several smaller-scale studies have generated specific molQTLs from osteoarthritis primary tissues and blood, focused on specific GWAS risk loci with the intention of prioritizing target genes (51, 72, 75–79). Rice et al. (77) investigated 42 osteoarthritis risk loci based on a UK Biobank GWAS (11). Using matched genotype and DNA methylation data from 71 osteoarthritis patients who have undergone joint arthroplasty for a knee or hip, the authors identified 24 methylQTLs in 10 osteoarthritis risk loci. Genes prioritized by this study include *COLGALT2*, *COL11A2*, and *WWP2*. A further study on methylQTLs using data from the same patient cohort prioritized *PLEC* and *GRINA* (79). The cartilage methylQTLs detected in this study were replicated across fat pad, synovium, and blood DNA samples from osteoarthritis patients in a study that prioritizes *RWDD2B* as a target of osteoarthritis susceptibility (72). Kehayova et al. (51) identified significant *cis*-eQTLs and *cis*-methylQTLs in osteoarthritis cartilage of the knee and the hip at three CpG sites within an enhancer of *COLGALT2*, supporting osteoarthritis association with this gene. The risk allele for the studied variant was associated with reduced levels of methylation and increased expression of *COLGALT2*.

Understanding the genetic basis of osteoarthritis through molQTL analyses may pave the way for personalized medicine approaches. To democratize this advancement, there is an urgent need to expand the collection of large-scale datasets from primary tissues to diverse populations (104). In addition, efforts should be made to advance the field from bulk to single-cell data by generating single-cell-resolution QTL maps from primary cell types.

CONCLUSION AND FUTURE DIRECTIONS

Advancements in the field of genetics and genomics for osteoarthritis have been notable over the past decade and have led to significant progress. However, it is imperative to sustain this momentum and continue pushing forward. One crucial aspect is the necessity for larger sample sizes in human genetic and functional genomics studies. With increased sample sizes, researchers can delve deeper into genetic discovery, enabling better patient stratification and improved resolution of heterogeneity. Ultimately, this would empower better predictions and clinical translation of the genetic discoveries.

A critical challenge in genomics revolves around achieving diversity in data collection across global populations. Large consortium efforts like the Genetics of Osteoarthritis consortium are the way forward to achieve the inclusion of more globally representative sample collections. However, since the vast majority of GWAS participants are still of European ancestry [94.77%,

according to the GWAS Catalog (81)], there is a pressing need for more inclusive studies. This requires further efforts of the scientific community to engage with diverse communities in an open and educational manner.

The integration of multiomic and genetic data for osteoarthritis into a more comprehensive and biologically interpretable prediction score introduces an opportunity to tackle the challenge of translating genetic findings into the clinic (53). Being able to stratify patients into more homogeneous groups is pivotal to prioritizing osteoarthritis cases for prevention and advancing targeted personalized approaches to treatment.

To further advance our knowledge of osteoarthritis progression and trajectory, longitudinal studies of all molecular levels are needed. In addition, despite the important insights into osteoarthritis molecular mechanisms provided by the different omics levels, the spatial aspect of how cells organize within primary affected tissues of osteoarthritis is still missing. Exploring this spatial aspect will provide further insight into tissue complexity, cellular microenvironments, and disease pathology. Spatial resolution can also shed light on how potentially pathological cell populations are distributed within tissues, paving the way for more effective—and eagerly anticipated—interventions.

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