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A GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A LOCUS ON CHROMOSOME 7Q22 TO INFLUENCE SUSCEPTIBILITY FOR OSTEOARTHRITIS
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Purpose: To identify novel genes involved in osteoarthritis (OA) we performed a Genome-Wide Association Study (GWAS) in Caucasians.

Methods: In total, 500,510 Single Nucleotide Polymorphisms (SNPs) were tested for association with osteoarthritis in 1341 OA cases and 3496 Dutch Caucasian Controls. SNPs nominally associated with at least two OA-phenotypes were analysed in 11 replication studies. In the meta-analysis, 14,938 OA cases and approximately 39,000 controls were analyzed using the program Comprehensive Meta-Analysis. A p-value < 1×10^-7 was considered genome-wide significant (0.05/500,510).

Results: The C-allele of rs3815148 on chromosome 7q22 (MAF 23%, 172 kb upstream of the GPR22 gene) was consistently associated with a 1.14-fold increased risk (95%CI: 1.09-1.19) for knee/hand OA (p=4.8×10^-12). In addition, immunohistochemistry showed absence of GPR22-protein in normal murine joint tissues, but detectable levels in articular cartilage in both the articular cartilage and osteophytes.

Conclusions: Our findings reveal a novel common variant to influence susceptibility for osteoarthritis.

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CYTOKINE-INDUCED CHANGES IN GENE EXPRESSION IN SHORT-TERM CULTURES DO NOT FULLY MIMIC ABERRANT EXPRESSION IN OSTEOARTHRITIS: ADVANTAGES OF LONG-TERM CULTURES
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Purpose: Cartilage destruction in osteoarthritis (OA) is due, in part, to cytokine-induced expression of proteases. In vivo, this aberrant expression is maintained even in the absence of cytokines and is transmitted to daughter cells, suggesting that epigenetic changes result in activating previously silenced genes. Current in vitro models used for studying transcriptional regulation consist of treating chondrocytes (primary or the cell line C28/I2) with cytokines (IL-1β, TNF-α) and investigating gene expression after a few hours. Many studies have shown that the cytokines up-regulate catabolic and down-regulate anabolic genes in vitro, but to what extent does this mimic the situation in OA? We asked i) whether aberrant expression in vitro was maintained after cytokine withdrawal; ii) whether it was possible to reproduce epigenetic changes in vitro and iii) to what extent the cytokine-induced expression in vitro correlated with in vivo expression by OA chondrocytes.

Methods: C28/I2 cells were treated with IL-1β and harvested after 1, 4, 8, 24 and 72 h. Non-OA chondrocytes, obtained after hemi-arthroplasty, were passed (P1), then treated once with IL-1β and harvested after 24 and 72 h. P1 chondrocytes were also cytokine-treated twice a week for 2-3 weeks, then passaged again and cultured without cytokines for another 2-3 weeks. Expression levels of catabolic (IL-1β, TNF-α, MMP-3, -13) and anabolic (COL2A1, DNM1T1) genes were quantified by qRT-PCR. DNA methylation was quantified at a key CpG site of IL-1β promoter (Epigenetics, 2007; 2: 86-95). mRNA expression was compared between superficial-zone OA chondrocytes and aged non-OA chondrocytes.

Results: In C28/I2 cells, induction of catabolic genes by IL-1β rapidly peaked at 1-8 h, depending on the gene investigated, then declined again. In primary chondrocytes, expression of catabolic genes increased considerably 24 h after IL-1β treatment, but decreased after cytokine withdrawal. COL2A1 expression was virtually abolished by IL-1β and not regained after 72 h. The % DNA methylation did not change during 72 h. Repeated treatment with IL-1β in long-term culture increased expression of IL-1β and the MMPs more markedly than single treatment and induced loss of DNA methylation. Expression and DNA de-methylation was maintained after cytokine withdrawal and passage. While induction of catabolic genes was also found in superficial-zone OA chondrocytes, the repression of anabolic genes was not. Compared with non-OA ‘old’ chondrocytes, which expressed very low levels of COL2A1, BMP-7 or IL1R antagonist, these genes were actually activated in OA chondrocytes.

