increased in severity and 13% decreased in severity over 10 years (Fig-1). Offspring showed a significantly higher increase in the mean score compared to the controls at the medial tibial and femoral sites in participants with a cartilage defect at baseline (Fig-2A). Offspring also showed a significantly higher number of incident cartilage defects at the medial femoral site only in the participants without a defect at baseline (Fig-2B). Presence of tibiofemoral cartilage defects was independently associated with higher odds of presence of effusion (Odd ratio (95%CI) = 2.19 (1.07, 4.47)) at baseline. Presence and severity of JSN, osteophytes and BMLspredicted change in total knee cartilage defects in unadjusted analysis, however these associations only persisted for presence (β = 0.83 (+0.32, +1.60)) and severity (β (per grade) = +0.86 (+0.02, +1.70)) of JSN and severity of BMLs (β (per unit area) = +0.64 (+0.10, +0.20)). Change in total knee cartilage defects was associated with change in BMI, pain and BMLs in the unadjusted analysis but these associations persisted only for BMI (β = -0.13 (-0.03, -0.25)) in the fully adjusted model. Change in cartilage defects also independently correlated with change in JSN (p = 0.16, p = 0.04) but not osteophytes. Furthermore, change in total knee cartilage defects was independently associated with absolute (β = -123.87 (-193.24, -54.50)) and percentage per annum (β = -0.08 (-0.11, -0.04)) cartilage volume loss (using mixed model analysis).

Conclusions: Data from this midlife cohort suggests that cartilage defects are on the OA causal pathway both for symptoms and structure. Unlike meniscal pathology, cartilage defects have the capacity to heal over time thus may be amenable to intervention.

53 ADDITION OF CLINICAL MEASURES IMPROVES RISK-PREDICTION MODELS OF 30 DAY POST-TJR READMISSION

P. Franklin, T. Fehring, S. Odum, C. Lewis, D. Ayers, University of Massachusetts Med. Sch., Worcester, MA, USA; OrthoCarolina Research Inst., Charlotte, NC, USA; OrthoCarolina, Charlotte, NC, USA; OrthoCarolina Research Inst., Charlotte, NC, USA; Hartford Hosp., Hartford, CT, USA

Purpose: Total joint replacement surgery (TJR) is the most common and costly procedure in the Medicare budget and TJR use is rapidly expanding among adults under 65 years of age making TJR outcomes an important public health issue. In September of 2012, the Center for Medicare and Medicaid Services (CMS) issued preliminary hospital-specific reports of ‘risk-adjusted’ 30 day readmission rates after TJR and in 2013, CMS publicly reported hospital-specific TJR readmission rates. The release of publicly reported TJR outcomes illustrates the importance of accurate comparisons of post-TJR outcomes across hospitals and surgeons. However, the CMS risk-adjustment models only apply to patients over 65 years of age, are based solely on administrative billing data and ICD-9 co-morbidity codes, and lack clinically refined measures. We investigated whether the addition of key pre-operative clinical risk factors would improve the 30 day post-TJR readmission risk-adjustment model.

Methods: We merged CMS patient-level data for those over 65 years of age enrolled in the first 18 months (June 2011- November 2012) in a national cohort study (FORCE-TJR) of primary TKR and THR patients from more than 130 surgeons in 22 states. CMS data include date and ICD-9 codes for co-existing medical conditions up to 12 months pre-TJR and readmissions within 30 days after TJR to an acute care hospital. Additional clinical data from the FORCE-TJR cohort include BMI, medical and musculoskeletal comorbidities, and pre-op patient-reported pain and function (HOOS/KOOS; SF36).

Results: Five percent of 2560 patients across 31 sites were readmitted. Readmission rates were greater among men and patients with the following administrative risk factors: older age, history of infection, COPD, diabetes, disorders of fluid/electrolytes, rheumatoid arthritis, and renal, hematologic, and vascular conditions (all p<0.01). In addition, readmitted patients had lower pre-TJR function scores, a greater number of knee/hip joints with moderate-to-severe pain, and greater Charlson index (all p<0.01). In logistic multivariable models, the CMS model with the addition of clinical measures (number of painful joints and Charlson) outperformed the CMS core model based only on ICD codes [C=0.65 (ICD/CMS) vs 0.76 (ICD + Clinical model)].

