Results: Common among all impacted groups in comparison to CTRL groups at every time point was the increased gene expression associated with the IPA canonical pathway/functional category "Role of Osteoblasts, osteoclasts and chondrocytes in Rheumatoid arthritis" containing genes such as *BMP2, BMP4, MMP13, FRZB, TNSF11, and ITGP3* . The biological networks associated with impacts differed with impact magnitude and time. After 24 hours, gene networks associated with "Skeletal and Muscular system development and function, tissue development, connective tissue disorders" (*MATN3, MMP13, FGF18, DMP1, BMP2, BMP4, COL9A1, TGFB3*, etc.) were active after 17 MPa impacts as compared to the biological network "Cellular development, Cellular growth and proliferation, Skeletal and muscular system development and function" (*COL10A1, VCAN, WISP1, TGFB3*, etc.) which was differentially expressed after 36 MPa impacts. 36 MPa impacts also changed the expression of genes in the "Granulocyte adhesion and diapedesis" (*CCL20, CXCL14, CXCR2, PECAM1*, etc.) functional category indicating a greater inflammatory response. Three days after impact, both impact groups showed differential expression of genes in the "Cell cycle, Cellular Assembly and organization, DNA replication, recombination and repair" group represented by genes such as *CDK1, CENPE, CENPF, MATN3*, among others. Similar biological groups were also identified 7 days after impact in the 17 MPa group with representative genes such as *BMP4*, *MMP13*, *OMD*, *COL10A1*, *COL1A1*, and *DMP1*. In the 7-day, 36 MPa group, we observed markers such as *IBSP*, *BAMBI*, *FGF7*, *SP7*, and *POSTN*.
Conclusions: While we observed differential expression of catabolic genes such as *MMP13, MMP3* and anabolic genes like *COL2A1, COL1A1*, and *MATN3* in most impact groups and time points, we found that each condition was also characterized by the expression of specific sets of genes. 24 hours after impact we noticed the expression of pro-inflammatory genes (*e.g. IL-6*) following the high impact force. Three days following impact, the top functional pathway identified by IPA in both the 17 MPa and the 36 MPa groups contained genes involved in cell cycle and DNA repair, suggesting the activation of a common reparatory mechanism. At 7 days gene expression by cells in 17 and 36 MPa impact samples diverged between chondrogenic/hypertrophic and osteogenic functional groups. This difference may reflect a more advanced disease state in the high impact group, or a different response altogether. Many of the genes identified in this study were previously identified as markers of OA, indicating that further validation and functional analyses of novel loci identified here could lead to the development of new PT-OA therapeutic candidates.

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GENETIC ASSOCIATION ANALYSIS OF RADIOGRAPHIC HIP OSTEOARTHRITIS WITH ESTABLISHED LOCI FOR BONE MINERAL DENSITY: DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Previous studies have shown that high bone mineral density (BMD) is associated with an increased risk of knee and hip osteoarthritis. We recently completed an analysis evaluating the association of 56 established BMD loci (64 SNPs) for their association with radiographic knee osteoarthritis (rKOA) and identified four genetic variants associated with higher BMD that were also associated with higher odds of rKOA. With this in mind we conducted genetic association analysis to determine the relationship between these 56 BMD loci and radiographic hip osteoarthritis (rHOA).
Methods: The current analysis was conducted on European-American participants from the Osteoarthritis Initiative with genome wide genotyping (Illumina 2.5 M array) and pelvis radiographs obtained at the baseline and 48 month visits. Pelvis images were read for radiographic features (IRFs) of hip OA using the OARSI atlas, and hips were classified as definite rHOA (Croft grade $\geq$2 or definite osteophytes or definite JSN), possible rHOA (IRFs present but not definite, e.g. isolated grade 1 osteophyte or joint space narrowing) or normal. Cases had at least one hip with definite rHOA at either time-point and hips replaced during follow-up with an adjudicated diagnosis of OA or degenerative arthritis. Controls had bilateral normal hips at baseline and, if radiographs were available, at 48 month follow-up. Using these definitions we identified 350 cases and 2294 controls with genotyping data for analysis. In two sensitivity analyses we included subjects with possible RHOA as cases and used as controls only those who had a 48-month pelvis films. Association analyses included adjustments for age, gender, BMI and principal components to correct for fine-scale population substructure.
Results: We identified one variant (rs7217932) near the SOX9 locus that had a nominal association with rHOA ($p = 0.03$, OR = 1.20). The allele associated with higher BMD was associated with higher odds of rHOA. Of note, this variant was one of the few BMD loci associated with BMD in the hip (femoral neck) but not the lumbar spine. The association did not remain significant when subjects with possible OA were included as cases ($p = 0.13$, OR = 1.10) but did when we required 48-month images for controls ($p = 0.05$ OR = 1.18). None of the SNPs we identified from our association analysis of rKOA were associated with rHOA ($p > 0.05$). A BMD risk allele score based on 62 independent loci was not significantly associated with rHOA risk.
Conclusions: Despite a limited sample size we identified a nominal association between a variant associated with femoral neck BMD and rHOA, however this association does not remain significant after accounting for multiple testing. Replication analysis in additional cohorts is needed to confirm these findings, however the SOX9 locus may play a role in osteoarthritis in addition to BMD at the hip.

