

non-normally distributed biomarker outcomes (MMP3) and days between injury and ACLR.

Results: Days between ACL injury and surgery ranged from 9 to 67, with a mean 31.0 ± 14.4 days. Greater days between ACL injury and ACLR was associated with greater C2C:CPII ratios at the six-month follow-up exam after ACLR (0.15 ± 0.02 , $r = 0.46$, $P = 0.03$). All other associations between biochemical markers and days between injury and ACLR were weak (r ranged between -0.006 and -0.075 , all $p > 0.05$).

Conclusions: Individuals who waited a greater number of days between ACL injury and ACLR demonstrated higher C2C:CPII at the six-month follow-up exam. Greater C2C:CPII may be influenced by aberrant knee joint loading on an ACL deficient knee in the time in which patients await ACLR. Conversely, it is possible that individuals with worse injuries may wait longer to undergo ACLR, and they may have greater C2C:CPII due to the initial severity of joint tissue injury. It is also important to note that the 6-month assessment occurs at a later time post-injury for those with longer time between injury and ACLR. Caution should be exercised in interpreting the results of this small preliminary study. More research is needed to understand mechanisms that may influence the association between days between ACL injury and ACLR and increased C2C:CPII at 6 months after ACLR. It will be important to determine if there is an optimal time frame to perform ACLR that minimizes the risk of, or rate of progression to, future PTOA.

125 ANALYSIS OF CLUSTERIN EXPRESSION CHANGES AS A BIOMARKER OF OSTEOARTHRITIS

C.R. Fellows, C. Matta, H. Quasnichka, A. Mobasher. *Univ. of Surrey, Guildford, United Kingdom*

Purpose: The discovery and validation of arthritis-related biomarkers and establishment of methodology for proteomic studies in osteoarthritis (OA) are needed. Proteomics strategies have identified many proteins that may relate to pathological mechanisms of OA, however targeted approaches are required to validate the roles of these proteins. This study aimed to use mass spectrometry and western blotting to identify peptides from several proteins in the secretome of chondrocytes, cartilage explants and osteochondral biopsies treated with inflammatory cytokines over a 2-week period, to evaluate their potential as biomarkers of OA progression.

Methods: Healthy cartilage was obtained from fetlock joints of skeletally mature horses, euthanized for unrelated veterinary reasons. Cartilage explants were isolated using a 6 mm biopsy, with discs placed into wells (3 discs per 1 ml DMEM + 1% Pen/Strep) before incubation for 24 hours (37°C , 5% CO_2). After this equilibration period, the media was removed and replaced with either fresh DMEM + 1% Pen/Strep or DMEM supplemented with 1% Pen/Strep containing TNF α and IL-1 β both at 10ng/ml. Explants were cultured for 7–14 days with the cytokines replaced every 4th day. For cell based assays chondrocytes were isolated from tissue using 70U pronase for 1hr at 37°C and overnight digestion at 37°C using a 0.2% collagenase II solution. The cell suspension was filtered and washed before being seeded into culture flasks and cultured until confluence was reached (37°C , 5% CO_2). Once cultures were established cells were split into two groups: healthy control (DMEM supplemented with 1% Pen/Strep and 10% foetal calf serum) or stimulated cells (DMEM as above plus TNF α and IL-1 β both at 10ng/ml). Chondrocytes were cytokine-stimulated for up to one week. Cells were used in experiments up to the 2nd passage.

Results: Mass spectrometry data showed that peptides representative of clusterin were found to decrease following 7 days of inflammatory stimulation. Western blotting of secreted proteins in media of cartilage explants or chondrocyte showed that clusterin expression was reduced following 7 days of cytokine treatment. Catabolic matrix metalloproteinase enzymes MMP1, MMP3 and MMP13, as well the matrix component cartilage oligomeric protein (COMP) were all found to have an increased abundance in the media of the cytokine treated samples. This data was supported by qPCR for clusterin gene expression which showed initially mRNA levels increased 3 day after inflammatory stimulation but expression was lost after 7 days. Western blotting of media from the osteochondral biopsies showed an increase in clusterin expression after 7 days of inflammatory stimulation however clusterin protein expression could not be detected after 14 days of treatment, indicating a delayed response compared to cartilage tissue alone.

