75 GENERATION OF A TRANSGENIC KNOCK-IN MOUSE MODEL EXPRESSING ONLY THE IIA ISOFORM OF TYPE II PROCOLLAGEN
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Purpose: To generate a mouse model where alternative pre-mRNA splicing of the type II procollagen gene (COL2A1) is inhibited. Normally, chondroprogenitor cells synthesize predominantly exon 2-containing mRNA isoforms of COL2A1 (type IIA and IID) while differentiated chondrocytes mainly generate COL2A1 mRNA isoforms devoid of exon 2 (type IIB). The biological significance of this splicing switch with respect to cartilage/skeletal development and maintenance is not known. To address this issue, we synthesized a transgenic mouse expressing predominantly the IIA isoform of COL2A1 by altering the 5′ splice site sequence of exon 2.

Methods: A 4nt mutation was created at the 5′ splice site of exon 2 in a COL2A1 mini-gene construct. This mutation converts exon 2 from a weak splice site to strong consensus splice site. Wild type and mutant mini-genes were transfected into chondrocyte cell lines and spliced isoforms were analyzed by RT-PCR. To generate the mouse model, a targeting vector was made containing one short arm (~2kb) and one long arm (~7kb including the 4nt splice site mutation) of the Col2a1 locus. Targeting vector was electroporated into ES cells (129S6/SvEvTac) and Southern blotting identified homologously-recombined clones. Positive ES clones were injected into C57BL/6 blastocysts to generate chimeric mice. Further breedings were done to generate homozygote mice devoid of the neomycin cassette by crossing to Cre-expressing mice (EIIa-Cre). Resulting heterozygote mice were bred to generate WT, +/− and −/− mice of the neomycin cassette by crossing to Cre-expressing mice (EIIa-Cre). Southern blotting identified homologously-recombined clones. Positive clones were injected into C57BL/6 blastocysts to generate WT, +/− and −/− mice of the Col2a1 locus. A 4ntd mutation was created at the 5′ splice site of exon 2. Preliminary histological analyses suggest that cartilage development is normal even though there is persistent and high expression of IIA propeptide protein in these mice. Future studies will involve analyzing whether other cartilage matrix components have been altered as a result of changing type II procollagen splicing.

Results: Splicing of the mutant COL2A1 mini-gene by cells in vitro resulted in synthesis of predominantly the IIA isoform. Homologous recombinants were injected into C57BL/6 blastocysts to generate chimeric mice. Chimeric mice were generated following blastocyst injections of ES clones and germline transmission was confirmed by PCR. Homozygote mice were generated at the expected Mendelian ratio. No overt phenotype was noted in the +/− and −/− mice compared to WT littermates. RT-PCR confirmed that IIA is the predominant isoform produced in epiphyseal cartilage from homozygote mice while WT mice produced mainly IIB mRNA at all three time points (P7, P14, P28) as expected. Immunohistochemistry showed a dramatic increase in localization of the IIA propeptide in proximal tibial cartilage of +/− and −/− mice when compared to WT. Western blotting showed that procollagen processing occurs in the matrix of +/− and −/− mice at P7 although levels of unprocessed proc1 (IIA) and pN-α1(I)IIA were also identified.

Conclusions: Global knock-in mice have been generated that express predominantly the IIA isoform of type II procollagen by altering a 4ntt sequence of the 5′ splice site of exon 2. Preliminary histological analyses suggest that cartilage development is normal although there is persistent and high expression of IIA propeptide protein in the matrix at post-natal time points when this protein is not normally present. Future studies will involve analyzing whether other cartilage matrix components have been altered as a result of changing type II collagen isoform expression. Investigation of cartilage maintenance over time with normal ageing or as a result of osteoarthritis induction (DMM model) will also be carried out.