Conclusions: The widely used short-term cytokine-treated chondrocyte cultures do not fully model the in vivo situation for two reasons: i) Repression of anabolic genes by IL-1β, which is frequently observed in vivo, does not occur in OA, where activation is observed. ii) Induction of catabolic genes is not maintained in short-term cultures after cytokine withdrawal and does not correlate with DNA de-methylation, whereas in OA it does. However, long-term treatment can mimic the loss of DNA methylation and maintain aberrant gene expression of catabolic genes even after cytokine withdrawal. This model will facilitate studies on the mechanisms of DNA de-methylation, which might ultimately lead to novel therapeutic approaches for the treatment of OA.

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POLYMORPHISM IN THE RUNX1 GENE ARE ASSOCIATED WITH OSTEOARTHRITIS OF THE HIP BUT NOT OF THE KNEE IN THE UK
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Purpose: The Runx (runt-related) family of transcription factors are important regulators of cell fate decisions in early embryonic development, and in differentiation of tissues. In murine embryonic development RUNX1 is expressed in pre-chondrocytic condensations, in the perichondrium and periosteum, and in immature proliferating chondrocytes. Functional data from analysis of limb bud cell condensations in vitro suggests that RUNX1 mediates the onset of mesenchymal cell commitment towards chondrogenesis.
In blood cells RUNX1 directly stimulates G1 to S cell-cycle progression and regulates specific genes and cytokine receptors. In addition, genetic variation in the RUNX1 gene has been implicated in susceptibility to rheumatoid arthritis in Japanese populations. We hypothesized that variation in the RUNX1 gene could be involved in genetic susceptibility to osteoarthritis.

**Methods:** 1983 knee OA cases (1232 total knee replacement patients and 751 with radiographic disease only), 1283 hip OA cases (1119 total hip replacement patients and 149 with radiographic disease only) and 2317 controls from the UK were genotyped for three tagging single nucleotide polymorphisms (SNPs) in the RUNX1 gene and genetic association was tested.

**Results:** Differences in allele frequencies did not achieve statistical significance between hip OA and controls (p =< 0.065) nor between knee OA and controls (p <= 0.370). Nevertheless, we found that the rare homozygotes at both rs2834656 and rs2834662 were significantly associated with lower risk of hip OA OR=0.63 (95% CI 0.46-0.88) p=0.006 and OR=0.70 (95% CI 0.51-0.94) p=0.019 respectively. No such association was seen with knee OA (OR=0.88, 95% CI 0.67-1.15 and p=0.358)

**Conclusions:** Data indicate that polymorphisms in the RUNX1 gene may be involved in genetic susceptibility of hip OA but not knee OA. This work was supported by the Arthritis and Research Campaign and by the European Union FP7 large collaborative project grant 200800 TREAT-OA

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**DIO2 METHYLATION IN ARTICULAR CARTILAGE AND WHOLE BLOOD**

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**Purpose:** Gene activity can be regulated or silenced by epigenetic control mechanisms, for example methylation. An ENCODE dataset of genome wide transcriptional repressor CCCTC-binding factor (CTCF) binding shows a CTCF binding peak in the proximity of rs225014, an OA susceptibility SNP within the DIO2 gene. In this study we set out to explore differences in methylation status of DIO2 between blood and cartilage DNA as well as in healthy and OA cartilage.

**Methods:** We collected cartilage material from subjects undergoing total joint arthroplasty and whole blood from OA patients. Cartilage was snap frozen in liquid nitrogen and stored at -80°C. Cartilage was ground up in a Retsch MM200 sample disintegrator and RNA and DNA were extracted using Qiagen columns. Blood DNA was collected by use of a Qiagen column. DNA was bisulfite treated using the Zymo Research EZ DNA methylation bisulfite kit and 13 DIO2 CpG sites were PCR amplified and quantitatively assessed for DNA methylation using a mass spectrometry based method (Epityper, Sequenom).

