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Whole-genome RNA sequencing identifies distinct transcriptomic profiles in impingement cartilage between patients with femoroacetabular impingement and hip osteoarthritis

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Abstract

Femoroacetabular impingement (FAI) has a strong clinical association with the development of hip osteoarthritis (OA); however, the pathobiological mechanisms underlying the transition from focal impingement to global joint degeneration remain poorly understood. The purpose of this study is to use whole-genome RNA sequencing to identify and subsequently validate differentially expressed genes (DEGs) in femoral head articular cartilage samples from patients with FAI and hip OA secondary to FAI. Thirty-seven patients were included in the study with

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whole-genome RNA sequencing performed on 10 gender-matched patients in the FAI and OA cohorts and the remaining specimens were used for validation analyses. We identified a total of 3531 DEGs between the FAI and OA cohorts with multiple targets for genes implicated in canonical OA pathways. Quantitative reverse transcription-polymerase chain reaction validation confirmed increased expression of FGF18 and WNT16 in the FAI samples, while there was increased expression of MMP13 and ADAMTS4 in the OA samples. Expression levels of FGF18 and WNT16 were also higher in FAI samples with mild cartilage damage compared to FAI samples with severe cartilage damage or OA cartilage. Our study further expands the knowledge regarding distinct genetic reprogramming in the cartilage between FAI and hip OA patients. We independently validated the results of the sequencing analysis and found increased expression of anabolic markers in patients with FAI and minimal histologic cartilage damage, suggesting that anabolic signaling may be increased in early FAI with a transition to catabolic and inflammatory gene expression as FAI progresses towards more severe hip OA. Clinical significance:Cam-type FAI has a strong clinical association with hip OA; however, the cellular pathophysiology of disease progression remains poorly understood. Several previous studies have demonstrated increased expression of inflammatory markers in FAI cartilage samples, suggesting the involvement of these inflammatory pathways in the disease progression. Our study further expands the knowledge regarding distinct genetic reprogramming in the cartilage between FAI and hip OA patients. In addition to differences in inflammatory gene expression, we also identified differential expression in multiple pathways involved in hip OA progression.

Keywords

1 | INTRODUCTION

Femoroacetabular Impingement (FAI) is a common syndrome causing hip pain and dysfunction in athletes and young adults, while hip osteoarthritis (OA) typically affects older patients resulting from a process of global joint deterioration. The osseous deformities defining FAI are the cam and pincer lesions representing a femoral head–neck junction incongruence and acetabular overcoverage, respectively. While these deformities can exist in isolation, most patients with FAI have elements of both cam and pincer architecture presenting as a mixed-type FAI. The cam deformity causes focal damage to the hip joint by impinging on the capsulolabral soft tissues as well as underlying acetabular and femoral head cartilage with hip flexion and rotation.[1] In addition to impacting hip function in young adults, multiple studies have identified a strong clinical association with cam deformity and hip OA.[2–6] In a study of premature hip OA in patients younger than 50, Clohisy et al.[4] identified cam or mixed-type hip architecture in 96% of previously defined cases of "idiopathic" hip OA. Additionally, the cam deformity provides a biologic gradient for hip OA progression with larger deformities associated with a higher risk of developing end-stage OA requiring arthroplasty.[5,6] Despite the strong clinical associations, the specific pathophysiologic mechanisms responsible for OA progression are largely unknown, and the transition from a process defined by focal impingement to one of global joint deterioration remains an area of active investigation.

Several studies have evaluated the expression of inflammatory markers isolated from pathologic tissues in FAI and hip OA (Table 1).[7–13] In a preliminary study of articular cartilage harvested during cam resection for FAI compared to articular cartilage from osteoarthritic hips, Hashimoto et al. reported increased expression of inflammatory mediators and markers of cartilage turnover (IL8, ADAMTS4, ACAN).[7] In a subsequent study, Chinzei et al.[7] confirmed increased expression of inflammatory markers in FAI cartilage compared to OA, while OA labral and synovial samples had higher levels of inflammation compared to FAI. More recently, Haneda and colleagues identified distinct expression profiles between FAI and developmental hip dysplasia, where impingement cartilage had increased expression of inflammatory markers including IL1β, MMP13, and ADAMTS4 compared to dysplastic cartilage. Taken together, these findings suggest that there are distinct expression profiles of FAI and OA articular cartilage with regard to mediators of inflammation and cartilage homeostasis. Further insight into altered gene expression between FAI and OA cartilage may identify unique biomarkers associated with OA progression and yield insight into novel treatment strategies for early OA in at-risk hips.[8,9]

Over the past decade, transcriptomic analyses have significantly advanced the understanding of the genetic framework underlying OA, as well as epigenetic and transcriptional changes that occur with disease progression.[19] Whole-genome RNA sequencing is a powerful tool used to evaluate differential gene expression across different tissue types or disease states.[14,20] There have been several RNA sequencing studies of human articular cartilage in the setting of hip OA which have compared osteoarthritic cartilage obtained during elective total hip arthroplasty to nonarthritic cartilage obtained during arthroplasty for femoral neck fractures or macroscopically normal cartilage areas of otherwise osteoarthritic femoral heads.[14–16,21,22] Recently, Pascual-Garrido et al.[17] published an RNA sequencing study evaluating gene expression differences in FAI and OA cartilage samples where they found unique transcriptomic profiles between the two groups as well as multiple differentially expressed genes (DEGs) relevant to OA progression with a focus on the PPARγ signaling pathway. This approach has advantages over prior sequencing studies in the evaluation of hip OA progression as it evaluates prearthritic tissues at an earlier stage in the disease process. In their study, Pascual and colleagues report no differences in Mankin scores between cartilage specimens for the FAI and OA cohorts, and downstream analyses were on histologically similar cartilage specimens. The purpose of this study was to use whole-genome RNA sequencing to identify and subsequently validate DEGs in femoral head articular cartilage samples with a range of chondral damage (Osteoarthritis Research Society International [OARSI] 1–6) from patients with FAI and hip OA secondary to FAI. We hypothesized that FAI cartilage would have a distinct gene expression profile compared to osteoarthritic cartilage with significant differences in signaling pathways relevant to inflammation, cartilage metabolism, and OA progression.