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LSD1-MEDIATED DEMETHYLATION OF HISTONE H3 LYSINE 9 CONTRIBUTES TO INTERLEUKIN 1-INDUCED MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 EXPRESSION IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Purpose: Microsomal prostaglandin E synthase-1 catalyzes the terminal step in the biosynthesis of PGE2, which plays a critical role in the pathophysiology of osteoarthritis. To investigate the role of histone H3 (H3K9) methylation in interleukin-1b (IL-1)-induced microsomal prostaglandin E synthase-1 (mPGES-1) expression in human osteoarthritic (OA) chondrocytes.
Methods: Chondrocytes were stimulated with IL-1 and the expression of mPGES-1mRNA was analyzed using real-time reverse transcriptase-polymerase chain reaction. H3K9 methylation and the recruitment of the histone demethylase LSD1 to the mPGES-1 promoter were evaluated using chromatin immunoprecipitation assays. The role of LSD1 was further evaluated using the the amino oxidase inhibitor tranylcypromine (a potent inhibitor of LSD1 activity).
Results: Treatment with IL-1 induced mPGES-1 expression in a time dependent manner. The induction of mPGES-1 expression by IL-1 was associated with H3K9 demethylation at the mPGES-1 promoter. These changes were concomitant with the recruitment of the histone demethylase LSD1. Treatment with tranylcypromine inhibited IL-1-induced H3K9 demethylation as well as IL-1-induced mPGES-1 expression.
Conclusions: These results indicate that H3K9 demethylation by LSD1 contributes to IL-1-induced mPGES-1 expression and suggest that this pathway could be a potential target for pharmacological intervention in the treatment of OA and possibly other arthritic diseases.

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A CASE CONTROL STUDY TO EVALUATE THE ROLE OF GENETIC AND ENVIRONMENTAL RISK FACTOR IN DEVELOPMENT AND PROGRESSION OF OSTEOARTHRITIS KNEE

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Purpose: The present study was initiated to investigate the interaction of SNPs in Estrogen receptor-α (ESR-α), Calmodulin-1 (CALM-1) and Growth differentiation factor-5 (GDF-5) gene with osteoarthritis knee (KOA); correlation of these genetic variants with envoirmnental factor such as living standards and occupation.
Methodology: In a case-control study, 300 cases with KOA and an equal number of age matched healthy controls were included. Cases were diagnosed using the American College of Rheumatology (ACR) guidelines of knee osteoarthritis (KOA). Blood was drawn for genomic DNA isolation; polymerase chain reaction coupled restriction fragments length polymorphism (PCR-RFLP), TaqMan assay were carried out to

identify the SNPs. The haplotype analyses (haplotype frequency estimation and pair wise linkage disequilibrium between the SNPs) were carried out using Haploview (www.broad.mit.edu/mpg/haploview/). All statistical analysis was performed with the SPSS software package (version 16.0 for windows; SPSS Chicago, IL).

