hip- and knee-specific OA but none of the signals reached genome-wide significance, as expected given the smaller sample sizes.

Conclusions: Our study highlights possible novel OA susceptibility loci, which will require corroboration in sample sets of larger size. Stage 2 of the arcGEN GWAS, which involves the collection and genome-wide scanning of an independent set of at least 4,500 OA cases (defined by the severe endpoint of total hip and/or total knee replacement), is currently underway. A combined analysis of the 2-stage GWAS including up to 8,000 samples will provide us with sufficient power (90% power to detect an allele with frequency 0.20 and allelic OR of 1.2) to detect modest effects at common loci at the genome-wide significance level.

379 THE ROLE OF EPIGENETICS IN iNOS INDUCTION IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: Osteoarthritis (OA) is a complex disease of the joint, characterized by progressive degradation of the cartilage matrix by aggrecanases and collagenases. Nitric oxide (NO), the product of inducible nitric oxide synthase (iNOS), not only suppresses the synthesis of cartilage matrix, but also increases expression of proteases in OA. Although iNOS is readily inducible in almost all rodent cell types in culture, in contrast, normal human cells are recalcitrant to induction with cytokines due to epigenetic silencing by DNA methylation. It has been shown that transcriptional activation of the human iNOS gene requires the presence of cytokine-responsive elements upstream of ~3.8 kb in the 5'-flanking region of the human iNOS gene. However, it is not known whether the activation of iNOS in OA chondrocytes can be attributed to epigenetic "unsilencing" with implications for therapeutic intervention in OA.

Objective: To examine and correlate mRNA expression of iNOS with the DNA methylation status of specific CpG sites in the iNOS promoter and NF-κB enhancer elements regions, and to examine if the increased expression of iNOS in OA chondrocytes is attributable to loss of DNA methylation.

Methods: Control chondrocytes were obtained from human fetal femurs (7-11wpc) or from fracture neck of femur (#NOF) patients; the latter patients typically suffer from osteoporosis, which does not affect the cartilage. #NOF patients are widely used as a suitable non-OA control. OA chondrocytes were isolated from the femoral heads of patients undergoing hip replacement surgery due to OA. Genomic DNA and total RNA were extracted simultaneously. Expression of iNOS was quantified by qPCR and the DNA methylation status of the iNOS promoter and enhancer regions was determined by bisulfite modification, followed either by cloning and sequencing or pyrosequencer analysis to quantify percentage methylation.

Results: OA samples showed a 13-fold increase in iNOS expression compared to #NOF samples (n=14, P<0.05). NOF# samples (n=14) showed a 79-fold increase in iNOS expression relative to fetal cells (P<0.01) and OA samples showed a 2747-fold increase in iNOS expression compared to fetal samples (n=14, P<0.01). The iNOS promoter has only 7 CpG sites in over 1000bp, 6 of which were highly methylated in both controls and OA. The CpG site at ~289 was un-methylated in both groups. The sites in the coding region (~13, +33 and +38), were largely unmethylated in #NOF and OA patients, although hyper-methylated in the fetal samples. Two NF-κB enhancer elements regions were analyzed, the enhancer region at ~5.2 was found to be totally unmethylated in all samples (~100%); however, the enhancer region at ~5.8 was significantly de-methylated in OA samples (n=12, P<0.05) compared with #NOF samples, with an approximate 10% loss of methylation.

Conclusions: These studies indicate the CpG sites in the proximal coding region may be involved in epigenetic regulation of iNOS. However, upregulation of iNOS in OA chondrocytes is not a consequence of epigenetic unsilencing in this region, because these sites are un-methylated even in #NOF samples. The de-methylation of ~5.8 enhancer element region may be an important factor for iNOS overexpression in OA chondrocytes and, our studies demonstrate that the regulation of iNOS expression in OA chondrocytes appears to be the result of multiple epigenetic modifications in different physiologic conditions. Further understanding of the regulation and epigenetic modulation of iNOS will inform our understanding the pathogenesis of OA.

