INTRODUCTION: Successfully managing rotator cuff (RC) tears remains a challenge due to high failure rates following surgical repairs. There is increasing evidence for a multi-stage model of RC tendon tears, wherein healing is affected by tear size. Larger tears are associated with poorer scores and function, and are more likely to re-rupture after surgical repairs compared to smaller tears [1]. The aetiology of RC tears is not a purely mechanical phenomenon, but also involves underlying biochemical changes which are often classified as part of intrinsic degeneration. Recent evidence suggests that the cellular, vascular and extracellular matrix composition, as well as the metabolism and viability, of the torn tendon edge changes significantly as the tear size increases [2]. The underlying pathophysiology however is not fully understood. Changes in the production and remodelling of the RC extracellular matrix (ECM) are likely to be important determinants of RC tendinopathy as they affect structural integrity and the ability to transmit loads.

Microarray analysis allows identification of changes in mRNA transcription for thousands of genes. This technology has previously demonstrated upregulation of cytokines and apoptosis markers in torn human and rat tendons [3]. Understanding alterations in gene expression between tear groups may offer greater insight into tear pathogenesis, as well as differences in healing and failure rates. By identifying modifiable targets related to failure, this important knowledge may help to guide treatment strategies and future research.

It was hypothesized that massive human RC tendon tears will have different gene expression profiles when compared to normal RC tendons. We also hypothesized that massive tendon tears can be differentiated from smaller tendon tears using microarray analysis. We hypothesized that many of the altered genes will be related to the ECM composition, as it plays a fundamental role in tendon structure and transmission of loads. This study aimed to perform the first genome wide microarray analysis of differences in human gene expression profiles between normal, small and massive RC tendon tears.

METHODS: The gene expression profiles of 28 human RC tendons were analyzed using microarrays representing the entire genome. 11 massive (>5cm) and 5 small (<1cm) chronically torn RC tendon specimens were obtained intraoperatively from tear edges, and compared to 12 age and gender matched normal controls. Approval for the study was obtained from our institutional review board.

RNA was extracted from the small biopsy specimens. To ensure high quality RNA, only specimens with an RNA integrity number of >7 were used. Gene expression analysis was conducted using Agilent single color arrays. 28 microarrays were run with >44,000 probes which hybridized with fluorescent labeled cDNA, and were detected by scanning. Microarray data analysis was performed using GeneSpring GX and TIGR MeV software. Genes were considered to be significant if they had a more than 2 fold change and p < 0.05 (Mann Whitney test). To assess changes in specific pathways and functional groups, the microarray data was analysed using Gene Ontology terms and with Gene Set Enrichment Analysis.

Validated altered genes and pathways suggested by the microarray, real-time quantitative polymerase chain reaction (rtq-PCR) was used to confirm expression for selected genes: matrix metalloproteinase (MMP)-3, interleukin (IL)-8, a disintegrin and metalloproteinase (ADAM)-15, forkhead box (FOX)-F2 and aggrecan (ACAN). GAPDH was used as a housekeeper to assess the accuracy of the PCR technique. Altered protein expression was confirmed using immunohistochemistry, specifically for MMP-3, BMP-5 and SAA.

RESULTS: Numerous insightful gene changes and pathway changes were detected. 165 genes had altered expression between the normal and small tears group, 181 between small and massive tears, and the greatest number of 258 genes changed in massive tears compared to normals. We focused upon genes related to ECM components. Expression of genes encoding collagen 4,12 and 14 was downregulated in small tears rather than massive tears whereas the collagen 9 gene COL9A3 was downregulated in both small and massive tears. With regards to ECM remodeling, MMP-3,-10,-12,-13,-15, -21,-25 and ADAMs-12,-15,-22 were significantly upregulated in tears. Other key changes included upregulation of aggrecan in massive tendon tears compared to normal controls, but not in small tears (p < 0.05, > 2-fold change). Amyloid A1 was downregulated in the small and massive tear groups when compared to normals, whereas downregulation of IL-1,-8,-11,-27, was seen. Previously reported gene expression changes in heat shock proteins, IL-1, MMP-3 and cyclooxygenase (COX-1) were also detected by our microarray analysis [4]. Common tendon markers such as scleraxis, decorin and tenasin-C were not altered between normal and torn rotator cuff groups. However tenomodulin showed a >2-fold increase between normal and small tears, as well as between small and massive tears.

Gene changes suggested by the microarray were also detected using qRT-PCR. Immunohistochemistry confirmed increased BMP-5 expression in small tears, and increased MMP-3 expression in both small and massive tears compared to normal tendons.

DISCUSSION: This study is the first genome wide analysis of rotator cuff tears to our knowledge. The gene expression profiles of normal, small and massive RC tear groups suggested they are biologically distinct groups. We have identified a significant role for ECM related genes such as MMPs and ADAMs in RC tear pathogenesis. This suggests that upregulation of the reported ECM genes may play key roles in the development of tears, as they may reflect a disruption in the balance between ECM synthesis and degradation. Aggrecan was specifically upregulated in massive tears, suggesting a potentially important role for chondroplasia in the pathogenesis of massive tears. BMP-5 upregulation may contribute to the development or healing response to small tears.

Our study confirmed altered gene expression in pathways reported in previous studies, and has identified a number of novel pathways which are affected between the different tendon groups studied. The highlighted changes in MMPs in either pathogenesis or adaptation to tears, encourage further studies about the suggested role of MMP inhibitors in the treatment of RC tendon tears as MMPs are critical for healing and cell migration [5]. Further investigation is required to determine whether some of these genes may potentially have a role as biomarkers of failure. Modulating these ECM pathways may be a useful treatment strategy for improving clinical outcomes.

REFERENCES: