

## Regional Differences in Femoral Condyle Cartilage Biomarkers Related to Histological Degradation Severity in the Osteoarthritic Knee

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**Introduction:** The femoral condyles (FC) are the articulating surfaces in the knee with the highest reported prevalence of symptomatic articular cartilage lesions. The development of osteoarthritis (OA) in the knee is typically characterized by progression of articular cartilage lesions to involve the whole "joint organ". However, there is significant variation in OA-related changes in articular cartilage across the different anatomical surfaces of the knee within and among patients. While inflammation and degradation are mechanistic pathways that are consistently associated with the development and progression of OA, the complex relationships among localized changes in articular cartilage and clinically relevant protein biomarkers have not yet been well characterized. It is possible that there are site-specific relationships that influence knee OA "phenotypes" with respect to mechanistic pathways and severity of disease. Therefore, this study was designed to characterize relationships among OA-related changes in cartilage structure with the concentrations of clinically relevant protein biomarkers in the FC. It was hypothesized that FC OA cartilage with more severe histological degradation would be associated with significantly higher levels of inflammation-related, degradation-related, and bone turnover-related biomarkers.

**Methods: Tissue recovery and culture:** All procedures were performed with IRB approval (IRB#1208392) and informed patient consent. Femoral condyle (FC) articular surfaces were recovered from patients (n=19, 13 F, 6 M, age 65.1±9.2, BMI 34.6±5.8) undergoing TKA for symptomatic knee OA. Osteochondral explants (6mm diam, n=90) were created from the anterior (A), center weight bearing (C), and posterior (P) regions of the medial (M) and lateral (L) FC. The explants were cut in half and half was stored at -80°C for protein extraction, while the other half was formalin fixed for histological assessment. **Tissue Protein Extraction and Testing:** Protein was extracted from the cartilage tissue using the T-Per protein extraction reagent with protease inhibitors. The protein content of the extract was determined using the BCA assay, and the concentration of leptin, adiponectin, adipisin, CRP, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, GRO- $\alpha$ , MCP-1, MCP-3, IL-6, IL-8, MIP-1 $\alpha$ , VEGF, OPG, OPN, and SOST was determined using commercially available Luminex assays. **Histology:** Stained sections of each osteochondral explant were evaluated by one blinded pathologist using a modified OARSI system that assesses cartilage structure (STR), chondrocytes (CHON), proteoglycan content (PRO), collagen integrity (COL), tidemark (TIDE), and subchondral bone plate (SUB). The sum of all scores was used for a total score (TOTAL); the TIDE and SUB were used for a bone score (BONE); the STR, CHON, PRO and COL were used for a cartilage score (CART); and the STR, PRO, and COL were used for an extracellular matrix score (ECM). **Statistical Analysis:** Biomarker concentrations were standardized to protein content and log transformed for analysis. Significant (p<0.05) differences between samples grouped based side (FC-L, FC-M), region of recovery (FC-P, FC-C, FC-A), and the various OARSI system sum score groups using a one-way ANOVA and Tukey post-hoc test or T-test based on number of groups in the comparison. A two-way ANOVA was performed using the histology sum score groups and the side (FC-L, FC-M) or region (FC-P, FC-C, FC-A) of recovery to determine significant differences for each biomarker based on the interaction of the histology and location of recovery. Only significant differences between groups are discussed.

**Results: Differences between Regions of the FC (Fig. 1):** The FC-M samples had higher Adiponectin and lower IL-8 than FC-L samples. The FC-C had significantly higher IL-8 than the FC-A. **Differences based on Histology Sum Score Groups:** The concentration of GRO- $\alpha$  and IL-8 were higher at lower histology scores, and DKK-1 and SOST were higher at higher histology score, for most sum score groups. The concentration of adiponectin was higher at higher TOTAL, CART, and ECM scores, and leptin and OPN were higher at lower CART and PRO/COL scores. The concentration of MMP-9 was higher at higher TOTAL and ECM scores. **Differences based on interaction of FC-M, FC-L, and Histology Sum Score Groups (Fig. 2):** The FC-L had higher TIMP-1 (PRO/COL (6-7)), TIMP-3 (TOTAL (10-20), CART (8-9, 12-15), ECM (5-6, 9-11)), TIMP-4 (TOTAL (14-20)), and MCP-3 (CART (6, 8-9)) than the FC-M, and the FC-M had higher adipisin (CART (11-15), ECM (9-11), PRO/COL (6-7)) than the FC-L at specific histology sum scores. For the FC-M, IL-8 was higher in lower TOTAL (1-8) scores and adipisin was higher at higher CART (10-11) scores. For the FC-L, TIMP-3 was higher in higher TOTAL (14-20), CART (7-15), and ECM (9-11) scores; TIMP-4 was higher in higher TOTAL (14-20) scores; MCP-3 was lower in lower CART (5) scores; and adipisin was higher in mid-range PRO/COL (3-5) scores. **Differences based on interaction of FC-A, FC-C, FC-P, and Histology Sum Score Groups:** Within the FC-C had higher MMP-1 at lower TOTAL (1-8) scores than other scores. Within the FC-P had higher adiponectin at mid-range TOTAL (11-13) scores; lower MMP-2 at higher CART (12-15) and mid-range ECM (4-8) scores; and lower TIMP-2 at lower ECM (1-3) scores than other scores.

**Discussion:** The data from this study indicate important and clinically relevant relationships among regional differences in articular cartilage protein biomarker concentrations and severity of histologic degradation of osteoarthritic femoral condyles. When samples were assessed solely based on region of collection, no significant differences in protein biomarker concentrations between FC-L or FC-M groups were noted. However, when the histologic degradation severity grade of tissue was considered, the data indicated that the FC-L was associated with significantly higher TIMP concentrations than the FC-M for more severe levels of degradation. This difference in TIMP concentrations could be a contributing factor to the higher prevalence and severity of articular cartilage degradation reported for the FC-M compared to the FC-L. The only significant difference noted among FC-A, FC-C and FC-P regions was higher IL-8 in the FC-A compared to the FC-C, and consideration of severity of degradation did not identify consistent differences among the three regions. However, pooling the FC-L and FC-M samples could have confounded this analysis.

**Significance:** Taken together, the results from this study suggest that severity of cartilage degradation related to OA is an important factor in regional differences in femoral condyle cartilage protein content that may relate to knee OA "phenotypes" with respect to pathways and severity of disease. Ongoing studies in our lab are aimed at determining mechanistic relationships between changes in OA cartilage architecture and composition with proteins that may serve as biomarkers for disease development and progression.