2 | MATERIALS AND METHODS

2.1 | Subject recruitment

Institutional Review Board approval was obtained before the study with subjects consenting to cartilage sampling preoperatively. Inclusion criteria for the study included both clinical (groin pain with restricted hip motion and positive impingement testing) and radiographic diagnoses of FAI or end-stage OA secondary to Cam or mixed-type FAI (OA) with an alpha angle (AA) of >60° for all patients. Exclusion criteria included prior hip surgery, dysplasia (lateral center-edge angle, LCEA < 25°), avascular necrosis, and rheumatologic conditions. Subject demographics were collected by review of the electronic medical record. Anteroposterior (AP) pelvis radiographs were evaluated to determine AA and Tönnis grade and were performed by the lead and senior authors. Intraoperative cartilage assessment was performed using the Outerbridge classification and defined by the senior author.[23]

2.2 | Cartilage acquisition

Cartilage samples were harvested intraoperatively from both the FAI and OA cohorts. For FAI samples, the location of the cam deformity over the anterosuperior femoral head–neck junction was confirmed fluoroscopically. Care was taken to ensure that sample collection occurred over the macroscopically most severe area of chondral damage. The cam deformity was then arthroscopically resected en-bloc with osteotomes and transferred to the back table where any residual subchondral bone was then sharply debrided with a scalpel before flash freezing with liquid nitrogen for RNA isolation. For OA samples, once the femoral head was excised it was transferred to the back table where a 10 mm biopsy punch was used to obtain an osteochondral sample overlying the anterosuperior femoral head–neck junction. Subchondral bone was then sharply excised, and the cartilage specimen was flash-frozen in liquid nitrogen. Where possible the samples were divided to allow for additional histologic analysis and these samples were immediately transferred to 10% neutral buffered formalin for fixation.

2.3 | RNA isolation and sequencing

For the sequencing arm of the study, 10 patients (5 males, 5 females) were included in the FAI and OA cohorts. Articular cartilage RNA isolation was performed as previously described by Le Bleu et al.[24] (Supporting Information: Appendix 1). Briefly, cartilage samples were cryogenically pulverized followed by RNA isolation with TRIzol reagent (Invitrogen) with lysate purified through GeneJET RNA Purification Kit (Thermo Fisher Scientific). RNA integrity number (RIN) scores as well as average $A_{260/280}$ and $A_{260/230}$ ratios were used to determine RNA quality and evaluate fitness for downstream analysis. RNA sequencing was then performed in conjunction with the University of Rochester Genomic Research Center (Supporting Information: Appendix 2).

2.4 | Geno ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses

To determine potentially altered biological functions and signaling pathways during OA progression, upregulated genes in FAI and OA were input into DAVID Gene Functional Classification Tool (http://david.abcc.ncifcrf.gov; Version 6.8)[25] to perform Gene Ontology

(GO) enrichment (biological process) and KEGG signaling pathway analyses, respectively. The top 10 GO terms and identified KEGG signaling pathways as well as their corresponding *p* values were visualized using GraphPad Prism (version 9.0; GraphPad Software) and ggplot2 R package.[26]

2.5 | Transcription factor-binding motif analysis and gene regulatory network reconstruction

To obtain putative transcription factor (TF) and their associated binding motifs governing co-upregulated genes in FAI and OA cartilage, upregulated in DEGs FAI and OA were analyzed by RcisTarget R package with default parameters and *hg19-tss-centered-10kb-7species.mc9nr.feather* as the database.[27] For each disease condition, the top three TFs (i.e., the highest enrichment scores) that potentially regulate reprogramming gene expression profiles in FAI and OA with their corresponding binding motif were selected and visualized. Gene regulatory networks that are controlled by these TFs were then constructed and visualized by Cytoscape (version 3.9) with the size of the node (i.e., gene) representing degree and the edge connecting two nodes representing edge betweenness.[28]

2.6 | Validation of anabolic and catabolic markers

On the basis of our DEG results, we selected well-known catabolic markers (MMP13, ADAMTS4) involved in cartilage degradation and anabolic markers that have been identified as potential targets for OA treatments (WNT16, FGF18).[7,18,29] Validation consisted of both quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemical analyses. There were 17 patients included in the initial validation cohort with viable histology and qRT-PCR data obtained from 12 of these (8 FAI, 4 OA). For the qRT-PCR analysis, isolated RNA was treated with DNAse (Invitrogen) and transcribed into complementary DNA (cDNA; iScript cDNA Synthesis Kit; Biorad). Gene expression was then quantified by using qRT-PCR reactions performed using the QuantiTect SYBR Green PCR Kit (Qiagen; Hilden). We used gene-specific primers (Supporting Information: Table S1; Integrated DNA Technologies Inc.). Transcript quantity measurements were normalized to *GADPH* and gene expression levels were quantified using the $2^{-\Delta C_T}$ method.

2.7 | Histology and immunohistochemistry

Following fixation for 48–72 h, specimens were dehydrated and embedded in paraffin wax followed by sectioning at 5 μm. Sections were deparaffinized and stained for hematoxylin and eosin, safranin-O/fast green, and anti-FGF18, respectively (Supporting Information: Appendix 3).

Histologic cartilage quality was graded in a blinded fashion by two faculty investigators (Chia-Lung Wu and Jennifer H. Jonason) using the OARSI criteria which have previously demonstrated satisfactory intra- and interobserver reliability. The intraclass correlation coefficient between the faculty graders was 0.74 with grading differences resolved in the conference for the final analysis. FAI samples that had lower OARSI scores (1–3) were considered low-grade FAI while FAI samples with higher OARSI scores (4–6) were considered high-grade FAI. A postsequencing validation cohort of four low-grade and four

high-grade OARSI cartilage samples from FAI patients was compared to four OA cartilage samples with a qRT-PCR evaluation of select markers (Supporting Information: Table S1).

2.8 | Statistical analysis

The results of qRT-PCR and histological grading were analyzed with JMP and PRISM. Differential expression was determined using the false discovery rate or adjusted $p < 0.05$, as appropriate. A priori power analysis (G*Power; Universität Düsseldorf)[30] was performed using the aligned data from the RNA sequencing results on FGF18 which required three subjects in the FAI and OA cohorts to determine differential expression with adequate power $(1 - \beta > 0.8; p < 0.05)$.

3 | RESULTS

3.1 | Cohort demographics

Thirty-seven subjects (15 female; 40%) with a mean age of 46.5 ± 17.5 and body mass index (BMI) of 28.7 ± 5.9 were included in the study (Table 2). Cartilage samples from 20 subjects yielded high-quality RNA (RIN > 7) suitable for downstream analysis and were included in the RNA sequencing. Cartilage samples harvested from the remaining 17 subjects were used for RNA sequencing validation with qRT-PCR and immunohistochemistry (IHC) analyses with 12 samples allowing for both qRT-PCR and IHC. Subjects in the OA cohort were significantly older with a higher BMI; however, there was no difference in AA or LCEA (Table 2).

All patients in the FAI cohort had Tönnis Grades 0–1 while the OA cohort had predominantly Grade 3 degenerative changes (Table 3). Intraoperative arthroscopic cartilage evaluation of FAI patients identified Outerbridge Grades 0–4 degeneration on both the acetabulum and femoral head. There were no significant differences between individual OARSI grades for the FAI and OA cohorts ($p > 0.05$).

The OARSI grades for FAI ranged from 1 to 5 while the OARSI grades for OA cartilage ranged from 2 to 6 (Table 3). FAI Cartilage samples with mild histologic cartilage degeneration (OARSI Grades 1–3) were compared to high-grade FAI samples with severe cartilage degeneration (OARSI Grades 4–6) as well as osteoarthritic cartilage samples in the validation cohort (OARSI Grades 4–5; Table 4).

Low-grade FAI cartilage specimens had a greater proportion of arthroscopic femoral head Outerbridge Grade 0 changes (100%) compared to high-grade FAI samples (0%; $p = 0.008$; Supporting Information: Table S2. There were no differences in Outerbridge acetabular cartilage grading when comparing low-grade and high-grade FAI specimens.

3.2 | Transcriptomic analysis

Whole-genome RNA sequencing identified 3532 genes that were significantly differentially expressed between the FAI and OA sequencing cohorts ($p < 0.05$; Figure 1). Of these, there were 27 genes in the OA cohort that had a Log_2 fold change (FC) greater than 2 while there were 523 genes that had a Log_2FC greater than 2. The gene expression heat map (Figure 1A) and principal component analyses (Figure 1C) demonstrate distinct transcriptomic profiles

between FAI and OA cohorts, with the volcano plot (Figure 1B) depicting expression trends relative to FC.

3.3 | KEGG pathway and GO functional analysis

For KEGG analysis, we observed that calcium, cGMP-PKG, FoxO, cAMP, and extracellular matrix(ECM)–receptor interaction signaling pathways were significantly enriched in FAI patients, while OA patients exhibited increased signaling pathways regarding rheumatoid arthritis, protein degradation and absorption, and interleukin (IL)-17 signaling (Figure 2A). For GO analysis, we identified that OA patients had the lowest p value for collagen catabolic process, extracellular matrix disassembly, and proteolysis, while intriguingly we detected there were several muscle- and contractile element-associated GO terms enriched in FAI patients (Figure 2B,C).

Gene regulatory network analysis identified myogenic differentiation 1 (MYOD1), estrogen-related receptor gamma (ESRRG), and Maf Bzip transcription factor A (MAFA) as putative TFs controlling gene expression in FAI chondrocytes, while signal transducer and activator of transcription 1 (STAT1), BTB domain and CNC homolog 1 (BACH1), and RELA proto-oncogene, nuclear factor-κB subunit (RELA) were potential TFs governing gene reprogramming in OA chondrocytes (Figures 3 and 4).

When the differential expression data were analyzed with the ingenuity pathway analysis (Qiagen) and additional literature review, multiple genes were identified that were involved in OA signaling pathways (Table 5).

3.4 | Target validation

qRT-PCR analysis was performed on select genes (*FGF18*; *WNT16*; *MMP13*; and *ADAMTS4*) from low-grade and high-grade FAI impingement cartilage as well as the OA cartilage samples in the validation cohort. Low-grade FAI cartilage demonstrated a 343.1-fold increase in *FGF18* expression compared to OA cartilage, while high-grade FAI had an 11.0-fold increase in expression compared to OA cartilage (Figure 5). *WNT16* was increased 57.8-fold in low-grade FAI and 19.0-fold in high-grade FAI with respect to OA cartilage. OA cartilage had significantly increased expression of *MMP13* compared to early and late FAI cartilage and significantly increased expression of *ADAMTS4* over early FAI cartilage ($p < 0.05$). *MMP13* also had significantly greater expression in late, compared to early FAI ($p < 0.05$).

3.5 | Immunohistochemical target confirmation

Immunohistochemical staining against FGF18 has been performed on articular cartilage sections from the low-grade and high-grade FAI impingement cartilage as well as OA samples in the validation cohort. FGF18 expression was increased in patients with low-grade cartilage degeneration compared to FAI patients with high-grade cartilage lesions (Figure 6).

4 | DISCUSSION

The hypothesis that FAI and OA femoral head articular cartilage will have distinct genomic expression profiles was confirmed through whole-genome RNA sequencing of femoral head articular cartilage samples. Furthermore, we found increased anabolic signaling in the FAI cohort compared to catabolic signaling in the OA cohort. We identified greater potential chondroprotective gene expression (FGF18; WNT16) in FAI cartilage samples with low histologic OARSI grades compared to FAI cartilage with higher degradation and increased levels of catabolic gene expression (MMP13; ADAMTS4) in OA cartilage tissue. Taken together, these findings support alterations in gene expression as FAI progresses to OA, where early anabolic signaling is replaced with catabolic signaling that predominates later in the disease course.

Transcriptomic evaluation of articular cartilage in FAI offers an appealing avenue to study OA progression as diseased human tissues can be ethically accessed before the development of end-stage OA. Recently, Pascual-Garrido et al.[17] have published their results on RNA sequencing of FAI and OA articular cartilage using a similar methodology to the present study. They identified 50 DEGs with an FC ≤ -1.5 or ≥ 1.5 and found upregulation in the PPARγ signaling pathway in FAI compared to OA cartilage. On the basis of KEGG pathway analyses, we identified similar upregulation in, ECM–receptor interaction and, calcium signaling pathways for FAI, and lysosomal pathways in OA. In the present sequencing analysis, we found a greater number of DEGs (550 with FC ≤ -2.0 or ≥ 2.0) than previously reported. One explanation for the discrepancy is based on the degree of cartilage degeneration present at the time of tissue acquisition. In their study, Pascual-Garrido and colleagues harvested FAI cartilage that was similar in histologic appearance to OA cartilage with no significant differences in Mankin score ($p > 0.99$), while we found FAI cartilage with a range of degeneration (OARSI 1–5). In a previous study, Hashimoto et al. found differential expression of select genes (IL8, CXCL2, CXCL3, and ACAN) based on Beck's macroscopic grading system of FAI and OA cartilage.[7] It is possible that increased DEGs were identified in the present study by the inclusion of cartilage samples with a wider spectrum of degenerative pathology in the sequencing analysis.

While this study did not identify PPARγ as a hub gene, regulatory network analyses identified MYOD1, ESRRG, and MAFA as primary regulatory TFs enriched in FAI. MYOD1, a master regulator for myoblast determination has been found to be upregulated in chondrocytes as they differentiate to a hypertrophic phenotype.[48,49] MAFA, a gene involving insulin regulation, was found to be hypermethylated in arthritic knee articular cartilage and ESSRG has been identified as a catabolic regulator of OA through induction of MMP13 and MMP3 expression.[50,51] Pathways regulated by these TFs have not been well studied in the setting of hip OA and require further investigation. Regulatory TFs increased in OA cartilage in this study included RELA, STAT1, and BACH1 which have been previously shown to contribute to OA pathogenesis.[52–54] Upregulation of RELA and BACH1 in OA cartilage was particularly interesting as these genes have been previously shown to influence inflammatory-mediated disruptions to chondrocyte homeostasis in OA models.[54,55]

