Insights into the molecular landscape of osteoarthritis in human tissues

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Purpose of review
To provide an overview of recent developments in the field of osteoarthritis research with a focus on insights gleaned from the application of different -omic technologies.

Recent findings
We searched for osteoarthritis-relevant studies focusing on transcriptomics, epigenomics, proteomics and metabolomics, published since November of 2019. Study designs showed a trend towards characterizing the genomic profile of osteoarthritis-relevant tissues with high resolution, for example either by using single-cell technologies or by considering several -omic levels and disease stages.

Summary
Multitissue interactions (cartilage–subchondral bone; cartilage–synovium) are prevalent in the pathophysiology of osteoarthritis, which is characterized by substantial matrix remodelling in an inflammatory milieu. Subtyping approaches using -omic technologies have contributed to the identification of at least two osteoarthritis endotypes. Studies using data integration approaches have provided molecular maps that are tissue-specific for osteoarthritis and pave the way for expanding these data integration approaches towards a more comprehensive view of disease aetiopathogenesis.

Keywords
evolution, multiomics, osteoarthritis, single-cell

INTRODUCTION

Osteoarthritis is a prevalent, debilitating and complex disease affecting the whole joint organ with a high public health burden and no curative therapy [1]. Osteoarthritis primarily affects knee, hip and hand joints leading to alterations in a multitude of joint tissues. The pathophysiology of osteoarthritis is characterized by degradation of articular cartilage, thickening of the subchondral bone, osteophyte formation, degradation of ligaments and synovitis [2].

Osteoarthritis development depends on both environmental (older age, female sex, obesity, joint morphology and injury) and genetic factors, with heritability estimated to be over 50% [3]. To pinpoint the specific genes and pathways associated with osteoarthritis, large-scale genome-wide association studies (GWAS) have been carried out and have identified over 140 osteoarthritis susceptibility risk loci [4–8]. The vast majority of these variants are located in noncoding regions, for which the effector gene is not readily discernible. Therefore, it is important to establish functional links between genomics and disease-relevant alterations on multiple molecular levels.

Increasing scalability and affordability of methods to measure transcriptomic, epigenomic, proteomic and metabolomic alterations in health and disease have led to an increasing number of studies monitoring these alterations genome-wide and combining the different -omics levels to glean new insights into osteoarthritis mechanisms. The aim of this review is

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KEY POINTS

- The integration of molecular data in osteoarthritis-relevant tissues has generated novel insights into tissue-specific molecular profiles.
- Single-cell RNA-sequencing approaches have started to shed light in the cellular heterogeneity of osteoarthritis-relevant tissues and their communication.
- Omic approaches have successfully been utilized for the identification of osteoarthritis subtypes.
- Potential biomarkers from accessible tissues have been identified with the use of proteomics and metabolomics.
- Integration of multiomic data types can reveal functional mechanisms across molecular levels and thus improve our understanding of osteoarthritis.

to give a summary of the knowledge accumulated within the last 20 months in relation to osteoarthritis molecular mechanisms, primarily through using and combining different -omics technologies.

Search criteria

We searched PubMed for studies published in any language during the time frame 1 November 2019–30 June 2021 for the terms 'osteoarthritis' AND [('transcriptomics') OR ('gene expression') OR ('RNA-seq') OR ('methylation') OR ('genomics') OR ('genetics') OR ('proteomics') OR ('ATAC-seq') OR ('ChIP-seq') OR ('metabolomics')]. In this review, we consider studies solely on human samples using both untargeted and targeted approaches and excluding in-vitro systems. We focus on primary research excluding purely bioinformatic analyses (Table 1).

TRANSCRIPTOMICS

Several studies have explored alterations in gene expression transcriptome-wide utilizing RNA-sequencing technology (RNA-seq) in osteoarthritis-relevant tissues, mainly of the knee or hip joints.

Coding transcriptome

The majority of studies have focused on characterizing changes in cartilage and synovium. The largest study of this kind in osteoarthritis involved 115 patients and identified 2557 differentially expressed genes between high-grade (macroscopically degraded) and low-grade (macroscopically intact) osteoarthritis cartilage, 409 of which also demonstrated significant protein-level differences. Notably, this study reported 36 genes with therapeutic potential for osteoarthritis, highlighting the down-regulation of *IL11* as a likely intervention point. The authors additionally stressed extracellular matrix (ECM) remodelling by chondrocytes in an inflammatory milieu as a fundamental molecular hallmark during cartilage degeneration [9▪]. The latter is also in agreement with the hypothesis that tissue cross-talk is a central aspect of osteoarthritis pathophysiology [10]. A recent study explored this cross-tissue interaction by monitoring gene expression changes in subchondral bone ($n = 24$ patients) and cartilage derived from knee and hip osteoarthritis joints. This study compared subchondral bone underlying low-grade and high-grade cartilage, thereby identifying 1569 differentially expressed genes. Of these, 305 genes were also differentially expressed with the same direction of effect between low-grade and high-grade cartilage samples in the same patients. Among these genes, the authors highlight *CHADL* and *IL11* as potential therapeutic targets for knee osteoarthritis [11]. Together, these studies use transcriptome-profiles to indicate potential future therapeutic intervention points, with *IL11* being repeatedly highlighted as a putative gene of interest.