Results: The variant genotype of ESR-α, CALM-1 and GDF-5 genes were found to be present at relatively higher frequency in cases than in the controls. Risk increased in cases that carried combination of variant genotypes of ESR α (Btg-AA) and GDF-5 (TT); CALM-1 (ApeKI-TG) and GDF-5 (TT); CALM-1(ApeKI-TG) and ESR α (Btg-AA) resulting in 4.00-6.00 fold elevated risk to KOA. The haplotype C-G-G and T-G-G of ESR- α gene reduced the risk to OA. In contrast the haplotype T-G-C containing variant of all three polymorphism of CALM-1 gene was over represented in the cases, increasing the risk to 3.5 fold (OR = 3.49, 95% CI = 1.67–7.27, p = 0.000). A significant association of this variant genotype was also found with clinical scores of KOA (VAS, WOMAC). Significant increased risk of TT than CC was found among moderate occupational status, the risk of TT (OR = 2.07, 95% CI = 1.18-3.62, p = 0.01) was higher than CC of GDF-5 (BsiE1) genotype among MIG living standard in cases compared to controls. Likewise, ESR-α, Btg-I risk genotype also shows significant with moderate occupation (OR = 2.34, 95% CI = 1.26-4.33, p = 0.007) and MIG living standard (OR = 1.48 95% CI = 1.01–2.19, p = 0.04). However, the risk of GT (OR = 3.15, 95% CI = 1.48–6.68, p = 0.003) was significantly higher than TT of ApeKI genotype among MIG living standard in cases as compared to controls.

Conclusion: The results suggest that GDF-5, ESR-α and CALM-1 gene polymorphism is associated with Knee OA and SNP-SNP interaction influences the development of Knee OA. Likewise, association of these SNP with clinical scores has again demonstrated that these genetic markers could be effectively used for predicting development and progression of osteoarthritis. Moderate occupation and MIG living standard are higher prone to develop OA on the basis of GDF-5 and ESR-α, Btg-I risk genotype. It may be hypothesized that the presence of variant GDF-5 (BsiE1), ESR- α, Btg-I genotypes increases OA risk in moderate occupation and MIG living standards in our population.

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THE MTDNA HAPLOGROUPS ASSOCIATE WITH DIFFERENT METHYLATION PATTERNS IN ARTICULAR CHONDROCYTES

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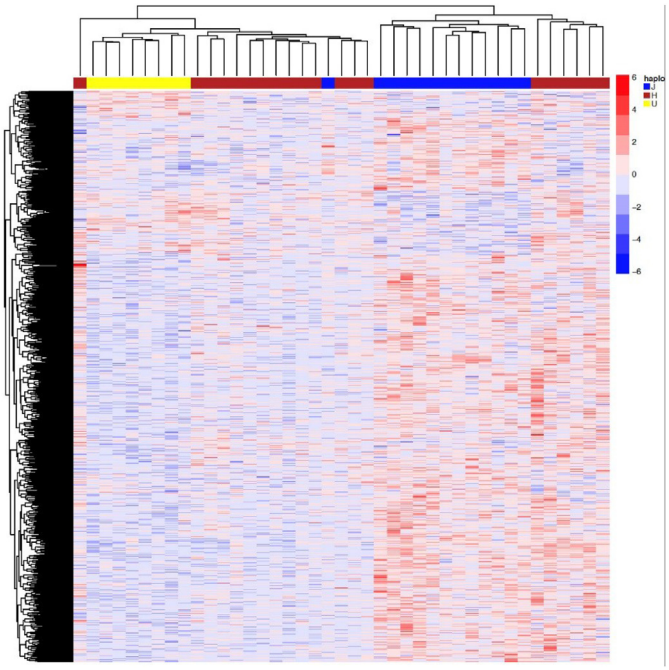
Purpose: To analyze the influence of the mitochondrial background on the methylation patterns of articular chondrocytes

