UK (n = 352) and Greece (n = 157) were also studied. Controls were selected to exclude clinical OA symptoms and were older than 44 years (from Greece, n = 183), or of 54 years (from Spain, n = 294, and the UK n = 454). Two ADAMTS5 non-synonymous SNPs identified by the software SIFT as probably damaging the enzyme function, rs226794 in exon 7 (P692L) and rs2830585 in exon 5 (R614H), were studied.

Results: SNP R614H did not show allelic frequency differences between controls and any of the three OA patient groups in the Spanish sample collection and was not studied further. The minor allele of SNP P692L, allele T, showed a trend to a decreased frequency in Spanish TKR patients (11.5% vs 8.2% in controls, p = 0.06). The Greek TKR samples did not show a significant difference (9.8% in TKR vs 8.0% in controls), neither so the UK TKR samples (10.9% in TKR vs 11.2% in controls). The combined analysis did not show any effect of the P692L SNPs (Mantel-Haenszel O.R. = 0.88, 95% C.I: 0.7-1.1, p = 0.25).

Conclusions: A first investigation of putative important SNPs in the main in vivo aggrecanase, ADAMTS5, has failed to show any relevant effect on OA susceptibility.

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PROMOTER SQUELCHING AS A TOOL TO DOWNREGULATE THE INFLAMMATORY CASCADE IN CANINE OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) characterised by cartilage degradation is a severe joint disorder affecting animals and humans alike. Canine species provide a useful model to study this disease process as it mimics the human form of OA both clinically and pathophysiological. It has been proven that IL-1β and TNFα are the inflammatory cytokines that accentuate the disease process. The absence of Th2 cells in the inflammatory synovial fluid points out that this disorder could be corrected by installing a balance between the Th1 and Th2-response. Previous work has shown that Interleukin 4 (IL-4) downregulates the inflammatory pathway and therefore is a good candidate to combat OA in canine species.

Methods: Present work focuses on simulating the OA disease process in-vitro using canine chondrocytes in monolayer and 3D cell culture systems. Our approach is based on dosage regulated IL-4 expression under cell culture conditions. To achieve this we are using promoter squelching as a method to self regulate IL-4 expression involving the canine COX-2 promoter which we partially sequenced.

Results: Our experiments have shown that canine chondrocytes can be passaged till the 7th generation without any change in morphology. We could also show by qPCR that these cells over express inflammatory cytokines such as IL-6, IL-8 and IL-18 in comparison to the house keeping gene GAPDH upon exogenous stimulation with either human recombinant IL-1β or TNFα.

Conclusions: We report on the optimization of our canine in-vitro model for OA therapy which includes IL-1β and TNFα synthesis from canine PBMC as well as a reporter assay for COX-2 promoter activity for our indigenously designed vector. Efforts are on the way to increase expression levels of canine IL-4 in cell culture.