Differences in inflammation and cartilage metabolism between FAI and OA cartilage tissues are well documented in the literature. In a landmark study by Hashimoto and colleagues, the authors demonstrated increased cartilage expression of ADAMTS4, IL8, and ACAN in FAI patients compared to OA patients that had preoperative FAI morphology.[8] In a subsequent study by Chinzei et al.,[7] the authors found increased expression of MMP13, IL1β, IL8, and ADAMTS4 in impingement cartilage in an FAI cohort compared to OA resulting from undefined etiology. While Hashimoto et al. did not find differences in IL1β or MMP13 expression between the FAI and OA cohorts, the differences in MMP13 and IL1β identified by Chinzei et al. may be secondary to cartilage sampled from osteoarthritic hips with non-FAI morphology.[7,8] The discrepancies in MMP13 and IL1β expression levels between these studies may possibly result from etiologic differences in the OA cohorts, and it is possible that OA secondary to FAI may have an increased inflammatory profile compared to OA resulting from dysplasia or other etiologies. This is supported by recent immunohistologic data from Haneda and colleagues who found no differences in staining of IL1β, ADAMTS4, and MMP13 in cartilage samples from patients with FAI or OA secondary to FAI, but both were increased compared to osteoarthritic cartilage obtained from dysplastic hips.[10] Consistent with these studies, we found that markers of cartilage breakdown (MMP13, ADAMTS4) were expressed in both FAI and OA cartilage tissues. While we observed increased expression of these markers in the OA compared to the total FAI cohort on the sequencing arm of this study, subsequent validation with qRT-PCR found similar expression between high-grade FAI and osteoarthritic cartilage, suggesting that expression of inflammatory markers increases as FAI progresses towards OA. These findings were supported in a recent study by Liang and colleagues who found differences in COL2A1, ACAN, and MMP3 immunostaining between early and late-stage FAI samples.[18] Taken together, these results suggest that as the degenerative processes of FAI become more advanced the articular cartilage expression profile parallels that of osteoarthritic cartilage.

Differences in gene expression between low OARSI grade FAI and high OARSI grade FAI may reflect progressive cartilage degradation. Impingement cartilage obtained from the FAI population in this study includes a spectrum of chondral pathology confirmed with macroscopic intraoperative grading (Outerbridge Grades 0–4) as well as microscopic immunohistologic evaluation (OARSI Grades 0–5). We found that FGF18 and WNT16 had greater expression in low-grade FAI compared to high-grade FAI and OA while catabolic genes (MMP13 and ADAMTS4) had greater expression in high-grade FAI and OA compared to low-grade FAI. These results are consistent with the recent studies reported by Haneda and colleagues that found FAI cartilage samples with higher OARSI grades to have similar expression of MMP13 and ADAMTS4, both of which were elevated compared to control samples without OA.[10] Understanding the evolution of cartilage gene expression as FAI progresses towards end-stage OA may yield insight into the specific molecular processes driving the transition from focal impingement to global joint destruction.

A recent genome-wide analyses study identified 10 OA-associated genes whose encoded proteins have targeted therapeutics in development or already in the market.[47] RNA sequencing results from this study found significantly differential expression in 4 of these 10 genes (TGFβ1, FGF18, CTSK, and MAPT). For this study, we focused on validating FGF18 expression results as FGF18 has been shown to have significant chondrogenic

effects in both in vitro and in vivo disease models of knee OA.[29,33,34,56–60] Acting through FGFR3 signaling, FGF18 has been shown to increase type II collagen production as well as stimulate cartilage repair, increase cartilage thickness, and prevent joint degradation in murine models of posttraumatic OA.[29,33,56,58] Further, sprifermin (the recombinant human FGF18 analog drug) has been shown to promote a dose-dependent increase in cartilage thickness, particularly in the lateral compartment, of patients with pre-existing knee OA in the double-blind randomized control FORWARD (*FGF18* OA randomized trial with administration of repeated doses) drug trial.[60] The finding from the present study that FGF18 is upregulated particularly in low-grade FAI, compared to OA cartilage indicates that altered FGF18 signaling may play a role in the progression of FAI-induced OA. Additional studies are required to further evaluate FGF18 signaling in FAI to investigate downstream signaling effects, additional therapeutic targets, and the role of sprifermin in hip cartilage repair and OA prevention.

5 | LIMITATIONS

This study has multiple limitations that may affect the interpretation of its findings. There was not a true negative control cohort of patients without FAI or OA, as harvesting cartilage from asymptomatic living donors without evidence of disease would be unethical. Second, while efforts were made to ensure that patients in the OA cohort had Cam morphology, we were unable to obtain radiographs before OA onset and it is possible that the increased AA was secondary to osteophyte formation. There were also differences in sampling techniques, as tissues from FAI patients were collected arthroscopically while tissues from OA patients were harvested after femoral head resection during total hip arthroplasty. Additionally, while both qRT-PCR and IHC specimens in the validation cohort were obtained from the same region of the Cam deformity at the anterolateral femoral head–neck junction, it is possible that there were geographic expression differences within the Cam deformity itself. Additionally, these findings should be limited to FAI and OA, as other etiologies of hip OA may be governed by separate pathologic processes. Future studies will be required to evaluate the homogeneity of gene expression and cartilage quality throughout the Cam deformity as well as confirm the expression changes identified in the current report. Despite these limitations, using an unbiased whole-genome sequencing technique, the present study identified distinct transcriptomic profiles between FAI and OA and DEGs in multiple signaling pathways relevant to OA.

