Purpose: In osteoarthritis (OA), articular chondrocytes undergo phenotypic change and acquire the ability to over-express genes such as matrix metalloproteinases (MMP)-3,9 and 13, ADAMTS-4 and interleukin-1 beta (IL1B). Previous studies have shown that, among the epigenetic changes, DNA methylation at CpG sites in the relevant promoters is correlated with the aberrant expression of different genes. Indeed, long-term treatment with inflammatory cytokines can cause aberrant and sustained gene expression along with loss of DNA methylation in vitro. More importantly, de-methylation on the CpG sites within the proximal MMP13 promoter was found to be relevant for its aberrant expression in OA chondrocytes. However, how CpG methylation status intervenes to determine the promoter activity of genes such as MMP13 is still not known.

The aims of the study were to determine 1) whether differences in CpG methylation status directly affect MMP13 promoter activity and 2) which CpG sites are responsible for changes in MMP13 promoter responses in chondrocytes.

Methods: A CpG-free luciferase reporter vector (pCpG-Luc) was generated according to the literature (Klug et al., Epigenetics, 127-130; 2006). The promoter region of MMP-13 was PCR-amplified to produce constructs containing different sequences (-372/+14, -214/+14 and -86/+14) of the proximal promoter with 7, 4 and 1 CpG sites, respectively. Each promoter construct was inserted in the pCpG-Luc backbone and treated with DNA methyltransferase (Sssl) to methylate all CpG sites in the inserted promoter sequences. Each Sssl-treated or untreated vector was co-transfected with a Renilla luciferase vector into the human chondrocytic cell line, C28/I2, by lipofection. The transfected cells were lysed 24 hours after transfection and transferred to a 96-well plate, and then Firefly and Renilla substrates were applied, followed by the light detection with LMax II 384 luminometer (Molecular Devices, CA). The plain pCpG-Luc vector (backbone) and the pCpG-Luc vector containing an active CpG-free promoter (pCpG-Luc-CMV) were also tested in methylated/non-methylated status.

Results: The backbone and pCpG-Luc-CMV activities were not changed before and after Sssl treatment, which indicates that the Ssxl methyltransferase treatment did not alter either the basal activity of the backbone or the CMV promoter activity. The reporter activities of the -372/+14 and -214/+14 MMP13 promoter constructs were decreased after Sssl treatment, whereas the -86/+14 MMP13 promoter activity was not significantly affected by methylation.

Conclusions: In CpG-free-Luc, which does not contain any CpG site, allows for evaluation of the role of CpG methylation in various promoters. Our results indicate that the three CpG sites within the region spanning -214 to -86 bp of the proximal MMP13 promoter could be responsible for the promoter activity in chondrocytes. Indeed, our results are consistent with our previous finding, showing that de-methylation of the CpG site at -110 bp of the MMP13 promoter correlates with its increased and abberant expression in OA chondrocytes.

Methods: Bilateral hand radiographs of 543 Finnish female dentists and teachers aged 45-63 years and living in the Helsinki metropolitan region were examined and classified for the presence of OA using a modified Kellgren-Lawrence (K-L) system and reference images. Hand OA was defined as at least mild (K-L ≥ 2) hand OA in at least 2 finger joints. The genotypes were determined by PCR-based methods. Data regarding other risk factors were collected by questionnaire. Association between the genotypes/diplootypes and hand OA were studied by logistic regression with SPSS statistical package Version 15.0.

Results: The prevalence of hand OA in at least 2 joints was 42.4% (45.7% in dentists and 54.3% in teachers). The genotype frequencies were in Hardy-Weinberg equilibrium in all of the studied polymorphic loci. There were no statistically significant differences in the frequency of the genotypes and carrier frequencies between different occupational groups, except in the ADIPOQ -10068 loci. The genotype frequencies did not differ significantly between women with or without hand OA. When taking age and BMI into account and stratified by occupation the LEP +19 and -2548 GA-genotypes showed a protective effect compared with the GG- and AA-genotypes (OR 0.47, 95% CI 0.27-0.84, p=0.01; 0.46, 0.25-0.86, p=0.02) respectively in the dentists whereas this effect was not seen in the teachers. On the other hand, the ADIPOQ -10068 GG- genotype increased the risk of hand OA compared to the AA-genotype (2.14, 1.04-4.41, p=0.04) in dentists. Again, this effect was not seen in the teachers. The ADIPOQ rs1501299, rs2241766, and rs17300539 polymorphisms did not show any statistically significant results. The haplotype analyses are currently underway. According to power calculations, this study had 80% power to detect ORs from 1.63 to 2.71 depending on the minor allele frequency (4-47%), based on a two-sided alpha of 0.05.