Methods: DNA methylation profiling was performed using the Infinium HumanMethylation27 beadchip. Previously, cartilage isolated DNA from 41 cartilage samples (13 from haplogorup J, 20 from haplogroup H and 8 from haplogroups U) was bisulfite-modified using EZ DNA methylation kit and hybridized according to the manufacturer's instructions. DNA methylation M-values were obtained and further compared between haplogroups using ANOVA and adjusting for cofounder effects of age, gender, disease status, and hospital origin. Post-hoc analysis was performed for analysing haplogroup pairwise differences. Enrichment in biological process and molecular function was tested by Gene set enrichment analysis using a conditional hypergeometric test. All statistical analyses were conducted in R software.

Results: ANOVA analysis showed a total of 1929 CpG probes with a p-value under 0.05 (Fig. 1); post-hoc analysis of ANOVA allowed us to identify 560 significant probes (adjusted p-value <0.05) between haplogroup H and haplogroup J, 440 significant CpGs for haplogroup H versus haplogroup U comparison, and a total of 1084 significant probes for the remaining pairwise comparison (haplogroup J versus haplogroup U). Gene set enrichment analysis showed a total of 38 biological processes and 3 molecular function processes significantly altered. DNA damage response (p-value 6.332061e-05), positive regulations of cell cycle process (p-value 2.491685e-06), RNA biosynthetic process (p-value 9.502843e-07) as well as mitochondrial electron transport, NADH to ubiquinone (p-value 9.111800e-04) were enhanced in carriers of the mtDNA haplogroup J.

Conclusions: The genome-wide methylation analysis shows a distinct epigenetic profile in articular chondrocytes attending to their mitochondrial background. The role played by the mtDNA haplogroups on Spanish patients with OA could be mediated by this particular epigenetic profile.



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VITAMIN D RECEPTOR GENE POLYMORPHISMS MODULATE THE CLINICO-RADIOLOGICAL RESPONSE TO VITAMIN D SUPPLEMENTATION IN KNEE OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is one of the most frequent causes of pain, loss of function and disability in the elderly. Knee OA is particularly common in Indian patients and there is currently no therapy that can slow its progression. A beneficial effect of vitamin D supplementation on symptomatic improvement in OA knee patients has recently been reported. Vitamin D receptor (VDR) gene plays an important role in regulation of bone mass and in human articular chondrocytes of cartilage. In view of the importance of the vitamin D in bone development the abnormalities in the VDR gene are viewed as potential contributors to OA. Therefore, this study was planned as a pilot study to find out to whether the clinico-radiological response to vitamin D was modulated by VDR gene polymorphisms.

Methods: This randomized placebo-controlled trial recruited 103 KOA cases as per American College of Rheumatology (ACR) guideline having vitamin D insufficiency (25(OH)D ≤ 50 nmol/L). Enrolled cases were randomly allocated in two groups to receive placebo (51) and vitamin D (52). Primary outcome measures: pain and functional disability which were recorded by knee specific WOMAC index and secondary outcome measure were radiological features (joint space width and osteophytes). The serum levels of vitamin D were assessed by a method Enzyme Linked Immunosorbent Assay using IDS, UK kit. Detection of VDR polymorphisms (Taq1 & Apa I) were done by PCR-RFLP technique. 25(OH)D levels, clinical and radiological features were recorded at baseline and at one year follow up.

Results: At one year, in vitamin D supplemented group, TT genotype of TaqI polymorphism showed the maximum increment in the level of 25(OH)D in comparison to Tt and tt genotype whereas in placebo group it remained same. No such association was observed for ApaI polymorphism. In clinical features, pain and functional disability improved in each genotype although least in tt genotype in vitamin D supplemented group whereas in placebo group it significantly worsened in Tt and tt genotype. Total WOMAC scores improved in each genotype of vitamin D supplemented group and was significant in case of Tt and TT