76 GREATER TROCHANTERIC PAIN IN HIP OSTEOARTHRITIS: INFLUENCE ON SYMPTOM SEVERITY
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Purpose: Patients with hip osteoarthritis (OA) often show a pattern with periods of increased pain (flares). Little is known about the association between the co-existence of a tendinitis of the gluteus medius muscle or a trochanteric bursitis, known as greater trochanteric pain syndrome (GTPS), and these fluctuations in pain. In this study we assess the frequency of GTPS in hip OA patients, the association with symptom severity in hip OA, and the association of fluctuation in presence of GTPS and fluctuations in symptom severity.

Methods: We used data from baseline and 24-months assessments of a RCT in which primary care hip OA patients meeting the ACR criteria were randomly assigned to receive either glucosamine sulfate or placebo. Symptom severity (0–100) was assessed with WOMAC scores for pain, function and stiffness and with a visual analogue scale (VAS) for pain in the past week. GTPS was defined as tenderness at the greater trochanter in combination with pain recognition, and with a painful resisted hip abduction. We used linear regression models to analyze the adjusted influence of GTPS on the symptom severity in hip OA patients. We also assessed the prognostic value of GTPS on symptom severity 24 months later, and whether individual changes in severity scores associated with changes in presence of GTPS.

Results: Of the 214 hip OA patients, 36 patients (17%) showed GTPS at baseline. Using adjusted regression models, the hip OA patients with GTPS at baseline showed a statistically significant higher mean WOMAC pain and stiffness severity (8.1 and 6.9 points respectively) and higher VAS pain severity (12.8 points). GTPS at baseline did not predict symptom severity 24 months later, but GTPS at 24 months follow-up was statistically significant associated with higher symptom scores at 24 months. In a subset analysis, GTPS was associated with pain severity, function, and stiffness, and of 12.1 points for VAS pain). Change in presence of GTPS associated with fluctuations in symptom severity with 13.1, 9.4 and 11 points for the WOMAC scores respectively, and with 15.6 points for the VAS-score.

Conclusions: Primary care hip OA patients have in five co-existent GTPS, which associates with higher symptom scores.

77 ASSOCIATIONS BETWEEN INFLAMMATORY BIOMARKERS AND CHANGE IN KNEE PAIN OVER 5 YEARS IN OLDER ADULTS
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Purpose: While increasing evidence supports the idea of inflammation in osteoarthritis (OA) pathogenesis, whether these inflammatory markers are predictive of knee pain is unknown. We investigated if both baseline and change over 2.6 years in serum levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and high-sensitivity C-reactive protein (hs-CRP) were associated with change in pain over 5 years.

Methods: A total of 149 randomly selected subjects (mean 63 years, range 52–78; 46% female) were studied. Serum samples were taken at baseline and 2.6 years later. TNF-α, IL-6 and hs-CRP levels were measured by radioimmunoassay. Knee pain was recorded using the Western Ontario and McMaster Osteoarthritis Index (WOMAC) questionnaire at baseline and 5 years later. Knee radiographic osteoarthritis (ROA) of both knees was assessed at baseline.

Results: After adjustment for confounding variables, both baseline (β = 0.27 mL/pg, p = 0.029) and change per annum (β = 0.38 mL/pg, p = 0.010) in TNF-α were positively associated with change in total knee pain. In addition, baseline and change in TNF-α was significantly positively associated with change in the standing and/or on stairs sub-scale of knee pain. Baseline IL-6 levels were positively associated with change in the standing sub-scale of pain (β = 0.18 mL/pg, p = 0.046), but change per annum in IL-6 was negatively associated with change in the sub-scale of pain lying in bed (β = −0.56 mL/pg, p = 0.023). Baseline hs-CRP was positively associated with change in total knee pain (β = 0.38/pg, p = 0.048), as well as change in the sub-scales for pain while lying in bed (β = 0.14/mL/pg, p = 0.019) and sitting (β = 0.13/mL/pg, p = 0.004).

Conclusions: While TNF-α and hs-CRP are predictive of increased knee pain, IL-6 is inconsistently associated with increases in knee pain over time. The underlying mechanisms need to be explored.