conchondrocytes (ACs) and its regulatory mechanisms remain unclear. This study aimed to explore epigenetic regulatory mechanisms of age-related SOX9 expression in ACs of mice, spanning from the embryonic stage to 18 months of age.

Methods: The hip and shoulder joints of wild type BALB/c mice were harvested at embryonic day 16.5 and 1, 2, 6, 12 and 18 months for histopathological and immunohistochemical analyses. Femoral and humeral head cartilage from the same age groups was used for chondrocyte isolation, gene expression, methylated DNA immunoprecipitation or chromatin immunoprecipitation assays to examine epigenetic changes in the promoter region of the SOX9 gene. siRNA-mediated knockdown of the histone lysine-specific demethylases-1 gene (Lsd1) and 5-azacytidine treatment were performed in cultured ACs.

Results: Sox9 mRNA and protein were highly expressed in ACs during joint development but significantly decreased at 2–18 months of age. No histopathological features of osteoarthritis were observed in examined joints by 18 months. Epigenetic DNA methylation and histone methylation are both associated with the age-dependent SOX9 expression. Knockdown of Lsd1 is sufficient to up-regulate SOX9 expression in ACs of adult mice through increased recruitment of H3K4me2 (a histone modification for transcriptional activation) in the promoter region of the Sox9 gene. However, the reduction of DNA methylation in the Sox9 promoter region induced by 5-azacytidine treatment in cultured ACs did not increase Sox9 expression. The data suggest that reduction of Sox9 expression in ACs of adult mice is primarily regulated by H3K4me2.

Conclusions: These results suggest that Sox9 expression in mouse ACs is significantly decreased after the completion of joint development due to reduced demands for SOX9. This developmental switch in Sox9 expression in mouse articular cartilage is primarily regulated by epigenetic histone methylation.

309 NOVEL SUSCEPTIBILITY LOCI FOR OSTEOARTHRITIS OF THE HAND: VARIANTS IN CODING AND GENE REGULATORY REGIONS

Purpose: Osteoarthritis (OA) of the hand, is to a large extend, genetically determined. However, despite the large GWAS efforts into OA over the last years only one locus has been found to be associated to hand OA. We here aim to identify novel genes and pathways involved in the etiology of hand OA.

Methods: We conducted a genome-wide association study of hand OA in a discovery set of 10,155 participants from the three Rotterdam Study cohorts (RS1, RS2 and RS3) using standardized age and gender adjustments. We have used a quantitative bilateral hand OA phenotype, summing all KL-scores of the hand joints for both hands (min score: 0, max score: 128). EasyQC was used to conduct quality control across cohorts. Results were combined in a jointed meta-analysis using inverse variance weighting. Genome-wide significant signals were analyzes for enrichment in genomic regulatory regions, using data from the ENCODE and Roadmap epigenetics project.

Results: The discovery analysis yielded 16 loci with suggestive evidence for association (P<10-6) and another 3 loci with genome-wide significant association (P<5x10-8). These novel genome-wide significant loci include SNPs in or near the MGP, PTHHL and APPB2 genes. The risk alleles of MGP, PTHHL and APPB2 genes had a higher KL-sum score compared to the reference allele (delta KL-score was 7, 6, and 23 respectively) MGP codes for Matrix Gla Protein and is important for mineralization of cartilage tissue. We here identified a SNP resulting in a protein-change to be associated with hand OA. The identified SNPs in the PTHHL-locus and the APPB2-locus co-localize with enhancer histone markers in osteoblast and chondrogenic cells from the Roadmap and ENCODE database, suggesting a potential gene regulatory function for these SNPs.

Conclusions: We identified 3 loci to be associated to hand OA, a coding variant in the MGP gene and two intronic variants in APPB2 and CCDC91 located near gene regulatory marks specific for bone and/or cartilage. In addition, we have also found 16 loci suggestively associated to hand OA. Future analysis will consist of validation of our findings in other cohorts and further functional assessment and pathway analyses of the identified variants.

310 SMAD3 IS UP-REGULATED IN HUMAN OSTEOARTHRITIC CARTILAGE INDEPENDENT OF PROMOTER DNA METHYLATION

Purpose: We previously reported that SMAD3 was associated with the total burden of radiographic osteoarthritis (OA). SMAD3 is a mediator of TGF-β signalling pathway that is known to be involved in the cartilage maintenance and repair. SMAD3 knock-out mice are deficient for Collagen and Aggrecan, and develop OA-like features in the joints. The aim of the present study was to investigate the SMAD3 gene expression in osteoarthritic and healthy human cartilage and to examine whether the gene expression is regulated by the promoter DNA methylation.

Methods: Osteoarthritic cartilage samples were collected from patients who underwent total hip/knee joint replacement surgery due to primary OA. Healthy cartilage samples were obtained from patients with hip fracture without any evidence of hip OA. DNA and RNA were extracted from the cartilage samples using Qiagen’s AllPrep DNA/RNA Mini Kit. Quantitative PCR experiment was done using ABI-7500 real time PCR system to measure RNA expression after cDNA synthesis by Thermoscript cDNA synthesis kit. DNA Methylation was assayed by Sequonome’s EpiTYPER after DNA bisulphate conversion using Qiagen’s EpiTect Bisulfite Kit. Mann-Whitney test was utilized to examine the association between OA cases and controls for SMAD3 expression and its promoter DNA methylation levels. Spearman’s rank correlation analysis was performed to examine the association between the promoter methylation and gene expression. A P-value less than 0.05 was considered as significant.