6 | CONCLUSION

RNA sequencing of articular cartilage in the impingement zone of FAI and OA patients revealed significantly differential expression of greater than 3000 genes. Our results also uncovered distinct gene expression profiles and signaling pathways between FAI and OA cartilage that may reflect hip OA progression. A detailed understanding of molecular mechanisms underlying transcriptomic reprogramming in chondrocytes from FAI to OA will provide significant insight into the development of an early therapeutic intervention for hip OA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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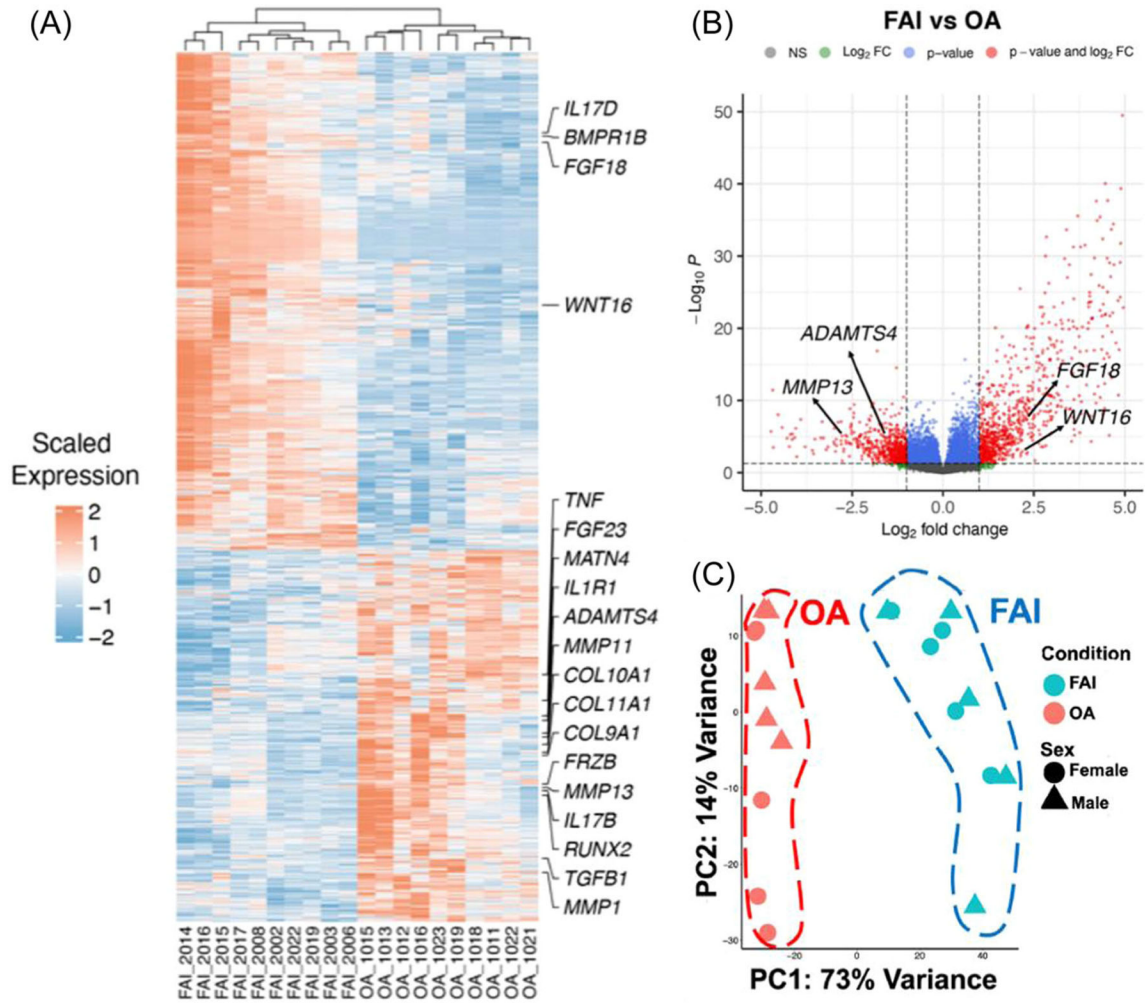
FIGURE 1.

(A) Heat map demonstrating sample-specific differential expression (FAI, red, positive log fold change), OA (Blue, negative log fold change). (B) Volcano plot demonstrating differential gene expression between FAI and OA cohorts. Negative fold change represents an increased expression of genes in the OA cohort while positive fold change represents an increased expression of genes in the FAI cohort. (C) Principal component analysis (PCA) demonstrating sequencing variation between OA and FAI samples. Log2FC, Log_2 fold change highlighting $p < 0.05$). FAI, femoroacetabular impingement; NS, not significant; OA, osteoarthritis.
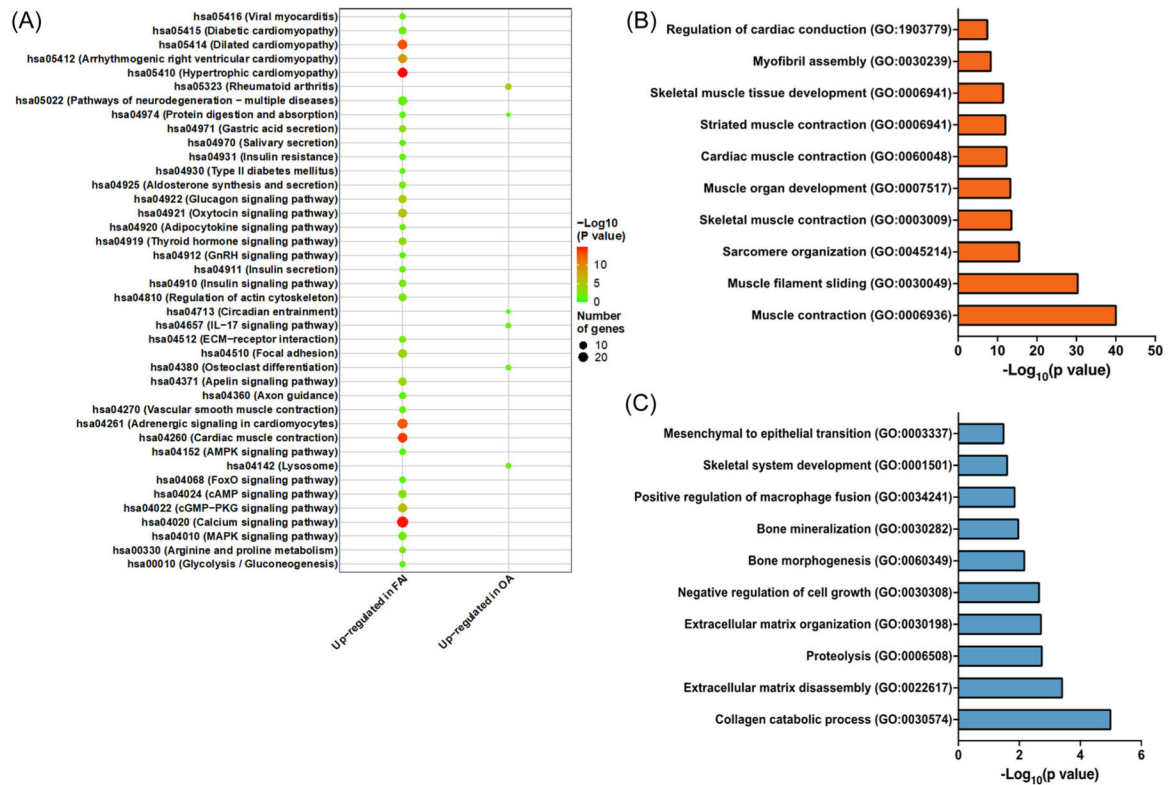
FIGURE 2.