Conclusions: The equine chondrocytes, cartilage explant and osteochondral biopsy models exhibited highest clusterin secretion in

untreated cultures. IL-1 β and TNF α treatment caused a reduction in clusterin secretion. Clusterin acts as a chaperone to aid protein refolding in situations of stress and is constitutively secreted by mammalian cells. IL-1 β and TNF α appear to interrupt clusterin secretion and therefore the protection it may offer healthy functioning cells. Previous studies have reported variable data, with some studies indicating a decrease in clusterin in OA, while others indicate an increase in clusterin expression. Our results suggest the clusterin increases immediately after inflammatory stimulation but is lost after prolonged exposure. Therefore, levels of secreted clusterin may be a candidate biomarker for OA progression.

126 URINE METABOLOMICS USING LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY INDICATES COMMON MARKERS OF DISEASE IN ALKAPTONURIA AND IDIOPATHIC OSTEOARTHRITIS IN HUMAN

B. Norman †, A. Davison †, P. Wilson †, G. Ross §, A. Milan †, N. Roberts †, L. Ranganath †, J. Gallagher †. † *Inst. of Ageing & Chronic Disease, Univ. of Liverpool, Liverpool, United Kingdom*; ‡ *Liverpool Clinical Lab., Royal Liverpool & Broadgreen Univ. Hosp. Trust, Liverpool, United Kingdom*; § *Agilent Technologies UK Ltd, Cheadle, United Kingdom*

Purpose: Osteoarthritis (OA) is associated with destruction of cartilage and is widespread in Alkaptonuria (AKU), a serious genioarthritic disease caused by a deficient enzyme, homogentisate 1,2-dioxygenase (HGD) and with morbidity caused by increased levels of homogentisic acid (2,5-dihydroxyphenylacetic acid; HGA) and resultant pigmentation, particularly of cartilagenous tissue. AKU is a severe, ultra-rare osteoarthropathy but there is considerable interest in using it as a model system to study pathological changes in common idiopathic OA (iOA). In support of this, some of the striking pathological features characteristic of AKU-associated cartilage destruction are observed at lower frequency in iOA. These include thinning of the subchondral plate, loss of collagen fibril integrity, high-density mineralised protrusions and aberrant bone remodelling. It was predicted that shared underlying biochemical processes underlie these common features of disease, and that these are detectable in biological matrices such as urine and serum. A non-targeted metabolomic profiling experiment using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) investigated this hypothesis using urine from human AKU and iOA patients. Spectra were compared with those from healthy controls.

Methods: Human urine samples were from AKU patients ($n = 15$), iOA patients ($n = 7$) and age-matched healthy volunteers ($n = 30$). All samples were analysed using reverse phase Ultra-High Performance Liquid Chromatography (UHPLC) on an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6550 Quadrupole Time-of-Flight mass spectrometer in positive and negative polarity Electrospray Ionisation (ESI). The data were processed using a feature finding algorithm (*Molecular Feature Extractor*). This interrogates the 3-D data space extracting chromatographic peaks representing compounds and the associated MS spectra including isotopes, adducts and multimers. The extraction and data review were performed using *Mass Hunter Profinder* software for alignment and elimination of false positives/negatives. The resulting ‘found’ entities were compared across the three sample sets using *Mass Profiler Professional*.

Results: Various quality control checks indicated that the LC-MS operating parameters and feature finding across samples were reproducible. A total of 109 and 275 and metabolic entities passed quality control filtering and were aligned across all sample groups in positive and negative ESI respectively. Interrogation of the variance across all datasets using Principal Components Analysis (PCA) showed clear separation between the urine metabolic profiles of AKU, iOA and controls. However, a number of entities were identified as up or down regulated in both AKU and iOA compared with controls indicating that there are common mechanisms of disease in these two conditions. In positive ESI, 17 and 4 entities were up- and down-regulated respectively in AKU and iOA compared with controls. In negative ESI, 38 and 9 entities were up- and down-regulated respectively in AKU and iOA compared with controls. These metabolic entities span a relatively broad range in mass (100–800 Daltons) and retention time, suggesting diverse chemical identities. Putative identities for these entities can be derived based on accurate mass and retention time using web-based databases (e.g. METLIN), supporting further investigation using a more-targeted metabolomics approach.

Conclusions: Analysis of urine samples through metabolomic profiling indicated presence of a number of entities that were up or down-