Regulatory transcriptomics

In addition to alterations in the transcription of protein-coding genes, an increasing number of studies have focussed on noncoding RNAs (ncRNAs). Depending on their length in nucleotides, ncRNAs can be divided into two subclasses: small ncRNAs (20–30 nucleotides) and long ncRNAs (lncRNAs; >200 nucleotides) [12]. MicroRNAs (~20 nucleotides) are the most well studied among small ncRNAs due their established role in posttranscriptional gene silencing [13]. LncRNAs are much less studied and their mechanism of action is unclear; however, they can be therapeutically targeted [14]. With regard to osteoarthritis, a recent study characterized for the first time changes in lncRNA transcriptome-wide, and studied the effect of lncRNA on mRNA expression in low-grade and high-grade cartilage from 98 osteoarthritis patients. This study pointed out 191 differentially expressed lncRNAs in degraded cartilage and demonstrated the importance of intergenic and antisense lncRNA in osteoarthritis pathophysiology. The authors also indicated *P3H2-AS1* lncRNA as a potential preclinical target through regulation of its sense gene *P3H2* [15]. Another study focused on microRNAs and their interactions with mRNAs by comparing the synovium between five osteoarthritis patients and three healthy controls. This study identified 395 miRNA–mRNAs pairs and implicated PI3K–Akt signalling in osteoarthritis pathophysiology in synovium [16].

Table 1. List of osteoarthritis -omic studies discussed

Reference	Year	Approach	Joint	Tissue	Type of study	Technology	Design
Richard *et al.* [27▪▪]	2021	Untargeted	Developmental knee components	Long-bone chondrocytes	Epigenomics	ATAC-seq	Characterization of the open chromatin profile (one developmental sample), followed by evolutionary analyses and comparisons with GWAS results
Dunn *et al.* [28]	2019	Untargeted	–	Blood	Epigenomics	450k methylation array	58 osteoarthritis progressors vs. 58 nonprogressors
Duffy *et al.* [30]	2020	Untargeted	Knee	Cartilage	Epigenomics	ChIP-seq	Target-site characterization of cartilage samples of osteoarthritis patients ($n=3$)
Kehayova *et al.* [44]	2021	Targeted	Knee and hip	Cartilage	Resolution of GWAS signals	Pyrosequencing, targeted epigenome editing	Functional characterization of cartilage samples ($n=137$) of osteoarthritis patients
Rice *et al.* [45]	2021	Targeted	Knee and hip	Cartilage, synovium, blood	Resolution of GWAS signals	Pyrosequencing, targeted epigenome editing	Functional characterization of cartilage ($n=177$), synovium ($n=63$) and blood ($n=50$) samples of osteoarthritis patients and cartilage samples of femoral head fracture controls ($n=29$)
Parker *et al.* [46]	2020	Targeted	Knee and hip	Cartilage, fat pad, synovium, blood	Resolution of GWAS signals	Pyrosequencing, epigenome editing	Functional characterization of cartilage, synovium and blood samples of 348 osteoarthritis patients
Sorial *et al.* [48]	2020	Targeted	Knee and hip	Cartilage, fat pad, synovium, blood	Resolution of GWAS signals	Pyrosequencing, RT-qPCR and genome editing	Functional characterization of low-grade cartilage ($n=36$), fat pad ($n=68$), synovium ($n=81$) and blood samples ($n=55$) osteoarthritis patients
Steinberg *et al.* [9▪]	2021	Untargeted	Knee and hip	Cartilage, synovium	Genomics, transcriptomics, proteomics, resolution of GWAS signals	Illumina Human CoreExome-12v1-1, RNA-seq, LC–MS	115 osteoarthritis patients (102 knees, 13 hips), transcriptomics: matched low-grade and high-grade cartilage from 83 patients and synovium from 77, proteomics: 103 patients

Table 1 (*Continued*)