Results: A total of 49 OA patients (38 hip OA and 11 knee OA) and 51 controls were included in the study. Mean age was 64.4 in OA patients and 78.7 in controls. Four CpG sites, located −450bp upstream of the first SMAD3 exon, were assayed, and we found no difference in methylation between OA cases and controls after adjusting for age. The expression experiment was performed for 38 patients with OA (32 hips and 6 knees) and 28 healthy controls, and we found that the SMAD3 gene was expressed in OA cartilage on average 1.8 times higher than controls (p<0.0005). Similar results were obtained when we looked at hip and knee OA separately (p<0.01). We found no association between SMAD3 expression and the methylation at the promoter region of the gene.

Conclusions: Our study demonstrated that SMAD3 is up-regulated in OA. This up-regulation, however, cannot be explained by the changes in the promoter DNA methylation. Given that TGF-β/SMAD3 pathway may play a protective role in the cartilage, the up-regulation of SMAD3 is more likely due to the consequence of OA, thus reflecting repairing activity stimulated by the cartilage lesion in OA.

311 EFFECTS OF HYPOXIA ON ANABOLIC AND CATABOLIC GENE EXPRESSION AND DNA METHYLATION IN OA CHONDROCYTES
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Purpose: Osteoarthritis (OA) is a clinical syndrome characterized by joint pain, functional limitation and reduced quality of life. An essential feature of the underlying pathogenesis of OA is an imbalance of anabolic and catabolic activity leading to progressive loss and destruction of extracellular matrix of articular cartilage. Cartilage is an avascular and aneural tissue. Chondrocytes thrive in this restricted environment of low oxygen tension and poor nutrient availability, which has led to suggestion that hypoxia, may be a protective mechanism against the development of OA. There is a growing body of evidence to support the role of epigenetic factors in the pathogenesis of OA. However, few studies have investigated 1-beta (IL-1β) plus oncostatin M (OSM), 5-azadeoxycytidine (5-aza-dC) or media alone (control) were added twice weekly to the incubated samples. After 5 weeks, levels of Collagen type
Validation of the association of a functional microsatellite in macrophage migration factor with hip OA

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Purpose: Microsatellites are not amenable for high-throughput genotyping and have been excluded from GWAS. However, several microsatellites have been found to affect expression of nearby genes and a couple of them, in the asporin and BMP5 genes, have been associated with susceptibility to OA in multiple studies. In a recent exploration of functional microsatellites we found association of the -794 CATT microsatellite in the MIF gene with hip OA in 1782 patients compared with 1878 healthy controls of European ancestry. Replication in other patient collections was hampered by the lack of a method to obtain the genotypes without actually performing the laboratory tests. Therefore, we aimed to develop an imputation methodology for this microsatellite using the genotypes of SNPs in linkage disequilibrium and to apply it for validation.

Methods: In our previous study of the MIF microsatellite, we had included 1090 samples that were also in the arCOGEN GWAS. Genotypes of the 130 SNPs in the linkage disequilibrium region surrounding the microsatellite in these samples were used as source of haplotype information. Imputation was done with Impu2 with modifications allowing estimation of the probabilities for the number of copies of each microsatellite allele and their posterior combination. Performance of this procedure was evaluated in 10 replicates of training, with 90% of the samples, and testing of accuracy in the remaining 10% of the samples with known microsatellite genotypes. Once concordance of imputed genotypes with tested genotypes was established, the approach was applied for the imputation of the MIF microsatellite in 5667 population controls from Wellcome Trust Case-Control Consortium (WTCCC) and in 2406 hip OA cases from arCOGEN (all of them of European UK ancestry). In addition, we have validated the functional effect of the MIF microsatellite on the plasma levels of MIF in 361 healthy control samples by ELISA (R&D Systems) from subjects that were either homoygous for the 5 repeat or the 6 repeat alleles. MIF microsatellite allele frequencies were compared with POWERMARKER and differences in plasma levels of MIF were analyzed with ANOVA using Statistica 7.0 (StatSoft).

Results: There was a microsatellite allele with frequency lower than 1% that was not included in the imputation. The genotypes of the other three alleles were imputed with sufficient accuracy (91.6%) and call rate in the reference samples (98.8%). However, other microsatellites showed that the procedure will require further refinements to attain this performance for microsatellites with more alleles. Application of this procedure to the WTCCC and arCOGEN samples produced genotypes for 99.0% of the samples. Comparison of the allele frequencies showed significant differences between hip OA and controls in women (Table 1) following the same pattern found in our previous study, with the five repeats allele less frequent in the patients than in the controls (OR = 0.88, 95% CI 0.79 to 0.98, P = 0.018). However, no difference was appreciated in men or between control women and men as it was in our previous study. This contrast between studies could be attributed to the use of OA-free controls in the previous study and population controls here.

Analysis of MIF in plasma of healthy controls showed higher levels in the homozygous for the 5 repeat allele (3.6 ng/mL) than for the 6 repeat allele (2.7 ng/mL, P = 0.00025) following the direction previously reported by other authors and without differences between women and men.

Conclusions: A new method to impute genotypes of microsatellites with three alleles based on SNP genotypes has been developed. It has shown good call rate and accuracy. This procedure allowed us to validate the association of the MIF microsatellite with hip OA in a large number of cases and controls. Concordance with our previous study was obtained in women, but not in men. Higher MIF levels were found in the subjects with the hip OA protective allele. These results should contribute to define the role of MIF, an important cytokine, in the OA pathology.