activities of SOX9, SOX6, and type II collagen (COL2A1) genes were determined by luciferase assays in human non-chondrogenic HeLa cells and mouse chondrogenic ATDC5 cells transfected with a reporter gene containing the respective promoter fragments. Protein-DNA binding was examined by electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay. Chondrogenic differentiation was assessed by endogenous SOX9, SOX6, and COL2A1 mRNA levels using real-time RT-PCR, and by Alcian blue staining and alkaline phosphatase activity. For the functional analyses, we established stable lines of HeLa and ATDC5 cells with retroviral overexpression of RelA or the control empty vector (EV). To know the physiological role, we compared the skeletal phenotypes histologically between homozygous deficient (-/-) mice and the wild-type littermates.

**Results:** Exhaustive comparison of the sequences of the SOX9 promoters among species found that the 1.0 kb upstream of the transcriptional start site was about 80% conserved, in which the binding motifs of nuclear factor kappaB (NF-κB), NFAT, AP-1, Sp-1, CREB, CCAAT, and GATA were identified. When expression vectors of 31 putative transcription factors for the seven identified motifs were co-transfected in HeLa and ATDC5 cells transfected with a reporter construct containing the 1.0 kb SOX9 promoter, RelA (NF-κB p65), an NF-κB family member, most strongly activated the promoter activity. RelA and SOX9 were well co-localized in resting, pre-hypertrophic and hypertrophic chondrocytes of the limb cartilage. The deletion, mutagenesis, and tandem-repeat analyses of the luciferase assay in HeLa and ATDC5 cells co-transfected with RelA or the control EV identified the core region responsive to RelA between -266 and -228 bp relative to the transcriptional start site of the human SOX9 gene. This region contained the NF-κB motif which was the only fully-matched sequence in the proximal SOX9 promoter. EMSA revealed the complex formation of the in vitro-translated RelA protein with the 39 bp NF-κB motif oligonucleotide probe, and the specificity was confirmed by the probe mutagenesis, the cold competition with excess amounts of wild-type and mutated unlabelled probes, and the supershift by addition of an antibody to RelA. ChIP assay confirmed the in vivo specific binding of RelA and the NF-κB motif, which was abolished when using non-immune IgG or a primer set that does not span the NF-kB motif. The RelA overexpression in HeLa and ATDC5 cells stimulated the chondrogenic differentiation parameters and promoter activities of SOX6 and COL2A1 genes, as compared to the control EV. Finally, the RelA-/- mice exhibited dwarfism from embryonic stages with about a half lengths of limbs and trunks compared to those of the wild-type littermates (E15.5), which was associated with suppression of several steps of chondrogenic differentiation.

**Conclusions:** We have identified RelA as a transcriptional factor for SOX9 induction via binding to an NF-κB binding motif in the promoter. The RelA/SOX9 signal is essential for chondrogenic differentiation and skeletal growth.

## 009

### EARLY SUBCHONDRAL PLATE THINNING IN DIFFERENT EXPERIMENTAL CANINE MODELS OF OA IS INTRINSIC TO CARTILAGE DEGENERATION WHILE TRABECULAR CHANGES ARE RELATED TO UNLOADING

**F. Intema**[1], S.C. Mastbergen[1], J.W. Bijlsma[1], H.A. Hazewinkel[2], H.H. Weinans[3], F.P. Lafeber[1]
*[1]Rheumatology & Clin. Immunology UMC Utrecht, Utrecht, Netherlands; [2]Faculty of Vet. Med., Univ. of Utrecht, Utrecht, Netherlands; [3]Orthopaedics, ErasmusMC, Rotterdam, Netherlands*

**Purpose:** Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage degeneration, synovial inflammation, and bone changes. In clinical end stage OA subchondral bone sclerosis is frequently present, represented by an increased volume of trabecular bone and cortical plate. However, in animal models where early features of OA are studied, trabecular bone volume and plate thickness decreases. It is unclear whether these early changes are intrinsic to the osteoarthritis process and independent of the cause of OA induction. Alternatively, they might be caused by a change in joint mechanics and are related to kind of model used. In this study, early bone changes in different canine models of OA are evaluated in relation to cartilage changes and mechanical variables.

**Methods:** Epiphyseal and metaphyseal bone changes were evaluated by micro-CT in the canine bilateral ACLT model (n=6), the bilateral Groove model (n=6) and the unilateral ACLT medial meniscectomy model (n=13), after 20, 20 and 12 weeks of OA induction respectively. Cartilage damage was evaluated by histology and proteoglycan content levels. In addition, in the two bilateral models hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) levels in urine were evaluated over time as indication of bone resorption.

**Results:** The subchondral plate thickness decreased in both bilateral models, reflected by an increased bone resorption shortly after operation. However, trabecular bone changes occurred only in the ACLT model and extended to the metaphyseal bone, which suggests a causative relation with joint (un)loading. Cartilage damage was similar in the bilateral ACLT and Groove model. In the unilateral ACLT medial meniscectomy model, cartilage changes were more severe on the medial side, while on the lateral (less loaded) side trabecular bone changes were more severe. Interestingly, thinning of the subchondral plate was not present on the lateral side, but did accompany the severe cartilage degeneration on the medial side.

**Conclusions:** These results show that thinning of the subchondral plate is intrinsic to the process of cartilage degeneration. The induction of OA seems to disrupt bone production locally, either biochemical or biomechanical, which might eventually lead to subchondral sclerosis. In addition, trabecular bone volume decreased when joint loading is disturbed. The significance of an early decrease of plate thickness and trabecular volume in OA development is still unclear. Previous studies postulated a protective role of the softening of bone which results in secondary unloading of cartilage. This concept is effectively applied in osteoarthritis treatments like joint distraction and bisphosphonate therapy.

**Acknowledgements:** This study was financially supported by the Anna Fund and the Dutch Arthritis Association.

## 010

### THE UNDERLYING MOLECULAR MECHANISMS OF PRIMARY HIP OSTEOARTHRITIS AND ITS INVERSE RELATIONSHIP WITH FRACTURE COHORTS

**D.D. Kumarasinghe**[1,2], B. Hopwood[2,3], G.J. Atkins[3,4], N.L. Fazzalari[1,2]
*[1]Discipline of Pathology, Univ. of Adelaide, Adelaide, Australia; [2]Bone & Joint Res. Lab, SA Pathology, Adelaide, Australia; [3]Hanson Inst., SA Pathology, Adelaide, Australia; [4]Discipline of Orthopaedics & Trauma, Univ. of Adelaide, Adelaide, Australia*

**Purpose:** Primary hip osteoarthritis (OA) is a common cause of disability, and costs the US and Australian economies billions of dollars each year. There is increasing evidence that systemic changes in the trabecular bone are an underlying cause. Differential gene expression observed between OA and controls through microarray analysis of trabecular bone from the intertrochanteric (IT) region has raised several candidates, including genes from the WNT and TGFβ signalling pathways; both have established roles in osteoblast cell biology. As such an investigation in primary