BtgI did not show any significant ($p>0.05$) association with these clinical features nor with WOMAC pain. The association of CALM-1 gene with radiological severity was significant in all the three SNPs studied; CALM-1 ApaLI ($F= 9.36$, $p<0.001$), CALM-1 ApeKI ($p<0.001$) and CALM-1 rs2300496 ($F= 3.96$, $p<0.001$). Further, the Tukey test showed significantly ($p<0.001$) different and higher mean radiological severity in CT and TT genotypes of CALM-1 ApaLI as compared to CC genotype; in CALM-1 rs2300496 the mean radiological severity in CC genotype was significantly ($p<0.05$) different and higher as compared to AA genotype and in CALM-1 ApeKI, the mean radiological severity in TG genotype was significantly ($p<0.001$) different and higher as compared to TT genotype. However GDF-5* rs 331383 and ESR-α BtgI rs* 2228480 did not show any significant association with mean radiological severity.

Conclusions: In this study we found that in cases of knee osteoarthritis, GDF-5* rs 331383 and CALM-1 ApekI genotype had significant association with WOMAC pain; all the three SNPs of CALM-1 genotype had significant association with radiological severity and ESR-α BtgI genotype did not show any association with either clinical or radiological features. These finding validate our contention that genetic variants influence knee pain and radiological changes in knee osteoarthritis and different genotypes being responsible for the severity in these two parameters may be responsible for the clinical and radiological discordance being reported by several authors.

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DIFFERENTIAL DNA METHYLATION AND EXPRESSION OF INFLAMMATORY AND ZINC TRANSPORTER GENES DEFINES SUB-GROUPS OF OSTEOARTHRITIC HIP PATIENTS

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Purpose: Using the Infinium HumanMethylation450 BeadChip (Illumina), which measures the level of DNA methylation at approximately 480,000 CpG sites throughout the human genome, we have previously shown that the cartilage DNA methylome delineates two groups, or clusters, of osteoarthritic (OA) hip patients. These two clusters, which we designated clusters 1 and 2, are characterized by differential methylation of genes involved in inflammation. In that study we had also analyzed a group of age-matched patients who were free of hip OA but who had undergone hip replacement due to a neck of femur (NOF) fracture. OA hip cluster 1 was more similar at the methylation level to the NOF patients than was OA hip cluster 2. In this study we aimed to investigate the two OA hip clusters in more detail to ascertain what are the key methylation and gene expression features that distinguish them.

Methods: Using our HumanMethylation450 BeadChip data we searched for CpG sites that were significantly differentially methylated between OA hip cluster 2 and both OA hip cluster 1 and the NOF patients (Kruskal-Wallis test, $p<0.05$, 10% or greater difference in methylation). Gene ontology (GO) term analysis was used to identify pathways hypo or hyper methylated. Gene expression was measured by real time quantitative RT-PCR and relative to the housekeeping gene 18s rRNA.

Results: We identified 10071 differentially methylated loci (DMLs), of which 4313 were hypomethylated and 5758 were hypermethylated in OA hip cluster 2 compared to both NOF and OA hip cluster 1. GO term analysis revealed a striking enrichment of terms related to inflammation and immunity. Cluster 2 was characterized by the promoter hypomethylation and increased expression of inflammation-associated genes including TNF, IL6, CXCR2, CCL5, IL1A and CCL2 (all p-values <0.01). Expression of these genes inversely correlated with methylation levels at the differentially methylated CpG sites. Recently it has been shown that elevated intracellular zinc levels are involved in the up regulation of metalloproteinases in OA and that inflammatory factors such as IL1 and IL6 regulate the ZIP group of zinc transporters. We found that the increase in expression of the inflammation-associated genes in hip cluster 2 was accompanied by an increase in the expression of several of the zinc transporter genes including ZIP3, ZIP4, ZIP7, ZIP8, ZIP11 and ZIP14 (all p-values <0.05). Furthermore, ZIP4, ZIP7, ZIP11 and ZIP14 were differentially methylated between our two OA hip clusters (all p-values <0.05). In addition the zinc responsive transcription factor MTF1 was also up-regulated in cluster 2 ($p<0.001$), which was accompanied by an increase in the expression of its downstream targets the metalloproteinase genes MMP13 and ADAMTS5 (both p-values <0.001).

