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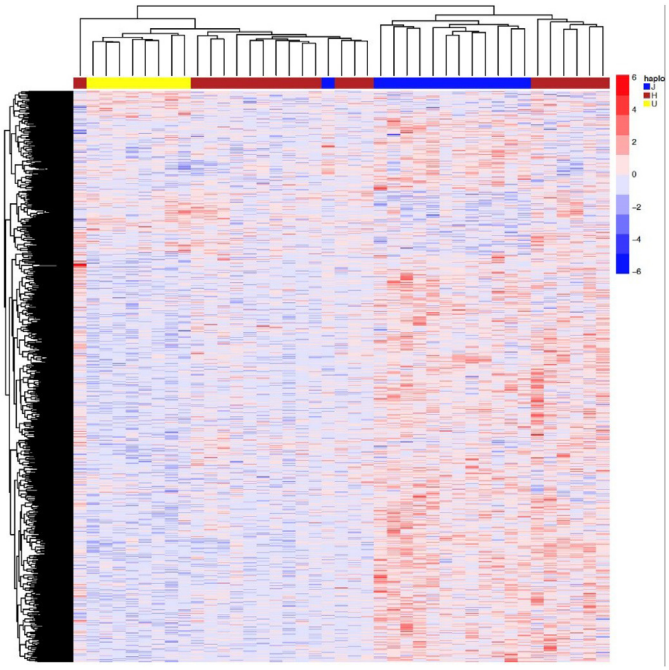
Purpose: To analyze the influence of the mitochondrial background on the methylation patterns of articular chondrocytes

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