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:** Our 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 off 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 going total joint arthroplasty and whole blood from OA patients. DNA and RNA were extracted using Qiagen columns. Blood and RNA and DNA were extracted using Qiagen columns. Cartilage was snap frozen in liquid nitrogen and stored at -80°C. Cartilage was ground up in a Retsch MM200 sample disintegrator.

**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:** Our 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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**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 LDLH, its etiology and pathogenesis are for the most part unknown. The 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 LDLH 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.
root for >3 months. We extracted genomic DNA from peripheral blood leukocytes of affected individuals and controls using standard protocols. Written informed consent was obtained from each subject.

Genotyping and Statistical Analysis.

We genotyped SNPs using the multiplex PCR-based Invader assay, TaqMan SNP genotyping assays, or by direct sequencing of PCR products. We assessed association and Hardy-Weinberg equilibrium using the \( \chi^2 \) test. We estimated haplotype frequencies using the expectation-maximization algorithm.

Functional Studies.

Allelic difference in the function of the associated SNPs was examined in vitro and in vivo according to their possible roles in pathogenesis of LDH and available experimental resources.

Results: We selected candidate genes based on our prior knowledge on their function, expression, animal models, and human monogenic diseases, etc. CILP (cartilage intermediate layer protein), ASPN (asporin), COL11A1, THBS2 (thrombospondine 2), MMP9 (matrix metaloprotease 9) and SKT (sickle tail) were found to be associated with LDH. CILP-susceptibility allele was a missense SNP whose predicted protein product had a stronger inhibitory effect on TGF-\( \beta \) function for cartilage differentiation. COL11A1-susceptibility allele showed decreased COL11A1 mRNA expression in vitro and in vivo. ASPN and SKT associations were replicated in different ethnic groups. THBS2-susceptibility allele was located in a polypyrimidine tract upstream of the 3'-splice site an intron 10 and exerted allelic differences on exon skipping rates in vivo, with the susceptibility allele showing increased skipping. The exon skipping resulted in decreased THBS2 interaction with MMP2 and MMP9.

Conclusions: Through a large-scale candidate gene approach, we have found several LDH-susceptibility genes, which would help in clarifying the pathogenesis of LDH and in developing innovative treatment for LDH.

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ASSOCIATION BETWEEN IL-4 RECEPTOR \( \alpha \) AND TGF-\( \beta \)1 POLYMORPHISMS AND HAND OA

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Purpose: Osteoarthritis (OA) is a common disease characterised by the degeneration of the cartilage of synovial joints such as the hip and knee. Available evidence suggests that genetic factors play a major role in etiology of OA. The gene product of IL-4 receptor (IL-4R) regulates cartilage chondrocyte differentiation and survival. Transforming growth factor (TGF)-beta, on the other hand, regulates the function of fibroblasts, and has been shown to have a role in the pathogenesis of rheumatoid arthritis. These enzymes may therefore play an important role in development of OA. Both IL-4R and TGF genes have been shown to exhibit genetic polymorphisms with functional consequences. We examined whether these gene polymorphisms modified individual susceptibility to hand OA in Finnish women.

Methods: Radiographs of both hands of 543 Finnish women aged 45-63 years were examined and classified for the presence of OA using reference images. Hand OA was defined by the presence of radiographic findings of grade 2 or more in at least two joint pairs (symmetrical OA) or in at least two DIP joint pairs (symmetrical DIP OA). The IL-4R Ser503Pro (rs1805015) and TGF\( \beta \)1 Leu10Pro (rs1982073) genotypes were determined using TaqMan-based methods. Data regarding anthropometric measures and other risk factors were collected by questionnaire.

Results: No significant association was found between the IL-4R Ser503Pro polymorphism and hand OA. However, the TGF-\( \beta \)1 10Pro allele posed a 1.6-fold (95\% CI 1.0-2.5) and a 1.8-fold (95\% CI 1.0-2.6) risk of symmetrical OA and symmetrical DIP OA, respectively. Moreover, the risk of symmetrical OA was almost 6-fold (OR 5.6, 95\% CI 1.3-24.7) among carriers of the combination of IL-4R 503Pro and TGF-\( \beta \)1 10Leu alleles.

Conclusions: Our results suggest that the studied IL-4R- and TGF-\( \beta \)1-gene polymorphisms may play a role in the etiology of polyarticular hand OA.

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FUNCTIONAL ANALYSIS OF THE GROWTH AND DIFFERENTIATION FACTOR 5 REGULATORY POLYMORPHISM THAT IS ASSOCIATED WITH OSTEOARTHRITIS SUSCEPTIBILITY


Purpose: Over the past decade, a genetic component for the multifactorial disease osteoarthritis (OA) has been established. Among others, a consistent and reproducible association with OA was found for the single nucleotide polymorphism (SNP) rs143383 (T/C) in the 5'UTR of the growth and differentiation factor 5 (GDF5) gene with a lower expression of the risk-associated T-allele in vitro and in vivo, the latter in cartilage tissue from OA patients. To further characterize its role in OA susceptibility, we have expanded the analyses of the effect of this SNP on GDF5 allelic expression to more joint tissues, and searched for cis and trans factors that interact with the SNP.

Methods: Tissues (cartilage, fat pad, meniscus, synovium, ligaments) were collected from OA patients undergoing joint replacement of the hip or knee. Nucleic acid was extracted and, using rs143383 and an assay that discriminates and quantifies allelic expression, the relative amount of GDF5 expression from the T and C alleles was measured. Electrophoretic mobility shift (EMSA) and luciferase assays were used to search for trans factors regulating transcriptional expression of GDF5 at rs143383.

Results: We observed a consistent allelic expression imbalance of GDF5 with a reduced expression of the risk-associated T-allele of approximately 20% in all tissues tested, implying that the functional effect rs143383 mediates on GDF5 expression is joint-wide. A differential binding of transcription factor(s) to rs143383 was revealed by EMSA. Among putative candidates tested, DEAF1 showed preferential binding to the T-allele of rs143383. Testing GDF5 promoter constructs in a luciferase assay confirmed DEAF1 as a trans-acting factor that merits further investigation as a tool for modulation of GDF5 expression and hand OA susceptibility.

Conclusions: The OA susceptibility being mediated by polymorphism in GDF5 is not restricted to cartilage, emphasising the need to consider the disease as involving the whole joint. DEAF1 is a trans-acting factor that merits further investigation as a tool for potentially modulating GDF5 expression, and the existence of an additional cis-acting regulatory polymorphism highlights the complexity involved in regulating the expression of this important OA susceptibility locus.