Reference	Year	Approach	Joint	Tissue	Type of study	Technology	Design
Tuerlings *et al.* [11]	2021	Untargeted	Knee and hip	Cartilage, subchondral bone	Transcriptomics	RNA-seq	24 matched preserved and lesioned cartilage and subchondral bone (18 knees and 6 hips)
van Hoolwerff *et al.* [15]	2020	Untargeted	Knee and hip	Cartilage	Transcriptomics	RNA-seq	98 matched preserved and lesioned cartilage (65 knees and 33 hips)
Zhou *et al.* [16]	2020	Untargeted	Knee	Synovium	Transcriptomics	RNA-seq	5 osteoarthritis patients and 3 normal controls
Steinberg *et al.* [21]	2021	Untargeted	Knee	Cartilage, synovium	Transcriptomics	RNA-seq	Low-grade and high-grade cartilage from 113 patients and synovium from 90
Coutinho de Almeida *et al.* [19]	2020	Untargeted	Knee and hip	Cartilage	Transcriptomics	RNA-seq	66 osteoarthritis patients (41 knees and 25 hips) preserved cartilage from 56 and lesioned from 45 patients
Yuan *et al.* [20]	2020	Untargeted	Knee	Cartilage, synovium, subchondral bone	Transcriptomics	RNA-seq	131 osteoarthritis patients 4 healthy controls, 131 osteoarthritis cartilage, 60 synovium samples and 65 subchondral bone. 54 matched along tissues
Chou *et al.* [23▪]	2020	Untargeted	Knee	Cartilage, synovium	Transcriptomics	Single-cell RNA-seq	3 osteoarthritis patients
Wang *et al.* [24]	2021	Untargeted	Knee	Cartilage	Transcriptomics	Single-cell RNA-seq	5 osteoarthritis patients, 5 Kashin–Beck patients, 5 healthy controls
Sun *et al.* [26▪]	2020	Untargeted	Knee	Meniscus	Transcriptomics	Single-cell RNA-seq	4 osteoarthritis patients, 3 healthy controls
Folkesson *et al.* [31]	2020	Untargeted	Knee	Meniscus	Proteomics	LC–MS	10 osteoarthritis patients, 10 healthy controls
Timur *et al.* [32]	2021	Untargeted	Knee	Cartilage, synovium, Hoffa's fat pad, meniscus	Proteomics	LC–MS/MS	14 osteoarthritis patients, 10 healthy controls (synovial fluid 13 patients, cartilage 4, meniscus 4, Hoffa's fat pad 4)
Styrkarsdottir *et al.* [33]	2021	Targeted	–	Plasma	Proteomics	SomaScan	37 278 individuals, of whom 12 178 individuals had osteoarthritis and 2524 had undergone joint replacement

Table 1 (*Continued*)

Reference	Year	Approach	Joint	Tissue	Type of study	Technology	Design
Camacho-Encina *et al.* [34]	2019	Targeted	–	Serum	Proteomics	NAPPA	327 participants (146 incident, 181 nonincident at 96-month follow-up)
Sarkar *et al.* [35]	2021	Untargeted	–	Plasma	Proteomics	2DE-iTRAQ with LC–MS/MS	58 osteoarthritis patients (45 undergoing total knee replacement and 13 undergoing unicompartmental knee replacement) and 40 healthy controls
Xiao *et al.* [36]	2019	Untargeted	–	Urine	Proteomics	iTRAQ with LC–MS/MS	4 osteoarthritis patients and 8 healthy controls
Meessen *et al.* [38]	2020	Untargeted	–	Plasma	Metabolomics	1H-NMR-metabolomics assay	1564 osteoarthritis patients and 2125 controls
Huang *et al.* [39]	2020	Untargeted	–	Plasma	Metabolomics	GC/Q-TOF-MS	12 osteoarthritis patients and 12 healthy controls
He *et al.* [40]	2021	Untargeted	–	Plasma	Metabolomics	GC–MS	12 osteoarthritis patients and 12 healthy controls
Tootsi *et al.* [41]	2020	Targeted	–	Serum	Metabolomics	AbsoluteIDQ p180 kit	70 osteoarthritis patients and 82 healthy controls
Abdelrazig *et al.* [42]	2021	Untargeted	–	Urine	Metabolomics	LC-HRMS	74 knee osteoarthritis patients and 68 healthy controls
Werdyani *et al.* [43]	2021	Targeted	–	Plasma	Metabolomics	AbsoluteIDQ p180 kit	615 primary osteoarthritis patients and 237 healthy controls

2DE-iTRAQ, two-dimensional gel electrophoresis followed by liquid chromatography with tandem mass spectrometry; GC/Q-TOF-MS, quadrupole time-of-flight mass spectrometry; GC–MS, gas chromatography–mass spectrometry; GWAS, genome-wide association studies; LC-HRMS, liquid chromatography high-resolution mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; NAPPA, nucleic-acid programmable protein arrays.

Transcriptomics and genomics

Integrating genetics with molecular profiles can identify molecular quantitative trait loci (molQTL). Steinberg *et al.* [9▪] provided the first molQTL map in three osteoarthritis primary tissues: low-grade and high-grade cartilage, and synovium. This study identified 1891 genes targeted by an expression trait locus (eQTL) in at least one of these primary tissues. In addition, the authors reported 172 genetic variants involved in differential regulation of gene expression between high-grade and low-grade cartilage (differential eQTLs) targeting 32 genes. Closer examinations of these genes revealed involvement in regulation of gene expression, nervous system development, response to stress, immune response, cell adhesion and catabolic processes [9▪].

Identification of osteoarthritis molecular subtypes using transcriptomics

Osteoarthritis is a disease of high heterogeneity both in its clinical manifestation and its molecular characteristics [17]. Molecular subtyping can help disentangle this heterogeneity by clustering samples with a similar molecular profile into groups [18].