Conclusions: We have identified a sub-group of OA hip patients that is epigenetically and transcriptionally characterized by a cartilage inflammatory phenotype with concurrent differential expression of zinc regulators. The emerging role of zinc in the induction of matrix-degrading enzyme expression in chondrocytes has led to the suggestion that zinc regulation could offer therapeutic opportunities in OA. Our data supports this suggestion but very clearly highlights that this may be particularly applicable to a sub-group of OA individuals. The identification of sub-groups therefore enhances stratified phenotyping of OA patients and has important implications for future therapeutic applications. For example, the administration of anti-inflammatories may be more efficacious for patients who fall into cluster 2 than those who fall into cluster 1.

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GENOME-WIDE DNA METHYLATION STUDY OF HIP AND KNEE CARTILAGE REVEALS EMBRYONIC ORGAN AND SKELETAL SYSTEM MORPHOGENESIS AS MAJOR PATHWAYS INVOLVED IN OSTEOARTHRITIS

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Purpose: The objectives of this study were to describe the genome wide DNA methylation changes in hip and knee osteoarthritis (OA) and to identify novel genes and pathways involved in OA by comparing the DNA methylome of the hip and knee osteoarthritic cartilage with those of healthy individuals.

Methods: OA cartilage samples were collected from the tibial plateau and femoral head articular surfaces of patients with severe OA undergoing total hip/knee replacement surgery. Healthy cartilage samples were obtained from individuals with femoral neck fractures, but no OA. Chondrocyte DNA methylation was assayed by Illumina Infinium HumanMethylation450 BeadChip array, allowing for the analysis of >480,000 CpG sites. A sub-analysis was also done to compare hip and knee OA. An independent sample T-test was conducted for each CpG site and those sites with at least 10% methylation difference and a P-value of <0.0001 were selected as differentially methylated regions (DMR). DAVID v6.7 was used for the functional annotation clustering of the DMR genes.

Results: The study population comprises 5 patients with hip OA, 6 patients with knee OA and 7 hip samples from healthy individuals. The comparisons of hip, knee and combined hip/knee OA patients with controls resulted in 26, 72, and 103 DMRs, respectively. The comparison between hip and knee OA revealed 67 DMRs. The overall number of the sites after considering the overlaps was 239, among which 151 sites were annotated to 145 genes. 28 of these genes were reported in previous methylation studies of OA. The functional annotation clustering of the identified genes revealed clusters significantly enriched in skeletal system morphogenesis (bonferroni corrected p=8.4e-9) and development (bonferroni corrected p=9.0e-6), embryonic organ (bonferroni corrected p=1.4e-5) and skeletal system morphogenesis (bonferroni corrected p=9.9e-7), as well as Homeobox genes (bonferroni corrected p=3.1e-4). A sub-analysis after the removal of the genes differentially methylated between hip and knee OA highlighted the same pathways excluding Homeobox genes.

Conclusions: We demonstrated a number of CpG sites and genes across the genome differentially methylated among OA patients and controls, a remarkable portion of which seem to be involved in potential etiologic mechanisms of OA. Genes involved in skeletal developmental pathways and embryonic organ morphogenesis may be a potential area of concentration for future osteoarthritis genetic/epigenetic research.

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GENOME-WIDE DNA METHYLATION PROFILING OF OSTEOARTHRITIC ARTICULAR CARTILAGE

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Purpose: The aim of this study was to characterize the genome-wide DNA methylation profile of cartilage from knee joints obtained from patients with primary osteoarthritis (OA) who underwent arthroplasty, and providing the first comparison of DNA methylation among the cartilage representing early (outer lateral tibial plateaus, oLT),