KEGG pathway and Gene Ontology (GO) functional analysis for DEGs upregulated in FAI and OA. (A) Distinct KEGG signaling pathways were identified in the cartilage between FAI and OA patients. (B, C) GO functional analysis showing collagen catabolic process, extracellular matrix disassembly, and proteolysis GO terms were enriched in OA patients, while several muscle- and contractile-element-associated GO terms were observed in FAI patients. DEG, differentially expressed gene; FAI, femoroacetabular impingement; KEGG, Kyoto Encyclopedia of Genes and Genomes; OA, osteoarthritis.
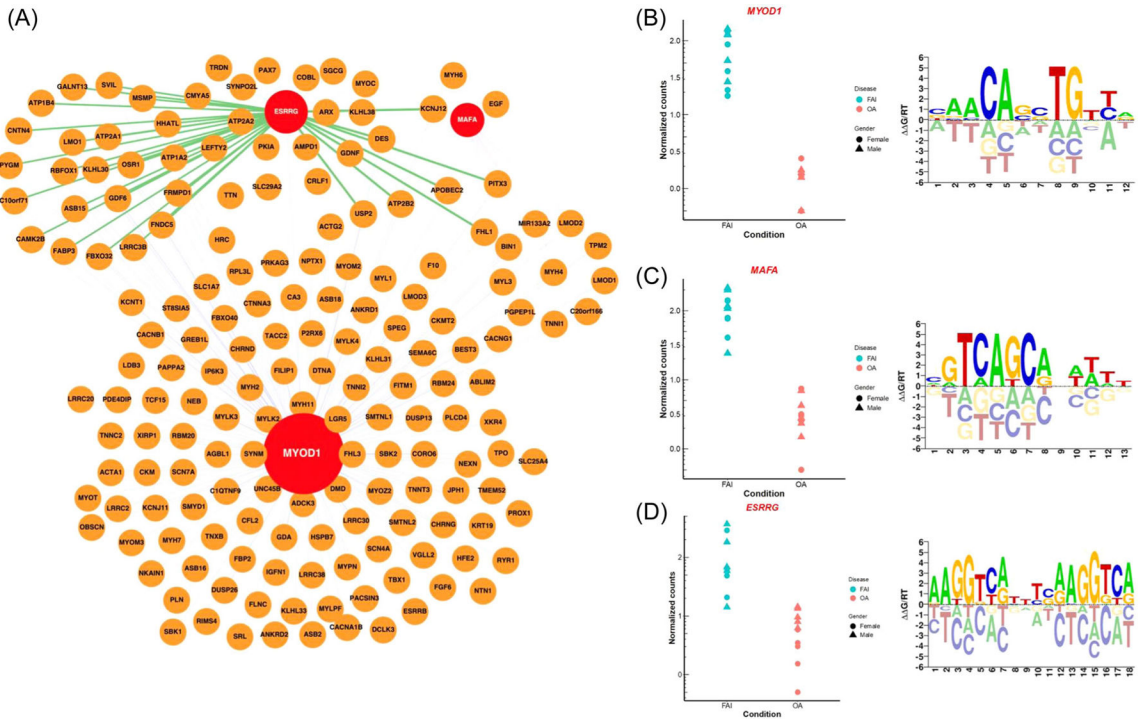
FIGURE 3.

Gene regulatory network for upregulated DEGs in FAI articular cartilage. The transcription factors MYOD1, MAFA, and ESRRG were the primary enriched network hubs for the pathway analysis (A). Normalized counts as well as the binding motif for each transcription factor are presented for MYOD1 (B), MAFA (C), and ESRRG (D). The size of a node represents the degree of a given gene, while the thickness of the edge connecting two nodes is positively associated with edge betweenness. DEG, differentially expressed gene; ESRRG, estrogen-related receptor gamma; FAI, femoroacetabular impingement; MAFA, Maf Bzip transcription factor A; MYOD1, myogenic differentiation 1.
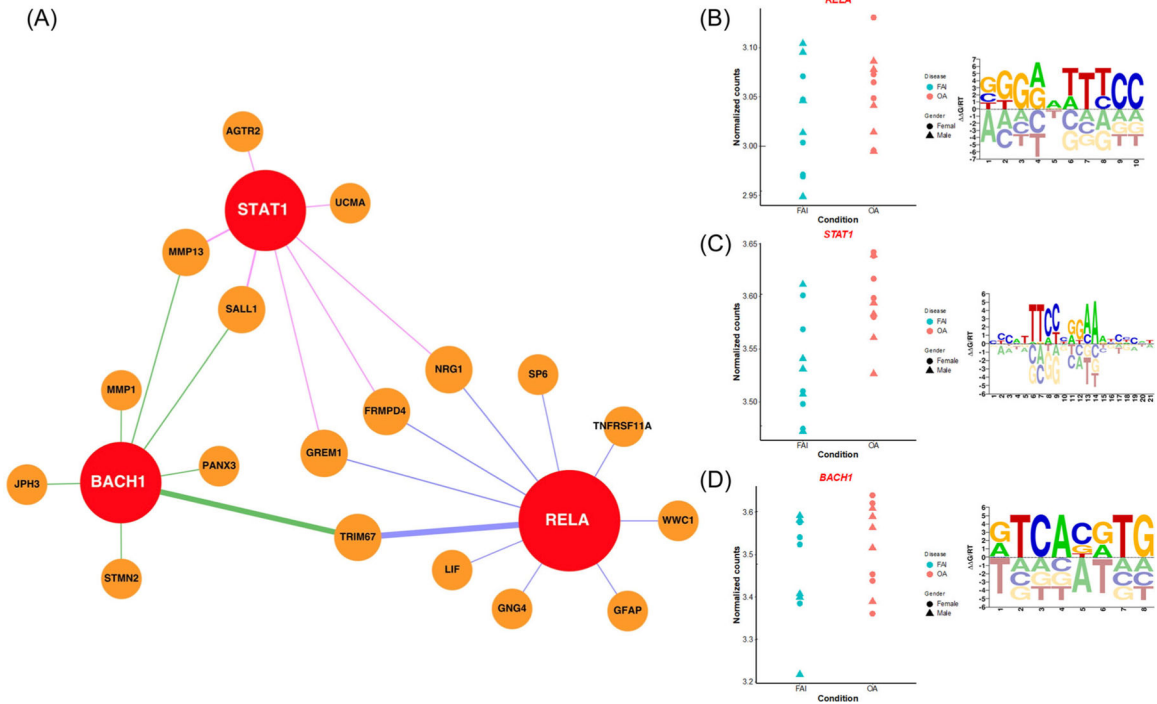
FIGURE 4.

Gene regulatory network for upregulated DEGs in OA articular cartilage. The transcription factors STAT1, BACH1, and RELA were the primary enriched network hubs for the pathway analysis (A). Normalized counts as well as the binding motif for each transcription factor are presented for RELA (B), STAT1 (C), and BACH1 (D). The size of a node represents the degree of a given gene, while the thickness of the edge connecting two nodes is positively associated with edge betweenness. BACH1, BTB domain and CNC homolog 1; DEG, differentially expressed gene; OA, osteoarthritis; STAT1, signal transducer and activator of transcription 1.

FIGURE 5.