Conclusions: Our results support the hypothesis that the LEP and ADIPOQ gene polymorphisms may play a role in the etiology of hand OA. There also may be an interaction between the Leptin-related individual susceptibility and hand workload.

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PROGRESSION OR INITIATION OF RADIOGRAPHIC KNEE OSTEOARTHRITIS AND THE INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE: THE JOHNSTON COUNTY OSTEOARTHRITIS PROJECT

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Purpose: There are currently no approved drugs for the treatment or prevention of osteoarthritis (OA), due in part to the complexities of clinical trials in which only a small subset of patients show progression of the disease during the studies. Mechanisms underlying the development or progression of OA are not well understood. Although OA is not a classic inflammatory disease, inflammatory mediators that degrade cartilage have been implicated in its pathogenesis. We previously reported (Attur et al. 2009) that interleukin-1 receptor antagonist gene (IL1RN) variations (SNPs) were associated with knee OA severity. In the present study, Caucasian participants (N=1,173; 38% men; mean age=60 years) in the Johnston County OA Project with 4-11 year follow-up data were selected to evaluate gene variations associated with radiographic knee OA progression or initiation.

Methods: Anterior-posterior standing knee radiographs were obtained with footmat positioning at both time points and read by a single musculoskeletal radiologist for Kellgren-Lawrence grade (K-L 0-4). Progression or initiation of knee OA was defined by an increase in KL grade or decrease in joint space width in at least one knee. For progression of OA, subjects who already had OA (KL ≥ 2 at either knee) at baseline were included in analysis. For initiation of OA, subjects without OA (KL ≤ 1 at both knees) at baseline were analyzed. A broad SNP panel was tested, including multiple genes and dense coverage of the IL-1 gene cluster. Logistic or linear regression with adjustment for age, gender and BMI was used to determine association between IL1RN gene polymorphisms and progression or initiation of knee OA.

Results: Specific SNPs and haplotypes of the IL1RN gene were significantly associated with progression or initiation of knee OA. There are 2 linkage disequilibrium (LD) blocks in the IL1RN gene, and markers in both blocks were significantly associated with initiation and progression of knee OA. Allele C of the IL1RN rs4251961, previously reported to be...
associated with reduced levels of the anti-inflammatory IL-1Ra protein, was associated with progression (OR=2.29, 95%CI:1.17-4.50) or initiation (linear regression, p=0.006) of knee OA. Other IL1RN SNPs within the 2 blocks that were associated with knee OA progression or initiation included rs419598, rs315952, rs9005, rs315931, rs315943, rs1794066 and rs579543. The association between haplotypes in the 1st block and the phenotype was explained by either IL1RN rs4251961 or rs419598, and the haplotype effect of the 2nd block is captured primarily by a single SNP (rs315943). The IL1RN (rs419598/rs315952/rs9005) TTG haplotype, previously shown to be associated with severity of knee OA, was associated with progression and initiation of disease in this cohort study.

Conclusions: These findings validate previous observations pointing to a genetic contribution of the IL1RN gene to knee OA progression and severity. This information could assist in guiding clinical development of new drugs for OA.

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ASSOCIATION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RN) TTG HAPLOTYPE WITH RADIOGRAPHIC KNEE OA SEVERITY IN META-ANALYSIS

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Purpose: Previously we have shown that radiographic severity of knee osteoarthritis is conditional on IL-1RN (CTA haplotype) variation. The objective of this study was to determine association between the common TTG (rs419598-rs315952-rs9005) haplotype of the interleukin-1 receptor antagonist gene (IL-1RN) and incidence and radiographic knee OA severity.

