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.
**Methods:** We generated BMP2 loss of function conditional mutant mice using BMP2 floxed alleles. The Gdf5-cre transgene was used to specifically delete BMP2 within synovial joints (BMP2-Gdf5 cKO) starting at embryonic day 12.5 (E12.5). We performed histology, immunohistochemistry (IHC), qRT-PCR, Xradia 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. Saf-O 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 decrease 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 (lysl oxidase) and Runx2 were also down regulated. AFM nanoindentation testing of knee 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 KO 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, Xradia 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 + 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.

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## E11 PROTEIN STABILISATION BY PROTEASOME INHIBITION PROMOTES OSTEOCYTE DIFFERENTIATION AND MAY PROTECT AGAINST OSTEOARTHRITIS BONE PATHOLOGY

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**Purpose:** The mechanisms which govern osteoblast-to-osteocyte transitions (osteocytogenesis) are yet to be established, however their dysregulation is likely to contribute to osteoarthritic (OA) subchondral bone sclerosis. The transmembrane glycoprotein E11 is critical in early osteocyte commitment thus here we sought to determine the mechanism regulating its expression during osteocytogenesis and to examine whether this was compromised 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 also examined the natural OA model, the STR/Ort mouse, with regards to E11 expression.
**Results:** We reveal 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.

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## PATHOGENESIS OF CAM MORPHOLOGY IN ENGLISH PREMIERSHIP FOOTBALLERS

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**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).