**Results:** The DNA methylation levels in cartilage (N=3) and blood (N=7) could be analyzed for 12 out of 13 DIO2 CpG dinucleotides. Overall, our preliminary data show that DNA performed better upon bisulfite treatment as compared to cartilage DNA, giving more robust data for blood. However, overall the methylation status of the DIO2 in cartilage appeared higher as compared to blood. For blood the average DIO2 methylation was 0.67, with methylation status per CpG dinucleotide ranging from an average of 0.1 to 0.96 (mean standard deviation 0.23 ranging from 0.04 to 0.17).

**Conclusions:** These preliminary data indicate that CpG methylation within the DIO2 gene varies between individuals and that we can consistently measure this variation. The overall hyper-methylation of DIO2 in healthy cartilage as compared to blood might be indicative of epigenetic control of the DIO2 within different tissues. This should be substantiated through the ongoing collection of DNA from both healthy and OA affected cartilage, which may also provide insights in possible differential methylation of DIO2 in OA. Unraveling epigenetic regulation at developmental genes within cartilage may elucidate additional mechanisms of the complex etiology of OA.

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**ASSOCIATION OF LEPTIN GENE (LEP) WITH BODY MASS INDEX (BMI) IN HAN CHINESE**

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**Purpose:** Although the number of candidate genes has increased and several polymorphisms have been studied in human populations, knowledge about genetic factors underlying the susceptibility to obesity remains incomplete. This study assessed the contribution of leptin gene polymorphism to BMI in Chinese Han population.

**Methods:** 3 tag SNPs of LEP were selected by haploviewer software from Hapmap database. They cover all the SNPs of LEP whose heterozygosity rate are over 10%. We genotyped the selected SNPs in 615 individuals ≥40 years old live in and around Nanjing, China, compared allelic and genotypic frequencies and haplotype distribution between normal range (BMI = 18.50-24.99) and overweight (BMI ≥ 25.00) groups.

**Results:** Associations were observed in haplotype CAT (P=0.009) and AGC (P=0.005) between the two groups, with the sequence of loci chosen for hap-analysis is rs11761556, rs12706832, rs2071045. No association between the genotypes and allele frequencies was observed between the two groups.

**Conclusions:** These findings suggest there is an association between LEP and BMI and it's a first report examined the relationship between LEP polymorphisms and BMI in Han Chinese.

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**LARGE-SCALE ASSOCIATION STUDY OF SUSCEPTIBILITY GENES FOR LUMBAR DISC HERNIATION**

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**Purpose:** Lumbar disc herniation (LDH) is a common disease, a predominant cause of low back pain and unilateral leg pain. Although many risk factors have been reported for LDH, its etiology and pathogenesis are for the most part unknown. Strong familial predisposition for LDH and lumbar disc degeneration has been established through a number of family and twin studies. Recently, several susceptibility genes have been reported to associate with LDH and related conditions. Most encode extracellular matrix (ECM) proteins in the intervertebral disc, suggesting the importance of ECM metabolism in LDH. The aim of the study is to examine susceptibility genes for LDH through a candidate gene approach for the cartilage genes.

**Methods:** Subjects.

Case-control association analyses were performed for LDH candidate genes by a two-step screening. We recruited a total of nearly 1,000 cases and 1,000 controls for the first screen and the replication study. Affected individuals with LDH were recruited from 19 collaborating hospitals. The diagnosis of LDH required the following three criteria: 1) diagnosis of LDH by MRI; 2) treatment and monitoring for >1 year by orthopedic surgeons; and 3) a history of unilateral pain radiating from the back along the femoral or sciatic nerve to the corresponding dermatome of the nerve.