In the past 20 months, three studies have used transcriptomics data to identify osteoarthritis patient subgroups. The first study utilized data from both knee and hip joint cartilage derived from 66 patients and described two osteoarthritis subtypes in low-grade osteoarthritis cartilage [19]. The first subtype corresponded to high inflammation showing upregulated chemokine signalling (*CCL2*, *CCL3*, *CCL4*), while the second one demonstrated increased expression of ECM-related components. The high-inflammation cluster was found to be associated with higher joint space narrowing (JSN) scores and low osteophyte scores [19]. Following that, a second study sought to identify osteoarthritis subtypes considering three different tissues including cartilage, synovium and subchondral bone from a total of 131 osteoarthritis patients (131 osteoarthritis cartilage samples, four healthy control cartilage samples, 60 synovium samples, 65 subchondral bone samples) [20]. The authors described four osteoarthritis molecular subtypes based on cartilage transcriptome: a glycosaminoglycan metabolic disorder subtype (C1), a collagen metabolic disorder subtype (C2), an activated sensory neuron subtype (C3) and an inflammation subtype (C4). Vasculature development was linked to clinical features including increased osteophytes in the metabolic disorder subtype and severe JSN in the inflammation subtype [21]. A third study utilized data from knee joint cartilage and synovium from a total of 113 (cartilage) and 90 (synovium) patients, respectively, and was the first study to explore clusters in synovium. In agreement with the first study [19], the authors identified two patient subgroups in low-grade cartilage, which were correlated with patient clinical characteristics. The two subgroups were different from each other in processes relevant to inflammation (cytokine and chemokine signalling), ECM interactions and cell adhesion pathways. The high-inflammation cluster correlated positively with female sex and prescription of proton pump inhibitors. Molecular subtyping in synovium identified two clusters which differed in similar pathways as the two clusters in cartilage. Patient cluster allocation in synovium and cartilage was different, indicating tissue-specific molecular processes active in osteoarthritis [21]. Despite differences in design among the three studies, they all highlight a high-inflammation molecular subtype and a subtype related to extensive remodelling of the ECM. In addition, the different studies also associate the high-inflammation subtype with more severe clinical symptoms including more apparent JSN.

Single-cell transcriptomics in osteoarthritis

Single-cell RNA-sequencing (scRNA-seq) has revolutionized the study of complex biological systems offering a greater view of cellular heterogeneity. Following the first single-cell study in osteoarthritis cartilage by Ji *et al.* in 2019 [22], Chou *et al.* [23▪] sought to characterize cellular and transcriptional heterogeneity of matched cartilage and synovium to glean further insight into the molecular crosstalk between these tissues in osteoarthritis. Profiling of 10 640 synoviocytes and 26 192 chondrocytes from three osteoarthritis patients revealed 12 distinct synovial and seven distinct articular cell populations. Synoviocytes were found to produce a plethora of cytokines relevant to osteoarthritis progression (55%) and a minority of key cytokines were found to be produced exclusively by chondrocytes (16%). To this end, this study identified 31 cytokines (12 uniquely expressed in synoviocytes) and 30 growth factors (seven uniquely expressed in synoviocytes) associated with phenotypic alterations in osteoarthritis chondrocytes. Chou *et al.* replicated the identification of five chondrocyte populations by Ji *et al.*, and characterized two additional distinct populations: the reparative chondrocytes characterized by ECM signalling (*COL2A*, *CLEC3A*, *CILP* and *COMP*) and the prefibrochondrocytes characterized by processes relevant to synthesis of ECM components and increased expression of *IL11*. These two populations were additionally enriched in osteoarthritis cartilage along with fibrochondrocytes and regulatory chondrocytes (RegC)

[23▪]. Increased fibrochondrocytes and RegC in osteoarthritis cartilage were also detected from a second scRNA-seq study comparing osteoarthritis (5834 cells) to healthy (4401 cells) and Kashin–Beck chondrocytes derived from osteoarthritis ($n = 5$) and Kashin–Beck ($n = 5$) patients, and from healthy ($n = 5$) healthy controls, respectively [24]. The authors additionally reported a novel chondrocyte population referred to as mitochondrial chondrocytes. This chondrocyte population was present in cartilage of both diseases but was absent in healthy cartilage indicating mitochondrial dysfunction in osteoarthritis and Kashin–Beck disease [24]. In addition to the roles played by cartilage and synovium, meniscus degeneration and weakening are common but much less studied in osteoarthritis [25]. A recent study on human meniscus samples identified seven cell populations in healthy meniscus, two of which were described for the first time. Comparison of healthy and degenerated meniscus pinpointed alterations in three cell populations: monocyte-derived dendritic cells, hypertrophic chondrocytes and degenerated meniscus progenitor cells (DegP). DegP was identified as a novel progenitor cell population and expression of its marker genes (*GAS1*, *RAB3B* and *CD318*) was associated with aberrant differentiation processes taking place during meniscus degradation. The authors described these differentiation processes in a trajectory from fibrochondrocyte progenitors to DegP, proposed as a marker of meniscus degeneration and as an intervention point [26▪].

