IX (COL9A1), IL1B, and matrix metalloproteinase-13 (MMP13) gene expression were measured using SYBR Green-based qRT-PCR and were correlated with methylation status analysed by pyrosequencing methodology.

Results: Hypoxia resulted in a >50-fold and >10-fold increase in relative expression of COL9A1 and IL1B respectively. This was inversely correlated to the DNA methylation status of these genes. Expression of MMP13 was reduced at 2% oxygen tension in control cells. Relative expression of MMP13 increased in cells stimulated with IL-1β and 5-aza-dC in normoxic conditions, and this effect was eliminated at low oxygen tension although no correlation with methylation status was observed.

Conclusions: These findings demonstrate a role for hypoxia in the regulation anabolic and catabolic gene expression and the influence of changes in DNA methylation. These results further support the role of epigenetics in OA and critically, highlight the complex relationship between the physiological environment of cartilaginous cells and the osteoarthritic process with implications for therapeutic intervention and our understanding of OA pathophysiology.

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VALIDATION OF THE ASSOCIATION OF A FUNCTIONAL MICROSATELLITE IN MACROPHAGE MIGRATION FACTOR WITH HIP OA

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Purpose: Microsatellites are not amenable for high-throughput genotyping and have been excluded from GWAS. However, several microsatellites have been found to affect expression of nearby genes and a couple of them, in the asporin and BMP5 genes, have been associated with susceptibility to OA in multiple studies. In a recent exploration of functional microsatellites we found association of the -794 CATT microsatellite in the MIF gene with hip OA in 1782 patients compared with 1878 healthy controls of European ancestry. Replication in other patient collections was hampered by the lack of a method to obtain the genotypes without actually performing the laboratory tests. Therefore, we aimed to develop an imputation methodology for this microsatellite using the genotypes of SNPs in linkage disequilibrium and to apply it for validation.

Methods: In our previous study of the MIF microsatellite, we had included 1090 samples that were also in the arcOGEN GWAS. Genotypes of the 130 SNPs in the linkage disequilibrium region surrounding the microsatellite in these samples were used as source of haplotype information. Imputation was done with Impute2 with modifications allowing estimation of the probabilities for the number of copies of each microsatellite allele and their posterior combination. Performance of this procedure was evaluated in 10 replicates of training, with 90 % of the samples, and testing of accuracy in the remaining 10 % of the samples with known microsatellite genotypes. Once concordance of imputed genotypes with tested genotypes was established, the approach was applied for the imputation of the MIF microsatellite in 5667 population controls from Wellcome Trust Case-Control Consortium (WTCCC) and in 2466 hip OA cases from arcOGEN (all of them of European UK ancestry). In addition, we have validated the functional effect of the MIF microsatellite on the plasma levels of MIF in 361 healthy control samples by ELISA (R&D Systems) from subjects that were either homozygous for the 5 repeat or the 6 repeat alleles. MIF microsatellite allele frequencies were compared with POWERMARKER and differences in plasma levels of MIF were analyzed with ANOVA using Statistica 7.0 (StatSoft).

Results: There was a microsatellite allele with frequency lower than 1% that was not included in the imputation. The genotypes of the other three alleles were imputed with sufficient accuracy (91.6 %) and call rate in the reference samples (98.8). However, other microsatellites showed that the procedure will require further refinements to attain this performance for microsatellites with more alleles. Application of this procedure to the WTCCC and arcOGEN samples produced genotypes for 99.0 % of the samples. Comparison of the allelic frequencies showed

significant differences between hip OA and controls in women (Table 1) following the same pattern found in our previous study, with the five repeats allele less frequent in the patients than in the controls (OR = 0.88, [95% CI] 0.79 to 0.98, P = 0.018). However, no difference was appreciated in men or between control women and men as it was in our previous study. This contrast between studies could be attributed to the use of OA-free controls in the previous study and population controls here.

Analysis of MIF in plasma of healthy controls showed higher levels in the homozygous for the 5 repeat allele (3.6 ng/mL) than for the 6 repeat allele (2.7 ng/mL; P = 0.00025) following the direction previously reported by other authors and without differences between women and men.

Conclusions: A new method to impute genotypes of microsatellites with three alleles based on SNP genotypes has been developed. It has shown good call rate and accuracy. This procedure allowed us to validate the association of the MIF microsatellite with hip OA in a large number of cases and controls. Concordance with our previous study was obtained in women, but not in men. Higher MIF levels were found in the subjects with the hip OA protective allele. These results should contribute to define the role of MIF, an important cytokine, in the OA pathology.

Comparison of the allele frequencies of the MIF microsatellite

	Controls				THR			
	Women		Men		Women		Men	
Allele	Count	Freq	Count	Freq	Count	Freq	Count	Freq
5	1402	25.4	1439	25.3	671	23.1	474	24.3
6	3453	62.5	3574	62.7	1853	63.7	1237	63.5
7	669	12.1	685	12.0	386	13.3	237	12.2
Total	5524		5698		2910		1948	

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ALTERNATIVE SURVIVAL ANALYSIS METHODS TO ESTIMATE TIME TO REVISION FOLLOWING HIP AND KNEE ARTHROPLASTY: CAN THE KAPLAN-MEIER METHOD COMPETE?

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Purpose: With increased longevity and frequency with which joint replacements are being performed on younger, more physically active patients, it has become common for patients to outlive the life of their prosthesis and require a revision. Measuring the cumulative incidence of revision (i.e., revision rate) provides a measure of the rate of failure of joint replacements and can be used to project future demand for revisions. Due to varying patient follow-up times and censoring, survival analysis is required to estimate revision rates. The Kaplan-Meier (KM) method is the most commonly applied survival analysis method. However, it does not account for the competing risk of death and consequently overestimates the cumulative incidence of revision. This is problematic given the high rate of competing risks, especially in older patients receiving joint replacements. Although alternative methods that account for competing risks have been developed, they are rarely used within the arthroplasty literature or among joint replacement registries. Our objective was to assess alternative methods for estimating the cumulative incidence of revision through application to population-based cohorts. We evaluated these methods