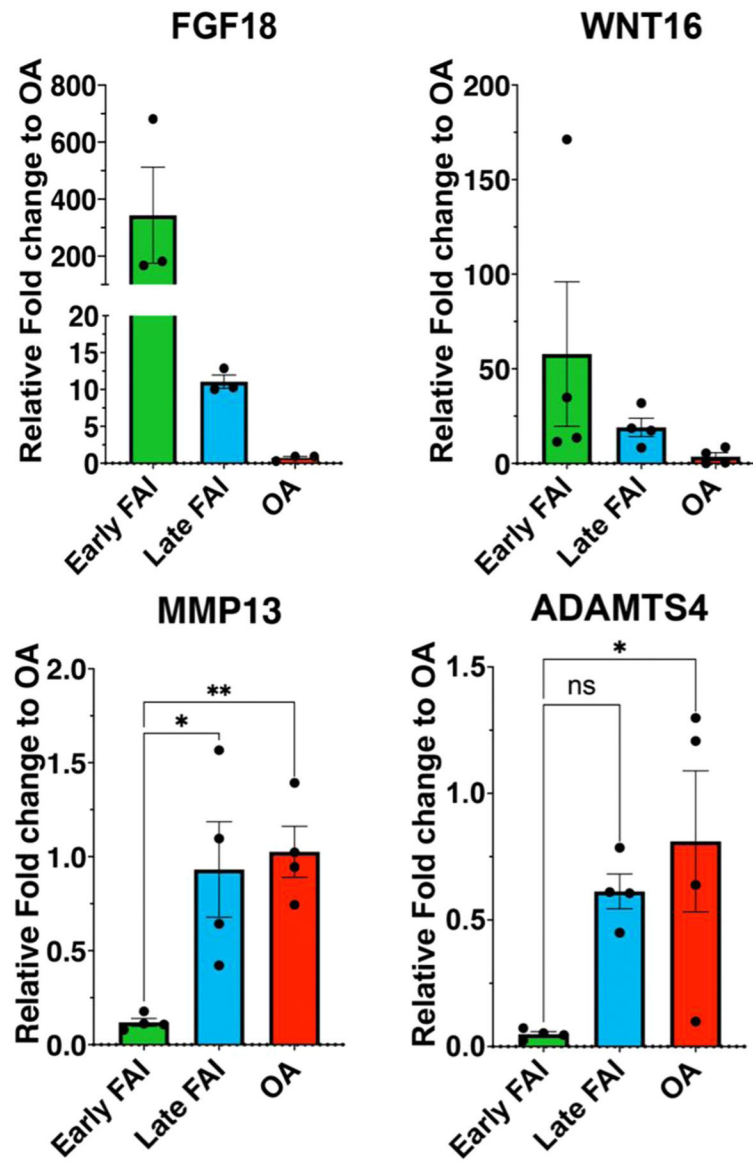qRT-PCR validation for specific markers identified in RNA sequencing. Cartilage expression of *FGF18* and *WNT16* were upregulated in FAI, particularly low-grade FAI, while *MMP13* and *ADAMTS4* were upregulated in osteoarthritic and high-grade FAI cartilage samples. FAI, femoroacetabular impingement; ns, not significant OA, osteoarthritis; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.
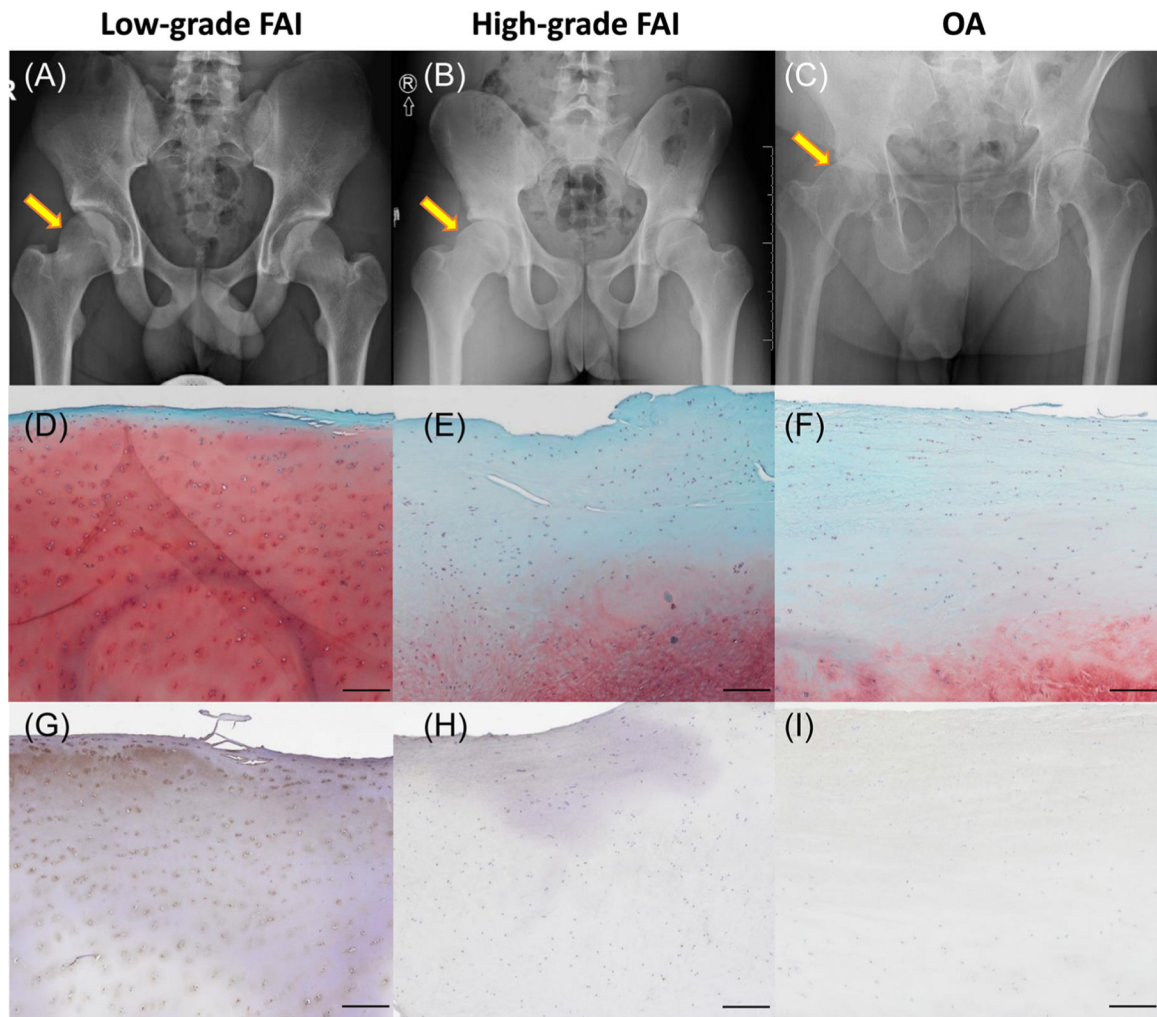
FIGURE 6.