Methods: A meta-analysis was performed on 4 studies with genotype data available for the rs419598, rs315952 and rs9005 IL-1RN polymorphisms. Knee ROA patients from NYUHJD (99 cases, 30 controls), RS-I (n=108 cases, 1283 controls), RS-III (n=152 cases, 1519 controls) and TwinsUK (n=56 cases, 145 controls) were included. Patients from NYUHJD were followed longitudinally for 2 years for progression studies. Genotyping was accomplished (Interleukin Genetics Clinical Laboratory; Waltham, MA, USA; CLIA certified) by polymerase chain reactions (PCR) targeting the sequence surrounding SNPs studied. Multiplexed single base extension (SBE) reactions were performed. RS-I and RS-III comprises men and women of the Rotterdam Study, which is a prospective population-based study on determinants of chronic disabling diseases. TwinsUK participants are white monogygotic and dizygotic twin pairs from the TwinsUK adult twin registry, a group used to study the heritability and genetics of age-related diseases. A meta-analysis was performed using fixed- and random-effects models, including all available data with allele/genotype counts with in total 303 cases with Kellgren-Lawrence (K/L) 3 or 4 compared to 1946 controls with K/L 1 or 2.

Results: In the current study we show that the TTG haplotype of IL1RN, formed by rs419598, rs315952 and rs9005, is significantly associated with radiographic severity of knee OA. In the meta-analysis, the TTG haplotype was associated with radiographic severity in both fixed and random effects model (OR=1.45; 95% CI 1.18-1.78; P=0.004, I²=28%) and (OR = 1.47; 95% CI 1.19-1.95; P= 0.007) respectively.

Conclusion: Common genetic variation in the Interleukin-1 receptor antagonist region (IL-1RN) TTG haplotype formed by rs419598, rs315952, rs9005) predicted high risk for knee OA radiographic severity in a meta-analysis of 4 studies. These genetic markers may be useful in identifying patients more likely to develop severe OA disease.

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INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE VARIATIONS PREDICT THE SEVERITY AND PROGRESSION OF KNEE OSTEARTHRITIS

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Purpose: We have previously shown that carriage of an IL1RN haplotype (CTA) was associated with substantially lower odds of radiographic severity (KL score, JSW) (Ann Rheum Dis. 2010). Now we assessed whether IL1-RN haplotypes predicted disease progression in patients with symptomatic knee OA.

Methods: One hundred seventeen (N=117) patients from NYUHJD who met ACR criteria for knee OA were genotyped for single nucleotide polymorphisms (SNPs) in the IL-1b and IL-1RN genes. Standardized semi-flexed radiographs were taken on 97 patients, in whom we determined progression of both signal and non-signal knee OA, by following change in JSW and KL score between visit 0 and 24 months.

Results: The signal and non-signal knees were analyzed separately. Excluding the KLAS knees at baseline, decreases in JSW ranged from zero to 3.7 mm over the 24-months. The study population was divided into tertiles based upon the distribution of observed joint space narrowing (JSN): lowest tertile < 0.4 mm; mid-tertile 0.5 - 1.2 mm; top tertile >1.3mm. For KL scores, a change in one grade was considered to represent progression. As shown in the Table 1, the CTCCG haplotype in the IL-1b gene was strongly associated with radiographic knee OA progression based on both JSW and KL score (OR = 4.1406 [p=0.0153], OR = 3.715 [p=0.0147]) and (OR = 3.1692 [p=0.0193], 1.492 [p=0.0176]) for the signal and non-signal knees, respectively However, we also observed that OA patients with IL1-RN (CTA haplotype) showed decreased risk for radiographic progression based on KL score in the signal knee (OR=0.366 [p=0.0137]). In contrast, patients with the IL-1RN TTG haplotype exhibited increased risk for radiographic progression based on KL score and JSW (OR=1.78 [p=0.0389]) and OR=2.6544 [p=0.048] and JSW OR=2.151 (p=0.0439) in both signal and non-signal knees respectively. In addition, VAS pain was higher in the TTG compared to the CTA group at both baseline and 24 months (p-value<0.01) (Table 1).

Conclusion: IL-1RN gene family polymorphisms predict the likelihood of progression in patients with radiographic knee OA.

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IDENTIFICATION OF THE GENETIC DETERMINANTS INVOLVED IN THE INITIATION OF OA

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Purpose: Osteoarthritis is the most prevalent form of arthritis and is characterized primarily by the degeneration of cartilage and subchondral bone remodeling. Cartilage is maintained by a delicate balance of cartilage destruction and repair; however, how this is shifted towards destruction is largely unknown. The aim of this study is to investigate the genetic determinants involved in the early changes in cartilage homeostasis and