Conclusions: The addition of patient-reported function and the clinical severity of musculoskeletal and medical conditions during the time of surgery improved the 30 day readmission prediction model for TJR. Public reporting based on risk models that do not address the complete comorbid profile, including musculoskeletal conditions, may disadvantage hospitals and surgeons treating patients with the most advanced disease. Further research should validate the enhanced risk models in both older (Medicare) and younger TJR patients.

54 TWIST1 PROMOTES CATABOLIC RESPONSES AND ABNORMAL DIFFERENTIATION IN HUMAN CHONDROCYTES

J. Hasei, T. Teramura, M. Olmer, T. Ozaki, H. Asahara, M.K. Lotz,1 The Scripps Res. Inst., La Jolla, CA, USA; 1Okayama Univ., Okayama, Japan; 2NIRCHD, Tokyo Med. and Dental Univ., Tokyo, Japan

Purpose: Dysregulated chondrocyte activation and differentiation in articular cartilage is an important mechanism in Osteoarthritis (OA) pathogenesis. Twist-related protein 1 (TWIST1) is known as class A basic helix-loop-helix protein 38 (BHLHa38), and involved in the negative regulation of cellular determination and in the differentiation of several lineages including myogenesis and osteogenesis. TWIST1 is also known as a factor contributing to dedifferentiation and chondrogenesis. We also found TWIST1 was upregulated in human OA knee cartilage tissue compared with normal knee through next-generation sequencing. In this study we revealed correlative relationship between TWIST1 and chondrogenesis-related genes (collagen type II alpha (COL2A1), SOX9).

Methods: Cartilage tissues and chondrocytes were obtained at autopsy from normal knee joints (n=7) and from OA joints (n=8) at knee arthroplasty. The expression level of TWIST1 in cartilage tissues were determined by qRT-PCR. TWIST1 protein expression in cartilage tissue was analyzed by immunohistochemistry in human and mouse OA model. Human normal cartilage derived chondrocytes, TC28 (immortalized human chondrocytes) and SW1335 (human chondrosarcoma) cells were used to study regulation of chondrogenesis-related genes by TWIST1. TWIST1 was knocked down with small interfering RNA (siRNA), and over expressed by pCMV6-Entry C-terminal Myc tagged TWIST1 ORF vector. To further investigate the linkage between TWIST1, COL2A1 and SOX9, we constructed luciferase reporters containing SOX9 3' UTR, COL2A1 promoter and COL2A1 enhancer segments.

Results: TWIST1 expression was significantly increased in human OA knee cartilages compared to normal knee cartilages (5-6-fold up-regulation). Immunohistochemistry analysis showed only few Twist positive cells in normal human and mouse cartilage and increased numbers of Twist1 positive cells in OA-affected tissues. Following TWIST1 siRNA treatment of chondrocytes, qRT-PCR results showed SOX9, COL2A1 and aggrecan gene expressions were up-regulated, and cartilage-degrading enzymes (MMP-13, ADAMTS4) were down-regulated. In contrast, over expression of TWIST1 suppressed COL2A1 gene expression, but not SOX9. Luciferase activities of SOX9 3' UTR and COL2A1 promoter-luciferase reporter were reduced after TWIST1 transfection. TWIST1 also reduced COL2A1 enhancer luciferase reporter activity, but there is no TWIST1 binding site in COL2A1 enhancer. This data shows that TWIST1 might affect COL2A1 enhancer indirectly through SOX9 down-regulation.

Conclusions: Overexpression of TWIST1 is a novel feature of OA affected cartilage, and promotes catabolic reaction and abnormal chondrocyte differentiation by interacting with COL2A1 promoter and SOX9 3' UTR. Thus, dysregulated TWIST1 may be an important factor in OA pathogenesis and a novel treatment target for OA.

55 ROLE OF BMP2 IN THE MATURATION AND MAINTENANCE OF THE KNEE JOINT

L. Gamer, K. Cox, Q. Lin, L. Han, V. Rosen, Harvard Sch. of Dental Med., Boston, MA, USA; Drexel Univ., Philadelphia, PA, USA