Radiographic (A–C), safranin-O (D–F), and anti-FGF18 immunostaining (G–I) for patients with low-grade FAI (OARSI 1), high-grade FAI (OARSI 4), and OA (OARSI 5). FGF18 IHC staining was increased in cartilage samples from low-grade FAI compared to high-grade and osteoarthritic samples. Histologic samples were taken at ×5 magnification, scale bar = 200 μm. FAI, femoroacetabular impingement; IHC, immunohistochemistry; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

TABLE 1

Differential expression for previously identified markers in FAI and OA[7,10,14–18]

Cartilage	Labrum	Synovium	Subchondral bone	Serum
IL1β	IL1β[a]	IL1β[a]	IL6	COMP
IL8	IL8[a]	IL8[a]	RANKL	CRP
CCL3L1	MMP3[a]	MMP3[a]	OPG	
MMP13	COL1A1[a]		ALP	
ADAMTS4				
ACAN				
COL2A1				
P21				
Bcl2				
FasL				

Abbreviations: FAI, femoroacetabular impingement; OA, osteoarthritis.

[a]Indicates increased expression in OA compared to FAI.

TABLE 2

Study cohort demographics

	FAI	**OA**	**p**
Study cohort			
N	21	16	
Gender (F)	8 (38%)	7 (44%)	0.75
Age	34.2 ± 11.5	62.7 ± 8.3	<0.001
BMI	26.6 ± 4.7	31.5 ± 6.2	0.01
AA	66.7 ± 5.3	65.4 ± 3.6	0.41
LCEA	36.2 ± 6.0	33.7 ± 5.1	0.2
Sequencing cohort			
N	10	10	
Gender (F)	5 (50%)	5 (50%)	1
Age	34.0 ± 13.4	63.1 ± 8.0	<0.001
BMI	25.8 ± 4.8	31.3 ± 6.3	0.04
Alpha angle	66.0 ± 3.5	65.1 ± 3.9	0.61
LCEA	35.9 ± 5.8	33.0 ± 4.1	0.22
Validation cohort			
N	11	6	
Gender (F)	3 (27%)	2 (33%)	0.79
Age	34.4 ± 10.1	62.0 ± 9.6	<0.001
BMI	27.2 ± 4.7	31.8 ± 6.6	0.11
Alpha angle	67.3 ± 6.6	65.9 ± 3.5	0.63
LCEA	36.4 ± 6.5	34.9 ± 6.7	0.66

Abbreviations: AA, alpha angle; BMI, body mass index; F, female; FAI, femoroacetabular impingement; LCEA, lateral center edge angle; OA, osteoarthritis secondary to FAI.

TABLE 3

Radiographic, intraoperative, and immunohistochemical cartilage assessment

	FAI	**OA**
Tönnis grade		
0	12 (58%)	0
1	9 (42%)	0
2	0	4 (25%)
3	0	12 (75%)
OARSI cartilage grade		
1	1 (6.5%)	0 (0%)
2	5 (33%)	1 (10%)
3	2 (13%)	1 (10%)
4	3 (20%)	3 (30%)
5	4 (26.5%)	4 (40%)
6	0 (0%)	1 (10%)
Outerbridge femoral head cartilage grade		
0	9 (43%)	
1	4 (19%)	
2	2 (9.5%)	
3	2 (9.5%)	
4	4 (19%)	
Outerbridge acetabular cartilage grade		
0	0 (0%)	
1	2 (9.5%)	
2	12 (57%)	
3	3 (14.5%)	
4	4(19%)	

Abbreviations: FAI, femoroacetabular impingement; OA, osteoarthritis secondary to FAI; OARSI, Osteoarthritis Research Society International.

TABLE 4

Demographic comparison of low-grade FAI, high-grade-FAI, and OA patients in the validation cohort

	Low-grade FAI	High-grade FAI	OA	p^*	p^{**}	p^{***}
N	4	4	4			
Gender (F)	2 (50%)	1 (25%)	2 (50%)	0.49	1.0	0.49
Age	31.7 ± 3.6	40.2 ± 14.8	65.3 ± 2.9	0.92	0.001	0.001
BMI	25.1 ± 6.5	29.4 ± 3.8	32.2 ± 8.1	0.58	0.39	0.19
Alpha angle	65.6 ± 3.1	66.6 ± 10.3	66.8 ± 3.9	0.26	0.62	0.37
OARSI grade	1.75 ± 1.43	4.75 ± 1.43	5.0 ± 1.71	<0.001	<0.001	0.34

Abbreviations: BMI, body mass index; F, female; FAI, femoroacetabular impingement; OA, osteoarthritis secondary to FAI; OARSI, Osteoarthritis Research Society International.

*
p low-grade FAI compared to high-grade FAI

**
p low-grade FAI compared to OA

***
p high-grade FAI compared to OA.

TABLE 5

Differential expression of select genes in the canonical osteoarthritis pathway

Gene	Function and relation to OA	P_{adj}	FC
FAI upregulated			
NOS1	Proinflammatory increased in OA[31]	1.87E–34	4.898
GDF6	Ligand augmenting BMP signaling[20]	1.08E–07	2.567
BMPR1B	BMP receptor, chondroprotective[32]	1.31E–22	2.036
FGF18	Anabolic growth factor chondroprotective against OA[33,34]	9.67E–09	2.038
HIF3A	Transcription factor inhibiting cartilage catabolism26 [35]	1.20E–08	1.901
WNT16	Wnt antagonist, chondroprotective[36]	1.93E–03	1.528
TAC1	Neurokinin receptor involved with pain signaling in OA[37]	4.87E–03	1.052
C/EBPβ	Transcription factor, increases type X collagen expression, increased in early OA[38,39]	2.32E–04	0.399
SMAD4	Mediates TGFβ signaling[39]	2.19E–03	0.181
OA upregulated			
GREM1	BMP antagonist, chondroprotective[40]	3.32E–12	–3.291
COL10A1	Short-chain collagen diffusely increased in OA[41]	1.64E–04	–2.222
MMP13	Cleaves type 2 collagen, proinflammatory, increased in OA[42]	2.77E–04	–1.935
RANK	Increased in response to IL-1β signaling in OA[43]	7.70E–08	–1.709
ADAMTS4	Enzyme degrading aggrecan in cartilage ECM, proinflammatory, increased in OA[44]	7.35E–03	–1.376
TNF	Proinflammatory cytokine, increased in OA[45]	6.12E–03	–1.129
RUNX2	Transcription factor, increased in OA[46]	4.76E–03	–1.114
TGFB1	Induces osteophyte formation and angiogenesis, increased in OA[47]	1.38E–03	–0.461

Abbreviations: BMP, bone morphogenetic protein; ECM, extracellular matrix; FC, fold change; IL-1β, interleukin-1β; OA, osteoarthritis; TGFβ, transforming growth factor β.