EPIGENOMICS

Gene expression is in part regulated by epigenetic processes, such as methylation and chromatin accessibility. Epigenomics defines the whole set of epigenetic modifications in a biological system. Characterizing the epigenomics landscape in disease-relevant tissues can expand our insights into osteoarthritis aetiology beyond gene and protein expression.

One study investigated the chromatin accessibility profile of chondrocytes of joint components in one human developmental sample (59 days old) [27▪▪]. In evolutionary analyses, knee-specific open chromatin regions (knee elements) showed signals for positive selection during hominin evolution and recent constraint and genetic drift, but also overlapped osteoarthritis risk variants. These evolutionary insights allowed the formulation of a model which suggests genetic variants that violate these constraints may not exert a negative effect during knee development, but may have a detrimental influence later, for example by contributing to an increased osteoarthritis risk. Considering this model and overlaying regulatory knee elements with GWAS results, enabled the prioritization of rs6060360 for further study. This is a variant located in the knee enhancer *R4* in the osteoarthritis risk locus for *GDF5*. Functional follow-up analyses linked *R4* loss and the risk allele 'T' of rs6060360 with lower *GDF5* expression and knee shape changes in mouse models, providing evidence in support of a causal role in osteoarthritis. This study shows how epigenomics data of a developmental sample can be used to investigate evolutionary aspects of osteoarthritis and how these insights can be used to identify likely causal variants [27▪▪].

Epigenetic profiles of peripheral but more accessible tissues might have the potential to be used as prognostic biomarkers [28]. A recent study trained a classifier to distinguish patients with progressing osteoarthritis ($n = 58$) from nonprogressors ($n = 58$). Here, progressors were patients with a consistent joint space width loss in affected knees across 48 months, based on radiographic data. Models that used DNA methylation from peripheral blood mononuclear cells and clinical information achieved an accuracy of 73% and outperformed models using solely clinical information. This suggests that DNA methylation can be a relevant resource for patient stratification [28].

Chromatin–protein interactions in osteoarthritis

Chromatin immunoprecipitation assay (Chip-seq) can help map the chromatin regulatory landscape of a given tissue. This has enabled the exploration of protein–chromatin interactions with a focus on transcription factors, and has offered a better view of gene expression regulation. In osteoarthritis, there has been evidence that the expression of the transcription factor forkhead box protein O1 (FOXO1) is increased in the intermediate layers of affected cartilage [29]. A recent study investigated this finding further by applying Chip-seq in primary chondrocytes of osteoarthritis patients ($n = 3$) to characterize FOXO1 binding sites at a genome-wide scale. The authors showed that osteoarthritis-linked pathways are more frequently regulated through FOXO1 binding to sites with a noncanonical motif, whereas in other (ubiquitous) pathways, FOXO1 interacts with the canonical binding sequence [30]. Follow-up analysis integrating cartilage RNA-seq data revealed 428 osteoarthritis-relevant target genes of FOXO1 to be differentially expressed in osteoarthritis. These genes were enriched in osteoarthritis-relevant pathways including senescence, ECM and circadian clock. This study highlights differences in the FOXO1-regulation between osteoarthritis-related and other pathways and

underlines its role in transcriptional changes during osteoarthritis.

Proteomics in osteoarthritis-affected tissues

Recent studies in osteoarthritis have monitored alterations in protein abundance in cartilage, synovium, meniscus and fat pad. Steinberg *et al*. reported differences in 2233 proteins between low-grade and high-grade osteoarthritis cartilage tissue from 115 patients. The main activated pathway among these proteins was ECM receptor interaction [9▪]. A further study examined differences between osteoarthritis and healthy menisci from 10 osteoarthritis patients and 10 healthy donors. The largest differences were observed for matrix metalloproteinase 3 (MMP3), metalloproteinase inhibitor 1 (TIMP1), asporin and versican [31]. Another recent study explored differences in the proteins secreted from cartilage, synovium, Hoffa's fat pad and meniscus from knee osteoarthritis patients ($n = 4$) and compared their abundance in the surrounding synovial fluid of osteoarthritis patients ($n = 10$) and healthy controls ($n = 10$). Using an untargeted mass spectrometry approach (LC–MS/MS), the authors identified 62 proteins that were significantly increased and 234 that were significantly decreased in synovial fluid of osteoarthritis patients compared with healthy donors. Thirty nine out of 62 and 56 out of the 234 were detected in the secretome of synovium, fat pad, meniscus and cartilage. The authors also reported tissue-specific secretion for antileukoproteinase [secretory leukocyte peptidase inhibitor(SLPI)] (highest in cartilage), MMP3 (highest in cartilage), complement C8 alpha chain (C8A) (highest in meniscus) and retinoic acid receptor responder protein 2 (RARRES2) (highest in cartilage). These findings stressed the differential contribution of the different joint tissues to osteoarthritis-relevant alterations in the synovial proteome of the knee and highlighted the fat pad and meniscus as additional important players [32].