Purpose: Osteoarthritis (OA) is the most common degenerative joint disease and knee OA often requires joint replacement to reduce pain and restore joint mobility. The role of bone morphogenetic proteins (BMP) in the pathogenesis of OA remains controversial despite many years of study. BMP signaling is required for synovial joint formation during development and is also involved in maintaining the normal function of synovial joints after birth. Paradoxically, too much BMP activity is also associated with synovial thickening and osteophyte formation, hallmarks of OA progression. Mice lacking BMP2 in all limb bud skeletal progenitors (BMP2-Prx1) develop OA of the elbows and
knees but have significant underlying skeletal defects including thinner bones that spontaneously fracture. The purpose of this study was to define the role of BMP2 in the formation and maintenance of the knee joint. In order to do this we removed BMP2 specifically from synovial joint cells using the Gdf5-cre transgene. At embryonic day 12.5 (E12.5) we performed histology, immunohistochemistry (IHC), qRT-PCR, Xrdia micro-CT, and Atomic Force Microscopy based (AFM) nanoindentation to assess both molecular and biomechanical changes in articular cartilage and meniscus during joint formation, maturation and maintenance using hindlimbs isolated from E17, 2 week, 8-10 week and 5 month old mice.

Results: Loss of BMP2 from synovial joint forming cells (BMP2-Gdf5 cKO) does not affect knee joint morphogenesis. At E17, the developing meniscus, ligaments and articular surfaces of the tibia and femur all appeared normal. At 2 weeks after birth, when joint structures are maturing, the meniscus of the BMP2-Gdf5 cKO knees appeared developmentally delayed when compared to controls. Sa-Fo staining showed reduced proteoglycan expression as well as decreased rounded chondrocyte like cells in BMP2-Gdf5 cKO meniscus. IHC analysis revealed reduced collagen type 2 (Col2) and altered aggrecan distribution in BMP2-Gdf5 cKO meniscus while picrosirius red staining showed a disorganized pattern of collagen fibers. This data was confirmed using qPCR analysis that showed a decreased in both Col2 and aggrecan expression in 2 wk old menisci from BMP2-Gdf5 cKO mice compared to controls and revealed that BMP target genes Lox (lysyl oxidase) and Runx2 (runx family) are not regulated. AFM nanoindentation testing of meniscal joints of 2 wk old BMP2-Gdf5 and control mice revealed no significant differences in the nanomechanical properties of the articular cartilages. However, menisci from BMP2-Gdf5 cKO mice appeared to have weakened mechanical function with significantly lower indentation stiffness when compared to controls. By 8-10 weeks of age, menisci from BMP2-Gdf5 cKO knees appeared to be less ossified and continued to have decreased expression of ECM components. In addition, knee joints of the BMP2-Gdf5 cKO mice started to show signs of early OA as the articular cartilage began to fibrillate. By 5 months of age, Xrdia micro-CT analysis showed loss of BMP2 results in joint space narrowing, flattened tibial epiphyses, menisci that are smaller and significantly less well mineralized, but no evidence of osteophyte formation. IHC analysis of 5 month old BMP2-Gdf5 cKO knees revealed distinct signs of progressive OA pathology including decreased expression of Col2, aggrecan and pSmad2 and increased expression of collagen type X (ColX) and ADAMTS5 when compared to controls.

Conclusions: BMP signaling provided by BMP2 is not required for knee joint formation during development but is necessary for the maintenance of knee joint function after birth. Our findings reveal an important role for BMP2 in the proper assembly and maturation of the meniscus ECM that appears to be essential for joint homeostasis as mice lacking BMP2 in Gdf5-cre + cells develop spontaneous OA as they age. Our data point to an important role for BMP2 production by cells in the knee joint and suggest that interventions that allow for the maintenance of adequate local BMP2 expression by these structures may be of benefit in the prevention of age related knee OA.

56 E11 PROTEIN STABILIZATION BY PROTEASOME INHIBITION PROMOTES OSTEOCYTE DIFFERENTIATION AND MAY PROTECT AGAINST OSTEOARTHRITIS BONE PATHOLOGY

K.A. Staines , M. prideaux, P. Hohenstein, D.J. Buttle , A.A. Pittsillides, C. Farquharson, Roslin Inst., Univ. of Edinburgh, Edinburgh, Midlothian, United Kingdom; Univ. of Adelaide, Adelaide, Australia; Univ. of Sheffield, Sheffield, United Kingdom; Royal Vet. Coll., London, United Kingdom

Purpose: The mechanisms which govern osteoblast-to-osteocyte transitions (osteocytogenesis) are yet to be established, however their dysregulation is likely to contribute to osteoarthritis (OA) subchondral bone sclerosis. The transmembrane glycoprotein E11 is critical in early osteocyte commitment thus we sought to determine the mechanism regulating its expression during osteocytogenesis and to examine whether this was preserved in OA.

