agreement. We now plan to compare its performance with other commercially accessible quantitative analysis software, and test its performance in a larger image dataset.

**Evidence of Faulty Matrix Repair Responses in Areas of Damaged Human Osteoarticular Cartilage**

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**Purpose:** Joint degeneration in osteoarthritis is characterised by damage and loss of articular cartilage. The pattern of loss is consistent with damage occurring only where the mechanical loading is high. In previous work using RT-PCR we established that major gene changes were similar in damaged and intact cartilage from the same OA patient and differed from age-matched healthy non-OA cartilage. We have now investigated using RNA sequencing (RNAseq) the gene expression changes that occur in damaged cartilage by comparing each patients sample with intact cartilage from the same joint. We have also recently developed a new analytical tool, PhenomeExpress, which incorporates published disease related ontology to identify processes most active in the damaged tissue.

Matrix cartilage was obtained with prior informed consent from patients undergoing total knee replacement. Samples from 8 patients were taken within 4h of surgery and tissue was removed from an area of damaged cartilage on the inferior medial femoral condyle (DMC) (adjacent to the area eroded down to bone) and a paired sample was taken from the central posterior area of the lateral condyle (PLC), which was invariably intact. Tissue was snap frozen for histology and scored using the OARSI standard score. Adjacent tissue was taken for RNA isolation. RNAseq libraries were generated from 1 μg of total RNA using the TruSeq® Stranded mRNA assay (Illumina, Inc.) according to the manufacturer’s protocol and sequencing was performed using the Illumina HiSeq 2500. The raw RNAseq reads were corrected for bias using standard bioinformatic tools, normalised to enable comparison and then further corrected for transcript length to reflect gene expression abundance.

**Results:** Histological grading confirmed the damaged cartilage (mean ± SD 3.8 ± 0.4) scored more than the intact cartilage (1.6 ± 0.7). The general features of the RNAseq datasets showed that the pattern of gene expression in the paired samples were similar. Superimposed on these similarities were significant changes in about 12% of the most expressed genes comparing paired damaged with intact samples. Analysing the whole data sets using PhenomeExpress identified 20 sub-networks of activity changes in damaged cartilage. The most significant of these included inflammation associated gene response, cartilage development, chondrocyte differentiation, cell proliferation and circadian rhythm, but with no evidence of inflammatory cytokine expression by chondrocytes, or of strong matrix protein expression. The 8 patients all showed very high expression of COL2A1 in damaged and intact sites and matrix protein gene expression was high in general, including strong expression of FN1 and 8 SLRPs; LUM, FMOD, DCN, OGN, BGN, CHI3L1, PREP and ASPN. There was also high expression of COL3A1, COL1A1 and COL1A2 expression. Changes associated with damage showed a decline in chondrocyte phenotype, with significantly less SOX9, COL9A1/2, COL11A2 and HAPLN1 and increases in COL1A1, COL1A2 and COL1A2 expression. Changes associated with damage showed a decline in chondrocyte phenotype, with significantly less SOX9, COL9A1/2, COL11A2 and HAPLN1 and increases in COL1A1, COL1A2, VCAN and also in FN1, LUM, OGN, ASPN, POSTN (Peristin), TNFRSF11B (Osteoprogerin) and TNFAP6 (TSG6). There was low expression of COL10A1, which did not vary with damage and there was no increase in ALPL (alkaline phosphatase) suggesting no progression to hypertrophy. Although there was cell proliferation in damaged cartilage there was only very low expression of the COL2A1 A isoform transcript, which is associated with chondroprogenitors. Comparison of 10 OA risk genes identified in GWAS studies revealed only low expression and no difference between damaged and intact cartilage. As these genes are each associated with only a very low risk of OA they would not be predicted to be linked to cartilage damage, or indeed to necessarily be expressed in chondrocytes.

**Conclusions:** The analysis reveals a pattern of gene expression with no evidence of very active matrix degradation, but strong sustained matrix protein and proteoglycan gene expression and particularly expression of collagens. The changes in damaged cartilage suggest a further decline in signals maintaining the chondrocyte phenotype. That this occurs at sites of cartilage damage and eventual loss shows that this response is unable to halt or reverse the damage and it may be the inappropriate matrix gene expression that causes a failed repair. Further detailed