Proteomics for biomarker discovery

The approaches described above offer an overview of alterations in osteoarthritis-relevant tissues. However, insufficient accessibility or invasiveness of the relevant tissues limits their utility for early diagnosis. Studies on blood serum or urine circumvent this obstacle and hold great potential in the identification of diagnostic biomarkers. The largest study of this kind from Styrkarsdottir *et al*. explored 4792 plasma proteins in 39 155 individuals, of whom 12 178 had osteoarthritis. This study identified CRTAC1 (cartilage acidic protein 1), a new potential biomarker for knee, hip and hand osteoarthritis, which correlates with disease incidence and predicts joint replacement surgery [33]. Camacho-Encina *et al*. [34] used a targeted proteomics approach (nucleic-acid programmable protein arrays) to explore the potential predictive role of autoantibodies in the serum of 327 osteoarthritis-free at the baseline participants in the development of radiographic knee osteoarthritis during a 96-month follow-up. The authors discovered that elevated serum concentration of autoantibodies against methionine adenosyltransferase 2 β can be used as a predictive marker for osteoarthritis development and validated their findings in an independent cohort ($n = 108$) [34]. Sarkar *et al*. compared plasma from healthy individuals and osteoarthritis patients to identify differentially expressed proteins in circulating blood. They highlighted 52 differentially expressed proteins with haptoglobin, a free haemoglobin (Hb)-scavenging protein, being the most significantly increased in osteoarthritis plasma. This finding, combined with lower abundance for haptoglobin tetramers and elevated autoantibodies against haptoglobin β (a cleaved precursor of haptoglobin), indicated that increased Hb levels may be associated with initiation of inflammation in osteoarthritis [35]. Xiao *et al*. explored urine proteomics differences between osteoarthritis patients and healthy individuals. This resulted in the identification of 102 proteins that had significant differences in their abundances (46 upregulated and 56 downregulated in urine from osteoarthritis patients). Among these proteins, collagen type IV (COL-4), matrix metalloproteinase 9 (MMP9), adiponectin and gamma-butyrobetaine dioxygenase 1 (BBOX1) were highlighted as potential biomarkers for early diagnosis of osteoarthritis in urine [36].

Proteomics and genomics

There have been two studies in osteoarthritis integrating genomics data with protein level abundance to discover genetic variants affecting protein expression (referred to as protein QTLs or protein quantitative trait locus (pQTLs)). Steinberg *et al*. [9▪] explored protein levels in cartilage (low-grade and high-grade) and synovial tissue. This led to the identification of 38 genes with a *cis*-pQTL effect in at least one of the osteoarthritis-relevant tissues. Styrkarsdottir *et al*. [33] studied the role of CRTAC1 variants in osteoarthritis pathogenesis. This study identified eight CRTAC1 pQTL variants in blood plasma which were, upon further testing, not associated with osteoarthritis. This indicated that CRTAC1, although a promising biomarker, is not causally involved in osteoarthritis pathogenesis.

METABOLOMICS

Metabolomics holds great potential for biomarker discovery and understanding of disease mechanisms. Metabolomics refers to the profiling of metabolites in biofluids, cells and tissues [37]. Global high-throughput (untargeted) or targeted MS-based metabolomics offer a global overview compared with greater selectivity and specificity, respectively [37].

Metabolomics for biomarker discovery

The majority of metabolic studies in osteoarthritis have been performed in blood samples comparing osteoarthritis patients to healthy controls. The largest study of this kind sought to identify metabolic signatures in the serum of 1564 osteoarthritis cases and 2125 controls using an untargeted approach (1H-NMR-metabolomics assay) [38]. The authors explored the association of 227 metabolites with radiographic knee/hip osteoarthritis prevalence and progression. They highlighted increased fatty acid chain length as the most strongly associated factor to end-stage osteoarthritis independent of patient BMI. This result indicated the presence of an altered systemic metabolic state in osteoarthritis and stressed the importance of measuring systemic factors in older age [38]. Three further studies, with smaller sample sizes, explored the metabolic changes in plasma or serum of osteoarthritis patients compared with healthy controls [39–41]. These studies identified panels of metabolites altered in the osteoarthritis plasma including cholesterol, lactic acid, stearic acid, alpha-tocopherol and oxalic acid [40], succinic acid, xanthurenic acid and L-tryptophan [39] and in the serum sphingomyelins, phosphatidylcholines, lysophosphatidylcholines, spermine, arginine and glycine [41]. Abdelrazig *et al.* examined changes in urine metabolites between osteoarthritis patients ($n = 74$) and healthy controls ($n = 68$), and identified perturbations in the tricarboxylic acid cycle, pyruvate and amino acid metabolism. This study also highlighted that perturbation of glutamine metabolism is associated with inflammatory osteoarthritis [42]. Together, these studies indicate that there is a complex interplay between chronic inflammation, oxidative stress and collagen destruction in osteoarthritis.

