Trapeziectomy is a surgical treatment for patients with intractable base of thumb OA and is a good source of hand OA cartilage.

Objectives: The aim of this study was to investigate the expression of atRA-dependent and inflammatory genes in the cartilage of patients and to examine these with respect to the presence/absence of polymorphic ALDH1A2 variants. We also investigate the regulation of atRA upon cartilage injury and its effect on injury-induced inflammatory gene regulation.

Methods: We collected 26 trapeziectomy samples, and dissected the cartilage within one hour of collection. Genomic DNA was extracted for the identification of the two common variants (SNP rs4238326 and SNP rs3204689). Expression levels of atRA-dependent and inflammatory genes were tested by RT-PCR.

Healthy cartilage was obtained from femoral heads of 5-week-old mice and porcine metacarpophalangeal joints of 3–6 months old pigs. Gene regulation upon cartilage injury was tested. To examine the role of specific injury-induced pathways, porcine joints were pre-injected with selective inhibitors to: TGF β R, FGF2R, TAK1 and CYP26, 1 hour prior to injury.

Results: Polymorphic variants in ALDH1A2 were common in this patient population and we identified 8 patients homozygous for both variants, and 5 patients who were wild type for both variants. mRNA levels of ALDH1A2 and atRA-dependent gene CYP19A were significantly lower in the homozygous group compared to wild type (figure 1). There were also trends in the regulation of several other atRA-dependent genes. Conversely, inflammatory genes such as HAS1, TSG6 and ADAMTS5 showed a general increase in homozygous patients.

Cartilage injury in both porcine and murine tissue led to a rapid down-regulation of atRA-dependent genes (CYP26s and RARs). Injecting joints with a potent TAK1 inhibitor prevented the drop of atRA-dependent genes. Prior injection of the joint with a CYP26 (enzymes that normally break down atRA) inhibitor, restored levels of atRA-dependent genes after injury and suppressed injury-induced inflammation genes.

Conclusions: Polymorphic variants in ALDH1A2 are associated with significantly lower levels of ALDH1A2 and CYP19A1 mRNA in hand OA cartilage. Adult articular cartilage constitutively produces atRA, and this is strongly suppressed by mechanical injury through TAK1 activation. Preventing the drop in cellular atRA upon injury, by pre-incubating the joint with a CYP26 inhibitor, restores atRA levels and leads to a reduction in inflammatory gene regulation. These results indicate that atRA plays an important anti-inflammatory role in cartilage and provides a potential novel therapeutic strategy to treat hand OA.

Disclosure of Interest: None declared

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OP0262 DISEASE MODIFYING EFFECTS OF THE CANINE IL4-10 FUSION PROTEIN IN THE CANINE GROOVE MODEL OF OSTEOARTHRITIS

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Background: An ideal disease modifying osteoarthritis (OA) drug should have analgesic, chondroprotective and anti-inflammatory effects. A fusion protein of Interleukin 4 and 10 (IL4-10FP) might have these effects.

Objectives: This study evaluates the effects of canine IL4-10FP (cIL4-10FP) in the canine Groove model of OA.

Methods: In 8 skeletally mature dogs, knee OA in the right leg was induced according to the Groove model. After 6 weeks of OA development, intra-articular injections in the affected knee with either PBS (500 μ l; n=4) or cIL4-10FP (10 μ g/500 μ l; n=4) were given weekly for 10 weeks. The contra-lateral joints served as a healthy control.

Force plate analysis (FPA) was used to determine joint loading as a surrogate marker of pain. The ratio of the affected over the contra-lateral control joint was calculated for each dog for each time point. FPA was performed before OA

induction to determine baseline values, and before and 24 hours after the 1st, 6th and 9th intra-articular injection. A linear mixed model was used to evaluate effects of injections. After 10 weeks of treatment dogs were euthanized and tissue samples were harvested. Serum Immunoglobulin G (IgGs) titers against cIL4-10FP were evaluated to check for potential antibody formation.

Cartilage proteoglycan content and release of proteoglycans were determined *ex vivo* by Alcian Blue assay. Synovial inflammation was evaluated by HE-staining using OARSI grading. Changes in outcomes in the affected/treated joints compared to contra-lateral control joints were calculated.

Results: After OA induction a clear reduction in joint loading (increase in pain) was found (standing force and braking force). After cIL4-10FP injections these forces increased toward normalisation (cIL4-10FP vs PBS group, p=0.002 and p=0.01, respectively). No IgG elevation was detected after 10 injections. Compared to contra-lateral controls, proteoglycan content of OA PBS injected knees suggested tissue degeneration (27 vs 34 mg/g). In the cIL4-10FP treated group proteoglycan content in right knees was not different from the contralateral controls (33 vs 31 mg/g). The mean change in proteoglycan content compared to contra-lateral controls was different with a p value of 0.057 (figure 1). A similar pattern was found for the change in release of proteoglycans from the cartilage, which was less increased in the cIL4-10FP group compared to the PBS group (0.4% vs 3.0%; p=0.029, figure 1).