380 GENE EXPRESSION ANALYSIS OF THE ARTICULAR CARTILAGE IN FRZB+/− MICE SUGGESTS ACTIVATION OF WNT AND BONE MORPHOGENETIC PROTEIN SIGNALLING

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Purpose: Frizzled related protein (FRZB/SFRP3) is a secreted WNT antagonist isolated from articular cartilage and expressed in developing skeletal elements. Polymorphisms in the human FRZB gene are associated with susceptibility for osteoarthritis. Induction of experimental osteoarthritis in FRZB−/− mice results in enhanced cartilage degradation associated with increased Wnt signalling. Mmp3 expression, Mmp activity and cortical bone thickness. In this study we used a whole mouse genome micro-array to investigate differentially regulated pathways between wild-type and FRZB−/− mice in tibial articular cartilage.

Methods: Articular cartilage from the tibia was isolated from 6 weeks old Frzb−/− mice and wild-type littermates. RNA was isolated using the RNeasy Fibrous tissue mini kit (Qiagen). RNA quality was assessed using an Agilent 2100 Bio-analyzer and RNA nanochips (Agilent technologies Inc). The transcriptional profiles were analysed by the VIB MicroArrays Facility using the whole genome Affymetrix GeneChip Mouse Genome 430 2.0 Array. Articular cartilage from one tibia from 3 wild-type mice and 2 Frzb−/− mice was used (3 vs. 2 chips comparison). Gene expression analysis was based on the RMA expression values and the MAS 5.0 detection calls. PANTHER Classification scheme, DAVID Bioinformatics resources and FUNNET transcriptional analysis were used for pathway analysis.

Results: Using Benjamini-Hochberg corrected P-values (p<0.01) in combination with a cut-off fold change (log2-ratio)>1 the analysis showed that 697 transcripts were significantly upregulated in the Frzb−/− sample group. PANTHER pathway analysis identified overrepresentation of genes linked to the Integrin, Wnt and Cadherin signalling pathways (corrected p-values 3×10−9, 5×10−8 and 5×10−8 respectively). Not surprisingly, genes linked to skeletal development and extracellular matrix were enriched in the analysis. DAVID analysis similarly identified the Wnt and Integrin pathways but also EGF signalling. FUNNET identified both Wnt and TGF/BMP signalling. For the Wnt signalling pathway different Frizzled receptors, Wnt9a ligand and distinct intracellular and extracellular antagonists were upregulated suggesting compensatory upregulation of this pathway. The link with integrin upregulation further supports our earlier data that identified a role for Frzb in mechanobiology.

Conclusions: In this study we demonstrated that loss of Frzb results in upregulation of the Wnt signalling pathway and a possible compensatory upregulation of BMP signalling. The upregulation of the Integrin and Cadherin signalling pathway suggest an important role for Frzb in the interactions of cells with surrounding cells and extracellular matrix.

381 HERITABILITY PATTERNS IN HAND OSTEOARTHRITIS: THE GHOST STUDY

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Purpose: To assess heritability of clinical and radiographic features of hand osteoarthritis (OA) in affected patients and their siblings.

Methods: As part of an ongoing genetic study (GHOST-Genetics of Hand Osteoarthritis Study), patients with hand osteoarthritis were recruited for a family-based study aimed at assessing genetic determinants of hand OA. A convenience sample of unrelated patients with symptomatic (i.e. pain or disability), idiopathic, clinical and radiographic hand OA and their siblings were evaluated by examination, and radiography. For inclusion, probands must have been ≥45 years of age, met American College of Rheumatology clinical classification criteria for hand OA and have radiographic evidence of OA in the form of osteophytes in either first metacarpal (1st CMC) joint or at least 2 distal interphalangeal (DIP) or proximal interphalangeal (PIP) joints. Radiographs were scored for hand OA features by radiographic atlas. The heritability of hand OA phenotypes was assessed for overall