**Results:** FGF9 had significant beneficial effects on multiple parameters used to assess cartilage damage. FGF9 treatment reduced the cartilage degeneration score for the outer region of the medial tibial plateau by 33% (p = 0.004), the width of significant cartilage damage by 38% (p = 0.018), and the depth of cartilage lesions by 20-44% (p = 0.003). Image analysis showed that FGF9 increased the total cartilage area by 24% (p < 0.001) and the viable cartilage area by 35% (p < 0.001). Proteoglycan loss was reduced by 43% (p = 0.003) and the area that showed minimal damage to the collagen was increased two-fold. FGF9 had no significant effect on the subchondral bone but increased the size of the chondrocytes/osteophytes by 29% (p = 0.001).

**Conclusions:** The local delivery of FGF9 in an OA model provided significant beneficial effect on the damaged cartilage. Our data indicates that FGF9 may be a disease-modifying drug candidate for the treatment of osteoarthritis.

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**65 MICROARRAY STUDIES OF SYNOVIAL SPECIMEN OF EARLY HUMAN (CHECK) AND EXPERIMENTAL OA IDENTIFY PATHWAYS AND PROCESSES ASSOCIATED WITH CARTILAGE DAMAGE**

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**Purpose:** Over 50% of osteoarthritis (OA) patients show synovial inflammation, even relatively early during the disease. However, the mechanisms through which this synovial activation contributes to the irreversible joint pathology that characterizes OA, are not known. In the present study we used microarray analysis of synovial tissue of early OA patients and of experimental OA, to identify common pathways that determine cartilage damage in this disease.

**Methods:** From a subpopulation of patients that entered the CHECK Cohort study (Cohort Hip and Cohort Knee), synovial biopsies were collected. CHECK is a prospective 10-year follow-up study that was initiated by the Dutch Arthritis Association on participants early osteoarthritis-related complaints of hip and/or knee. Radiographs are taken in a standardized manner and scored (Kellgren&Lawrence KL) at inclusion (n=18). In addition, biopsies of 7 control synovia were collected. A longitudinal expression analysis was performed on murine synovial tissue at day 7, day 21 and day 42 after induction of collagenase induced OA (CIOA). CIOA was induced by intra-articular injection of collagenase, which causes joint instability, and contra lateral knee joints served as controls. Initial analysis of microarray data was performed using Partek software and functional annotation clustering (FAC) and pathway analysis was done using DAVID.

**Results:** Gene expression profiles of control synovia were compared to CHECK synovia. Analysis using DAVID indicated enrichment of several biological processes and signaling pathways, including regulation of macrophage differentiation, innate immune responses, cell migration, TGFβ-, BMP- and wnt-signaling. This indicates clear activation of the synovium in the CHECK patients compared to controls. Next we compared synovial tissue of CHECK-patients with radiological damage (KL>1) with CHECK-patients without joint damage (KL=0). Among the top 30 genes that were strongest associated with cartilage damage were MMP-1 (18-fold), MMP-3 (10-fold), S100A8 (6-fold) and COMP (13-fold). Again, wound healing, innate immune response and metalloproteases were strongly and significantly enriched and thus associated with joint damage. Pathway analysis demonstrated that in the synovium of patients with joint damage the complement-activation pathway, TGFβ- and BMP-signaling and TLR-activation were significantly upregulated. These results were further underlined by analysis of synovium from experimental OA. Among the genes that were strongly upregulated on all 3 time points after induction were MMP-3 (6-fold), MMP-13 (16-fold), MMP-14 (6-fold) and COMP (13-fold). Again, wound healing, innate immune response and metalloproteases were significantly enriched, as were the complement pathway, the TLR-, TGFβ, BMP and wnt-signaling pathways. In a recent publication, complement was demonstrated to be essential in experimental OA. We therefore determined whether Island Searcher and the MethPrimer program, respectively. In bisulfite sequencing, primers were designed based on MethPrimer program and sequences were analyzed using 2BLAST.

**Results:** Methylation status of SOX9 gene promoter region increased in OA cartilage compared to normal cartilage. From MS-PCR, methylation status of SOX-9 for R3 (from -3653 to -3496, p = 0.0186) and R4-1 (from -3111 to -2983, p = 0.0014) significantly increased in OA cartilage compared to normal cartilage. When we analyzed regions (BSQ1-5) from -4548 to -2846 in the promoter of SOX-9 by bisulfite sequencing, methylated CpG sites significantly increased in all the examined regions: total methylated CpG sites increased about eight-fold in OA cartilage (14.04%) than in normal cartilage (1.66%).

**Conclusions:** Our study suggests that the increased methylation status in the SOX-9 promoter region may have a close relation to the progression of OA.
the synovium expressed complement activating proteins, and found a strong upregulation of COMP, lumican, osteomodulin, biglycan, decorin and fibromodulin. In addition, biglycan expression was strongly enhanced (2.5-fold) in human CHECK-samples from patients with joint damage.

**Conclusions:** All in all, these data suggest an active role for the synovium in OA pathology, and identifies pathways that are likely to be involved. One of the strongest associations was of the complement-pathway with cartilage damage. In addition, TGFβ-, BMP- and wnt-signaling in the synovium, may contribute to further joint damage. The enhanced expression of cartilage damaging MMP-1, MMP-3 and MMP-13 again suggests an active role of the synovium in OA pathology. Future studies will focus on association of gene expression patterns with progression of damage of CHECK-patients.

