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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 analyses 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 $<1\times10^{-7}$ 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 ($p=8\times10^{-8}$), and also with an 18% increased risk for OA progression (p=0.03). This SNP is in almost complete linkage disequilibrium with rs3757713 (located 68 kb upstream of GPR22) which is associated with GPR22 expression levels in lymphoblast cell lines ($p=4\times10^{-12}$). In addition, immunohistochemistry showed absence of GPR22-protein in normal murine joint tissues, but detectable levels in arthritic disease 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 changes *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 passaged (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, DNMT1) 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 passaging.* 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 vitro*, 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.