high blood glucose levels, suggesting a link between blood glucose and severe OA, as has been suggested previously. BMLs detected with specific MRI sequences may act as potential MRI biomarkers for identification of individuals at high risk of progressive OA and inform development and monitoring of new therapies. Figures A, B, C, D represent significant difference between groups.

479 GENE EXPRESSION ANALYSIS AND DNA METHYLATION IN THE PROMOTER REGION OF MATRIX METALLOPROTEINASE – 13 (MMP-13) AND OSTEOARTHRITIS


Purpose: Osteoarthritis (OA) is a progressive, chronic condition characterized by focal damage to articular cartilage, chronic inflammation and alterations to the extracellular matrix, leading to pain, stiffness and a loss of physical function. OA’s initiation and progression are caused by various environmental and genetic factors. One common factor believed to play a significant role in OA is environmentally-triggered epigenetic alterations, specifically DNA methylation. Of particular interest in the study of OA is the loss and degradation of collagen caused by matrix metalloproteinase – 13 (MMP-13). Several studies have examined the association between OA and methylation in MMP-13. Furthermore, MMP-13 has been suggested as a potential therapeutic target for OA treatment. However, these studies have reported conflicting results. The aim of this study was to determine the gene expression and potential corresponding methylation levels in the promoter region of MMP-13 in patients diagnosed with hip OA versus healthy controls.

Methods: A hospital-based case-control study design was utilized. Cartilage samples were collected from patients undergoing total hip replacement due to primary hip OA and hip fracture patients who required a hemiarthroplasty but did not have evidence of OA. RNA and DNA were extracted from the same cartilage samples. MMP-13 expression was examined from RNA using real-time quantitative PCR. Using DNA, methylation levels of seven CpG sites located in the promoter region of MMP-13 (up to 600 bp from the transcription start) were determined through bisulfite conversion and PCR. The CpG region in the promoter region was amplified using a bisulfite-based PCR technique. The amplified DNA was purified and used for the quantification of DNA methylation.

Results: A total of 38 subjects were included in the analysis, 23 patients diagnosed with hip OA (13 females, 10 males) and 15 healthy controls (12 females, 3 males). The mean age was 63 in OA cases and 77 in controls. Our gene expression analysis indicated that OA patients had a five-fold increase in MMP-13 expression compared to controls. This difference was statistically significant with a p-value of 0.0005, based on student’s T-test. Due to the skewed distribution of the gene expression data, this significance was verified using Mann-Whitney test. Among the 7 CpG sites in the MMP-13 promoter region analyzed, we found one site with significantly altered methylation pattern in OA patients versus controls. The CpG site 218 bp upstream of the first exon showed a trend towards lower methylation in OA patients at an average level of 44% versus controls at 57%, with a p-value of 0.009. This methylation level was significantly correlated with gene expression in OA cases, with a correlation coefficient of 0.55 and a corresponding p-value of 0.0068 based on Spearman’s rank correlation. This correlation was not seen in controls however. Furthermore, we found that methylation was confounded by age with a trend toward increasing methylation correlated with advancing age.

Conclusions: This study demonstrates a significant difference in the gene expression levels of MMP-13, with OA patients having a higher level of MMP-13 expression. In addition, a hypomethylated CpG site in the promoter region of MMP-13 was found in OA patients versus controls, implicating this CpG site as one potential regulatory site for MMP-13 gene expression. The finding was confounded by age, indicating that methylation at this site could be a potential intermediate factor in advancing OA. We hypothesize that increasing methylation with advancing age in MMP-13 may serve as a protective mechanism to prevent collagen degradation. A validation study is needed to confirm the findings.

480 EVALUATION OF TIBIOFEMORAL CARTILAGE CONTACT AREA ALTERATIONS IN ANTERIOR CRUCIATE LIGAMENT INJURED PATIENTS

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Purpose: Anterior cruciate ligament (ACL) injuries cause changes in tibiofemoral joint kinematics and can lead to early onset of osteoarthritis (OA) despite surgical reconstruction. Changes in kinematics may alter the contact area (CA) between the femoral and tibial cartilage surfaces. Evaluations of the tibiofemoral CA in ACL injured individuals can help understand the mechanism behind cartilage degeneration seen after ACL injury and reconstruction. The purpose of this study was to determine the changes in tibiofemoral CA and their positions at the time of injury and after reconstruction.

Methods: Subjects: Bilateral knee MRI scans of thirty healthy adults (15 males, age = 28.93 ± 7.81 years, BMI = 23.44 ± 2.74 kg/m²) with isolated unilateral ACL injuries were obtained within 6 months of their injury (i.e. baseline) prior to surgery and again at 1 year after ACL reconstruction. Fourteen healthy control subjects (10 males, age = 31.78 ± 4.80 years, BMI = 24.01 ± 2.26 kg/m²) with no history of knee injuries were recruited for comparison. Exclusion criteria included prior diagnosis of cartilage degeneration, inflammatory arthritis, or surgery in either knee.

MRI Protocol: Subjects’ knees were scanned with a 3 Tesla MRI scanner (GE Healthcare, Milwaukee, WI, USA) with an 8-channel phased array knee coil (Invivo, Orlando, FL, USA). Sagittal T2 fast spin-echo (FSE) images were acquired (TR/TE = 4000/49.3 ms, slice thickness of 1.5 mm, field of view of 16 cm, 512 x 512 matrix size and echo train length 9 were obtained at extension, loaded axially with 25% total body weight.

Image Processing: Regions of interest were delineated in FSE images with an in-house Matlab program using a spline-based semi-automated segmentation algorithm. The midpoint between the tibial medial and lateral posterior cortical border was defined as the origin of the tibial coordinate system. Tibiofemoral cartilage CA was defined as the weight bearing regions on the tibial surface (Figure 1). Fluids indicated by hyperintensity and menisci were excluded along with areas close to the tibial spine. CA centroid position (CP) was defined as the geometric center of CA projected onto the axial plane. The flexion angle (FA) was defined by the angle between the lines through the midpoint of femur and tibia in the anteroposterior (AP) direction. A single observer processed all MR images and all measurements were taken in the extended position.

Statistical Analysis: Reliability was measured using Intraclass Correlation coefficient (ICC) over three trials of 10 control subjects. Differences in mean CA and CP between groups and longitudinal changes from baseline to 1 year were measured with a Student’s t-test. Significance was defined as P<0.05 for all analyses.

Results: Reliability: The intra-operator ICC’s were 0.922 and 0.942 for medial and lateral CA respectively, and 0.996 and 0.996 for medial and lateral CP.

Contact Area: Medial CA was larger in injured knees compared to control knees at both time points and the difference reached significance at one year (P=0.036). For the medial CA, there were significant differences between contralateral and control knees at baseline and one year (P=0.043 and P=0.008). There was no significant difference between injured and contralateral knees at the two time points (Table 1).

Centered Position: CP along the AP direction was significantly more posterior for the injured knees compared to the contralateral knees at baseline for both medial and lateral regions (P=0.001 and p=0.043). No significant difference was found between injured and control knees or between contralateral and control knees (Table 2).

Conclusions: ACL injured subjects had increased localized medial CA for both injured and contralateral knees compared to the controls. The difference between contralateral and control knees suggests that there may be inherent differences in knee kinematics of ACL injured patients. Lack of significant difference in CA between the two time points indicates that reconstruction did not restore CA and knee kinematics. Overall, the result of the study shows altered knee kinematics in those with ACL injuries. Increased CA may indicate changes in knee kinematics that could lead to ACL tears and OA.

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