Synovial inflammation was mild (characteristic of this model) and did not change after intra-articular injections (0.4 and -1.3 points out of 18 for cIL4-10FP and PBS respectively).

Abstract OP0262 – Figure 1 Chondroprotective effects of cIL4-10 fusion protein. (A) change in proteoglycan content between left (control) and right (injected) knees. Values are expressed per animal, representing a mean of 8 samples, and as median $\pm$ IQR. (B) change in release of proteoglycans between left (control) and right (injected) knees. Values are expressed per animal, representing a mean of 8 samples, and as median $\pm$ IQR.

Conclusions: Repetitive intra-articular injection with canine IL4-10FP in dogs did not lead to antibody formation. Dogs treated with cIL4-10FP showed improved joint loading compared to PBS treated dogs, reflecting an analgesic effect. Recovery of proteoglycan content and normalisation of release in cIL4-10FP treated dogs indicate a chondroprotective effect. Synovial inflammation in the Groove model was too mild to be changed significantly. These results clearly warrant further research to develop IL4-10FP as a DMOAD.

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OP0263 CLAUDIN-11 REGULATES BONE HOMEOSTASIS VIA BIDIRECTIONAL EPHB4-EPHRINB2 SIGNALLING

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Background: Claudins (Cldns) are well-established components of tight junctions (TJs) that play a pivotal role in the modulation of paracellular permeability. Several studies have explored the physiologic aspects of Cldn family members in bone metabolism. However, the effect of Cldn11, a major component of central nervous system myelin, on bone homeostasis has not been reported.

Objectives: This study was performed to identify the effects of Cldn on bone metabolism via regulation of osteoclast and osteoblast differentiation and their function

Methods: We performed various *in vitro* and *in vivo* studies using gain- and loss-of function of Cldn11 that is belong to the Cldn group. Osteoclast formation from bone marrow cells (BMC) and Osteoblast formation was evaluated in specific condition with over-expression or down-regulation of Cldn11. The expression of osteoclast associated gene and osteoblast related gene mRNA were assessed

by RT-PCR. The levels of c-fos and NFATc1 protein were assessed by western blot. Also the mitogen-activated protein (MAPK)s and important signal pathways were measured using Western blot analysis. Osteoclast function was evaluated with resorption pit assay and osteoblastic effects of Cldn11 was evaluated with new bone formation of mouse calvaria. With LPS and co-treated Recombinant protein of Cldn11 on mouse calvaria, we evaluated the effects of Cldn11 on LPS induced bone loss by using histologic analysis.

Results: We found that Cldn11 played a negative role in receptor activator of nuclear factor kappa B ligand dependent osteoclast (OC) differentiation by down-regulating the phosphorylated form of extracellular signal-regulated kinase (ERK), Bruton's tyrosine kinase, and phospholipase C gamma 2, in turn impeding c-Fos and nuclear factor of activated T cells c1 expression. Osteoblast (OB) differentiation by positive feedback of Cldn11 was achieved through the phosphorylation of Smad1/5/8, ERK, and c-Jun amino-terminal kinase. Importantly, this Cldn11-dependent dual event arose from targeting EphrinB2 ligand reverse signalling into OC and EphB4 receptor forward signalling into OB. In agreement with these *in vitro* effects, subcutaneous injection of Cldn11 recombinant protein exerted similar effects on local calvarial regions in mice.

Conclusions: These findings suggest that Cldn11 is a novel regulator in bone homeostasis.

Disclosure of Interest: None declared

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OP0264 GLUCOSEPANE : A NEW BIOMARKER OF THE SEVERITY OF OSTEOARTHRITIS

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Background: Glycation, oxidation and nitration of proteins are reactions involved in accelerated ageing of tissues. The products of these reactions are used as biomarkers of chronic pathologies such as diabetes or chronic inflammatory states.

Objectives: In this work, we studied by mass spectrometry the levels of amino acids and glycated, oxidised or nitrated proteins in culture media of chondrocytes cultivated in multi-layers and in the blood of guinea pigs or osteoarthritis patients.

Methods: Sixty male, 3-week-old Dunkin-Hartley guinea pigs were used in this work. At 4-weeks-old and 8 week intervals until week 36, twelve animals were sacrificed and histological severity of knee osteoarthritis evaluated and cartilage rheological properties. Human patients with early and advanced osteoarthritis and healthy subjects were recruited. Human chondrocytes cultured in multilayers were treated for 10 days with interleukin (IL) -1 β . Amino acids and glycated, oxidised and nitrated proteins were analysed in the serum of guinea pigs, osteoarthritis patients and in the culture medium conditioned by chondrocytes by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry using the Acquity UPLC system.