Metabolomics for osteoarthritis subtyping

Metabolomics profiles in osteoarthritis can be used to identify subgroups of patients based on pathological factors including radiographic osteoarthritis progression, obesity, type 2 diabetes and coronary heart disease. A recent study used a targeted metabolomics approach (Biocrates AbsoluteIDQ p180) to identify osteoarthritis metabolic endotypes [43]. This study included 615 osteoarthritis patients and 237 controls and measured a total of 186 plasma metabolites. Three clinical endotypes of primary osteoarthritis (knee and hip) were identified based on distinct metabolic markers: these were characterized by muscle weakness, arginine deficiency and low inflammatory osteoarthritis. The clusters differed from each other in the plasma-levels of (butyrylcarnitine) C4, arginine and lysophosphatidylcholine. Notably, cluster A included more patients with higher BMI and incidence of type two diabetes, cluster B had the highest association with coronary heart disease and cluster C with osteoporosis [43]. Replication of the findings in an independent sample is important for validation of the observations.

RESOLVING GENOME-WIDE ASSOCIATION STUDIES SIGNALS

Large meta-analyses have revealed more than 140 genetic risk variants for osteoarthritis to date [4–8], but their impact on the molecular profile for the most part remains elusive. Therefore, recent studies sought to resolve GWAS risk loci and identify their effector genes in affected tissues.

Steinberg *et al.* provided the first genome-wide molQTL maps of primary osteoarthritis tissue types (see Transcriptomics and Genomics sections). Combining these maps with GWAS results for osteoarthritis traits using colocalization identified high-confidence effector genes of five risk variants (*ALDH1A2* and *FAM53A* in low-grade osteoarthritis cartilage, *NCP1*, *SMAD3* and *SLC44A2* in high-grade osteoarthritis cartilage) [9▪].

Candidate region-focussed functional follow-up studies examined the regulatory activity of a single genetic risk on nearby genes. Kehayova *et al.* showed that the osteoarthritis risk variant rs11583641 targets *COLGALT2*, which encodes a transferase that catalyses the transfer of beta-galactose to collagen, through methylation. Specifically, the authors identified associations between rs11583641 and close methylation sites of a *COLGALT2* enhancer in cartilage of osteoarthritis patients ($n = 137$). In chondrocyte cell models, they found these methylation sites to be negatively correlated with *COLGALT2* expression [44].

Rice *et al.* demonstrated that the risk variant rs75621460 influences *TGFB1* expression in a tissue-specific manner. Using chondrocyte cell models and cartilage samples of osteoarthritis patients ($n = 319$), this study revealed that the risk allele 'A' of rs75621460 is associated with increased methylation levels in nearby methylation sites. These sites were correlated with *TGFB1* expression and