### 66

**IDENTIFICATION AND CHARACTERISATION OF MICRO-RNAS INVOLVED IN CHONDROCYTE DIFFERENTIATION AND OSTEOARTHRITIS**

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**Purpose:** The majority of bones develop through the process of endochondral ossification where a cartilaginous template is calcified and remodelled into bone. During this process chondrocytes undergo proliferation and differentiation. Many of the signalling pathways and transcription factors which control this developmental programme have been established. MicroRNAs are 20-24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. There is strong evidence that they can influence both chondrogenesis and the initiation and progression of osteoarthritis (OA), however the exact mechanisms are still undetermined. In this study we aimed to profile expression of miRNAs in a cell model of chondrocyte differentiation and use this to prioritise miRNAs for functional analyses in OA. Using deep sequencing we also aimed to identify novel miRNAs expressed in human articular cartilage.

**Methods:** The ATDC5 murine embryonic carcinoma cell line was induced to differentiate through chondrogenesis and in vitro. An Exiqon miRNA microarray was used to profile the expression of all known murine miRNAs across this cell model. Expression of regulated miRNAs was verified in the mouse and chick embryo by in situ hybridisation. The 3' UTRs of potential target genes were subcloned downstream of a luciferase gene for experimental. Novel miRNAs expressed in human articular cartilage were identified using deep sequencing with Illumina's GAIIx system and validated in primary chondrocyte culture in vitro.

**Results:** The expression of a number of microRNAs was regulated across chondrogenesis. This includes 39 microRNAs co-expressed with microRNA-140, known to be involved in cartilage homeostasis and osteoarthritis. Of these, microRNA-455 resides within an intron of COL2A1 which encodes a cartilage collagen. Comparing human osteoarthritic cartilage with femoral neck fracture controls, both microRNA-140-5p and microRNA-455-3p show increased expression in osteoarthritic cartilage. In situ hybridisation shows microRNA-455-3p expression in the developing limbs of chicks and mice and in human osteoarthritic cartilage. The expression of microRNA-455-3p is regulated by TGFβ ligands and the microRNA regulates TGFβ signalling. **ACVR2B, SMAD2 and CHRD1** are direct targets of miR-455-3p and may mediate its functional impact on TGFβ signalling. Deep sequencing of the small RNA pool from chondrocytes extracted from primary human osteoarthritic cartilage identified 16 potential novel miRNAs. These have been validated in cultured chondrocytes using qRT-PCR, Northern blot and Dicer knockdown.

**Conclusions:** MicroRNA-455 is expressed during chondrogenesis and in adult articular cartilage where it can regulate TGFβ signalling, suppressing the Smad2/3 pathway. Diminished signalling through this pathway in ageing and osteoarthritic chondrocytes is known to contribute to cartilage destruction. We propose that the increase in microRNA-455 in osteoarthritis exacerbates this process and contributes to disease pathology. Novel miRNAs expressed in cartilage may regulate cartilage homeostasis and contribute to disease.

### 67

**THE ANALYSIS OF THE GENOME-WIDE DNA METHYLATION PROFILE OF HUMAN ARTICULAR CHONDROCYTES REVEALS DIFFERENT FORMS OF OA.**

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**Purpose:** To identify and analyze the genome-wide DNA methylation profiles of human articular chondrocytes from a population-based case-control study of OA.

**Methods:** DNA methylation profiling was performed using the Infinium HumanMethylation27 beadchip (Illumina Inc.), which allows interrogation of 27,578 highly informative CpG loci. Previously, cartilage isolated DNA from 23 OA patients and 19 healthy controls was bisulfite-modified, using the EZ DNA methylation kit (Zymo Research) and hybridized according to the manufacturer’s instructions. DNA methylation β-values were normalized using GenomeStudio v3.0 (Illumina Inc.). Appropriate bioinformatics analyses were carried out using both R bioconductor software packages and Babelomics suite v 4.2 (babelomics.bioinfo.cipf.es).

**Results:** A first approach based on an unsupervised clustering method for the most variable CpG loci (n=508) showed three distinct groups of samples, called cluster 1 (5 OA patients), cluster 2 (6 OA patients) and cluster 3 (12 OA patients and 17 healthy controls). Specifically, cluster 2 formed a particularly tight cluster with a characteristic DNA methylation profile (Figure 1). The analyses of the biological relevance of the differentially methylated genes in cluster 2 compared with non-cluster 2 by means of a gene set enrichment approach, showed that the biological processes significantly altered were those related to the superoxide metabolic process, morphogenesis/angiogenesis and regulation of cell proliferation, all of them hypermethylated in cluster 2; on the contrary, those mechanisms related to both IL-8 biosynthetic process and apoptosis appeared significantly hypomethylated in cluster 2.

**Conclusions:** The genome-wide methylation analysis shows a clearly distinct epigenetic profile for OA. The DNA methylation profile could be one of the reasons of the existence of different forms of OA and could also be related to both the prevalence and progression of this disease.