Results: Severity of osteoarthritis increased progressively in guinea pigs with age. Glycated, oxidised and nitrated amino acids were increased markedly at week 36. Glucosepane and dityrosine increased progressively from weeks 20

and 28, respectively. Glucosepane was positively correlated with the OA histological severity ($r=0.58$, $p<0.0001$) and the Young's modulus ($r=0.52-0.56$, $p<0.0001$). Oxidation free adducts were positively correlated with OA severity ($p<0.0009-0.0029$) and hydroxyproline with cartilage thickness ($p<0.0003-0.003$). In the clinical study, plasma glucosepane was increased 38% in patients with early osteoarthritis ($p<0.05$) and 6-fold in patients with advanced osteoarthritis ($p<0.001$) compared to healthy subjects. IL-1 β increased the release of glycated, oxidised and nitrated products from chondrocytes *in vitro*.

Conclusions: The glycation, oxidation and nitration of proteins are reactions related to the severity of osteoarthritis. The products of these reactions are measurable in blood by mass spectrometry and could be biomarkers of osteoarthritis. More specifically, glucosepane is an advanced glycation product very strongly increased in the severe form of the disease. In conclusion, serum glucosepane is a potential biomarker for diagnosis and progression of osteoarthritis.

Disclosure of Interest: None declared

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OP0265 LOCAL REACTIVATION OF GLUCOCORTICOIDS BY 11 β -HYDROXYSTEROID DEHYDROGENASE TYPE 1 MEDIATES THE DEVELOPMENT OF GLUCOCORTICOID-INDUCED BONE LOSS

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Background: Due to the potent immunomodulatory and anti-inflammatory nature of glucocorticoids (GCs) they are routinely used in the treatment of inflammatory diseases such as rheumatoid arthritis. However their therapeutic potential is limited due to the prevalence of adverse side effects associated with long term GC exposure such as osteoporosis, insulin resistance and obesity. 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is a bi-directional enzyme that primarily converts inactive GCs to their active counterparts. Previously, local reactivation of GCs by 11 β -HSD1 has been shown to play a major role in the metabolic side effects associated with GC excess.

Objectives: We aim to assess whether local reactivation of GCs by 11 β -HSD1 mediates the adverse effects of therapeutic GCs on bone.

Methods: Wild-type (WT) mice and transgenic mice with a global 11 β -HSD1 knockout (11 β KO) were treated with the active murine GC corticosterone (CORT) (100 mg/ml) for 4 weeks. Tibia and humerus bones were excised post-mortem for micro-CT analysis, gene expression analysis and three point flexure strength (TFS) tests. Serum was collected from mice for ELISA analysis of TRAcP-5b and P1NP.

Results: Micro-CT analysis of bone volume to tissue volume (BV/TV), trabecular thickness (TT) and trabecular number (TN) found no significant differences between untreated WT and 11 β KO mice (BV/TV: WT 8.5% $\pm$ 0.66 vs 11 β KO 7.5% $\pm$ 0.76, NS; TT: WT 96.5 μ m $\pm$ 3.8 vs 11 β KO 95.8 μ m $\pm$ 6.4, NS; TN: WT 0.0009 1/ μ m $\pm$ 0.00004 vs 11 β KO 0.0008 1/ μ m $\pm$ 0.00004, NS). Humerus TFS tests of WT and 11 β KO animals also showed no significant differences (WT 51.2 MPa $\pm$ 15.1 vs 11 β KO 49.2 MPa $\pm$ 4.9, NS). All bone parameters were decreased in CORT fed WT mice indicating the development of osteoporosis, whilst 11 β KO mice were protected against many of the detrimental effects of CORT (BV/TV: WT 4.2% $\pm$ 0.38 vs 11 β KO 7.2% $\pm$ 0.71, $p\leq 0.05$; TN: WT 0.0006 1/ μ m $\pm$ 0.00004 vs 11 β KO 0.0009 1/ μ m $\pm$ 0.00008, $p\leq 0.001$; HBS: WT 27.1 MPa $\pm$ 5.6 vs 11 β KO $\pm$ 50 MPa $\pm$ 5.1, $p\leq 0.05$). ELISA analysis of mouse serum showed no significant differences in the bone resorbing osteoclast marker TRAcP-5b amongst the groups, whereas analysis of the bone forming osteoblast marker P1NP revealed a significant increase in CORT fed 11 β KO mice compared with CORT fed WT mice (11 β KO 158.6 ng/ml $\pm$ 53.1 vs WT 31.4 ng/ml $\pm$ 7.4, $p\leq 0.05$). Gene expression of the mature osteoblast markers ALP (alkaline phosphatase) and BGLAP (osteocalcin) showed significant increases in CORT fed 11 β KO animals compared to CORT fed WT animals (ALP: 11 β KO 0.0074 AU $\pm$ 0.0012 vs WT 0.0022 AU $\pm$ 0.0007, $p\leq 0.01$; BGLAP: 11 β KO 0.27 AU $\pm$ 0.04 vs WT 0.02 AU $\pm$ 0.01, $p\leq 0.001$). No significant differences were observed between untreated WT and 11 β KO animals.

Conclusions: These data suggest that local reactivation of GCs by 11 β -HSD1 mediates the development of glucocorticoid-induced osteoporosis by inhibiting osteoblastic bone formation.

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