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DNA METHYLATION OF SPECIFIC CPG SITES IN THE MMP-13 PROMOTER REDUCES ITS ACTIVITY IN HUMAN CHONDROCYTES: LUCIFERASE ASSAY WITH CPG-FREE REPORTER VECTOR

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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(s), respectively. Each promoter construct was inserted in the pCpG-Luc backbone and treated with DNA methyltransferase (SssI) to methylate all CpG sites in the inserted promoter sequences. Each SssI-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 SssI treatment, which indicates that the SssI 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 SssI treatment, whereas the -86/+14 MMP13 promoter activity was not significantly affected by methylation.
Conclusions: The 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 aberrant expression in OA chondrocytes.

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GENETIC POLYMORPHISMS OF LEPTIN AND ADIPONECTIN GENES AND SUSCEPTIBILITY TO HAND OSTEOARTHRITIS IN FINNISH WOMEN

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Purpose: Osteoarthritis (OA) is a common disease characterised by degeneration of the cartilage of synovial joints. Available evidence suggests that genetic factors and overweight have a major role in the etiology of OA. Our aim was to study the role of the Leptin (LEP) and Adiponectin (ADIPOQ) genes in the individual susceptibility to hand OA in Finnish women. Two single nucleotide polymorphisms (SNPs) ("-2548" rs7799039 and "+19" rs2167170) in LEP and four SNPs ("+276" rs1501299, "+46" rs2241766, "-10068" rs182052 and "-11391" rs17300539) in ADIPOQ gene were studied. Since the enzymes encoded by these genes play a critical role in the regulation of body weight and energy homeostasis, they may also influence the development of OA.
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/diplotypes 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 six studied polymorphic loci. There were no statistically significant differences in the frequency of the genotypes and carriage rates between the 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 Johnson 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$\geq$2 at either knee) at baseline were included in analysis. For initiation of OA, subjects without OA (KL$\leq$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