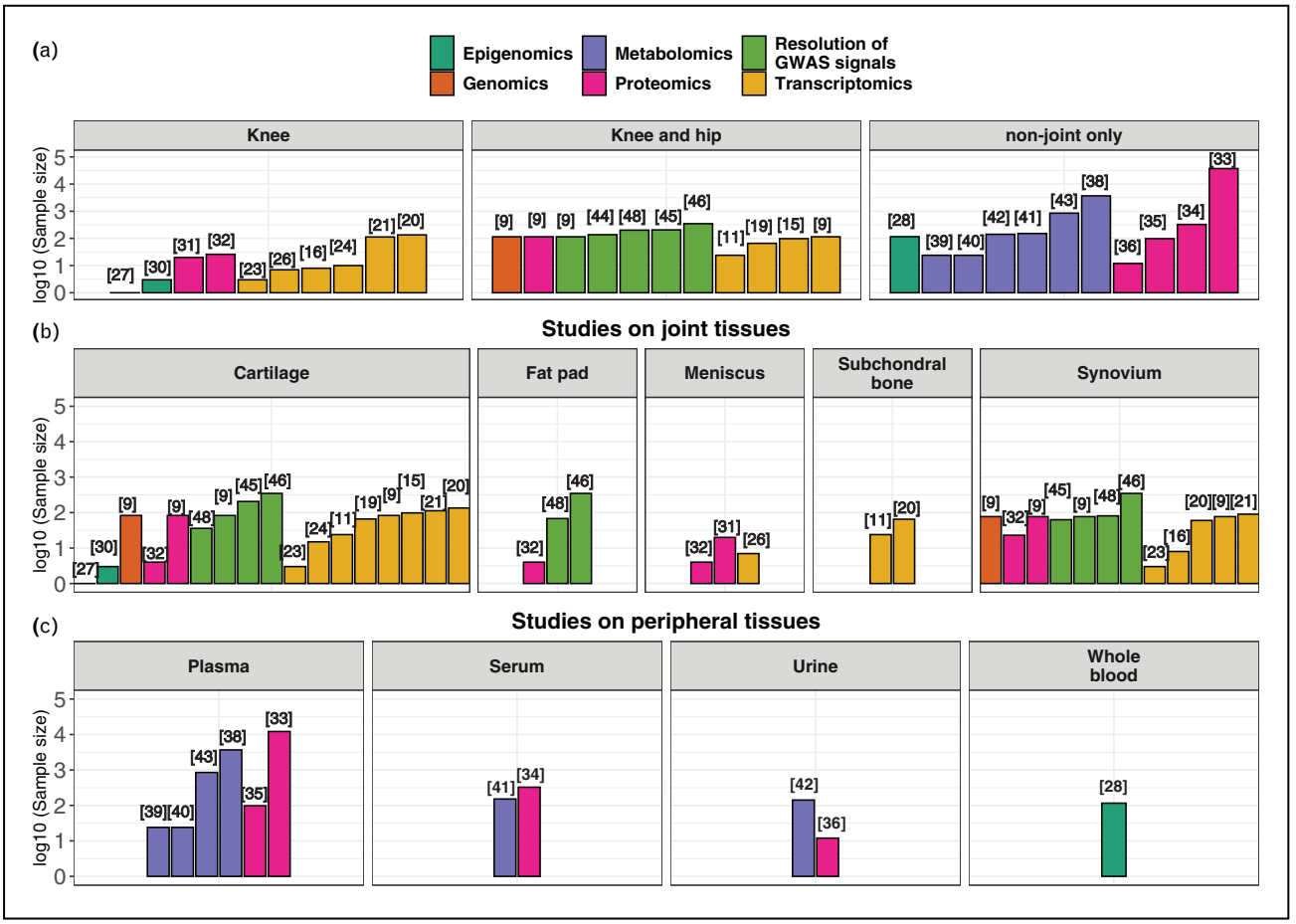

FIGURE 1. Type of -omic studies discussed and their distribution based on tissue. (a) Distribution of studies among -omic technologies and osteoarthritis-relevant tissues. Colours denote the different -omic approaches. (b) Distribution of studies among joint-specific tissues. (c) Distribution of studies among peripheral tissues.

showed opposing direction of effect in the synovium and cartilage of osteoarthritis patients [45].

Similarly, Parker *et al.* showed that the osteoarthritis risk variant rs6516886 targets *RWDD2B* through methylation. Rs6516886 was found to be associated with a nearby methylation site (cg20220242, located upstream of *RWDD2B*) across several tissues (cartilage, fat pad, synovium and peripheral blood) in osteoarthritis patients ($n = 348$). They found that the risk allele 'T' of rs6516886 correlates with reduced expression of *RWDD2B*, which was reversed in a chondrocyte cell model by increasing the methylation levels of *RWDD2B* methylation [46].

A previous study by Rice *et al.* [47] revealed rs11780978 to be an eQTL and an mQTL for *PLEC* expression and methylation in cartilage, respectively. In a recent follow-up study, Sorial *et al.* investigated these effects in additional tissues (fat pad, synovium, blood) of osteoarthritis patients ($n = 36$ in low-grade cartilage, $n = 68$ in fat pad, $n = 81$ in synovium and $n = 55$ in blood). They found tissue-specific differences in the associations between rs11780978 and *PLEC* methylation (present in all tissues, but stronger in joint tissues), between rs11780978 and *PLEC* expression (present in synovium in cartilage, but not in fat pad), and between *PLEC* expression and methylation in synovium. This study suggests that rs11780978 targets *PLEC* also in synovium, but not in fat pad, which highlights the tissue-specificity of these functional mechanisms [48].

CONCLUSION

The current review covers developments in the field of osteoarthritis molecular mechanisms through omics approaches over the last 20 months. Multiple lines of evidence have indicated that osteoarthritis is a complex disease characterized by significant matrix remodelling taking place in an inflammatory environment as a result of multitissue crosstalk. The majority of -omics studies have focused on the transcriptome, potentially due to the cost efficiency,

robust and high-throughput protocols associated with bulk RNA-seq (Fig. 1). Proteomics and metabolomics studies have started to emerge, but larger sample sizes are needed to gain a better understanding of functionally important alterations in osteoarthritis. As with many complex diseases, the vast majority of omics studies conducted in osteoarthritis have focussed on European-descent populations. There is an urgent need to increase the diversity of study participants going forward. Although individual -omics approaches offer important insights into osteoarthritis mechanisms, data integration approaches across -omics levels and combination with patient clinical data hold great promise both for the identification of disease mechanisms and for the discovery of potential therapeutic interventions. In the era of single-cell sequencing, scRNA-seq approaches have identified specific cell populations involved in osteoarthritis pathophysiology in a plethora of afflicted tissues (cartilage, synovium meniscus). The next step towards this direction would be to increase the number of cells and to apply combinatorial -omics approaches on a single-cell level, monitoring both expression and epigenetic regulation. A welcome step further would be to identify the effects of genetic variants on molecular profiles in single-cell resolution [49].

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Conflicts of interest

There are no conflicts of interest.

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