Methods: We have used immunohistochemistry, RT-qPCR and western blotting to examine the temporal and spatial localisation of E11 during osteocytogenesis. To examine the functional role of E11 we transfected the late osteoblast MLO-A5 cell line with E11 over-expressing and empty vector pLVX plasmids using Fugene HD. Using these cells we have investigated the post-translational regulation of E11, through addition of calpain and proteasome inhibitors (Z-Fa-FMK, E64d, calpeptin, ALLN and MG132, lactacystin, Bortezomib, Withaferin-A, respectively) and subsequent western blotting and RT-qPCR analysis. We have generated mice harbouring a conditional deletion of E11 in late osteoblasts (osteocalcin promoter driven) (OB-E11-/-) and analysed its bone phenotype through histology, RT-qPCR and micro-CT scanning. We have investigated the post-translational regulation of E11 in the natural OA model, the STR/Ort mouse, with regards to E11 expression.

Results: We revealed increased expression of E11 protein/mRNA (P<0.001) concomitant with extensive osteocyte dendrite formation and matrix mineralization (P<0.001) in MLO-A5 cell cultures. Whilst MLO-A5 cells transfected with E11 over-expressing pLVX plasmids exhibited significantly increased mRNA expression (P<0.001), western-blotting failed to detect any correlative increases in protein expression, suggestive of post-translational regulation. We therefore treated MLO-A5 cells with calpeptin and ALLN and found that both promoted E11 protein expression, with ALLN having the greatest effect. Treatment of MLO-A5 cells and osteocytic IDG-SW3 cells with ALLN also induced a profound increase in stellate cell morphology (50%, P<0.001) and increased E11 protein expression, whilst calpeptin treatment failed to promote similar osteocytogenic changes. Alternative calpain inhibitors E64d and Z-Fa-FMK also failed to modify MLO-A5 cell morphology or E11 protein expression. Unchanging calpain 1/2 levels upon osteocytic differentiation during 15-day MLO-A5 time course suggests lack of calpain contribution to osteocytogenesis. Due to the dual roles for ALLN in calpain and proteasome inhibition, this characterized proteasomal degradation as the key pathway in E11 post-translational targeting and degradation. This was supported by studies using the proteasome inhibitors MG132, lactacystin, Bortezomib and Withaferin-A which produced similar dose-dependent increases in E11 expression in MLO-A5 cells. These data implicate proteasome degradation in controlling E11 stability. Preliminary microCT analyses of OB-E11-/- mice revealed decreased trabecular bone volume/tissue volume (27%) associated with decreased trabecular number (16%) and thickness (7%) in comparison to control mice. This pilot data has also revealed that the conditional deletion of E11 in osteoblasts results in decreased cortical cross-sectional thickness (12%). Further analyses will enable a more thorough skeletal phenotyping of these mice which will include osteocyte number and dendrite formation. Examination of a natural model of OA, the STR/Ort mouse, revealed decreased E11 protein expression in the subchondral bone osteocytes in regions of the joint where OA pathology is observed.

Conclusions: Together these data suggest that proteasome-mediated E11 protein degradation limits acquisition of the osteocyte phenotype and that its deregulation may contribute to bone changes observed in OA.

57 PATHOGENESIS OF CAM MORPHOLOGY IN ENGLISH PREMIERSHIP FOOTBALLERS


Purpose: Femoroacetabular impingement (FAI) is a cause of pain and osteoarthritis. The pathogenesis of this condition remains poorly understood and this limits the ability to develop treatment strategies. Cam morphology is thought to develop during adolescence, often in association with intense sporting activity. Postulated mechanisms include a subclinical slipped upper femoral epiphysis (SUFE) or extension of the epiphysis along the anterosuperior femoral neck. Cam morphology has an extremely high prevalence amongst professional footballers making them an ideal cohort to study disease pathogenesis.

Methods: Players at an English Premiership Football (Soccer) Club Academy were invited to participate using a randomisation algorithm within each age group. The cross-sectional cohort was loaded towards the youngest age groups to enhance a future longitudinal study. 20 players were selected from the U10 and U11 teams, and 10 players from the U12, U13, U14, U15, U16, and U18 teams (n=100).