may be mediated, in part, by increased CGRP-expression. At latter stages (day 28) there is considerable tissue remodelling within the joint where CGRP is known to contribute. Whereas, during the intermediate stages of the model (day 14) the inflammatory component has subsided and tissue remodelling is not as pronounced as latter stages. The absence of any differential CGRP-P2X3 co-localization in total and joint-specific afferents, between groups and over time, suggests that P2X3 does not have a primary role in the pathophysiology associated with the first 14 days of the MIA model.

3

TYPE IX COLLAGEN KNOCKOUT IS ASSOCIATED WITH ALTERED GAIT BEHAVIORS AND MECHANICAL ALLODYニア IN MICE

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Purpose: Mice with inactivation of the Col9a1 gene (type IX collagen) prematurely develop osteoarthritis and intervertebral disc degeneration. Measures of gait, motor coordination, and nociceptive responses are reflective of disease pathology in other models, but have not been well-studied for genetic models with joint degeneration. Our objective was to determine if these measures can serve as minimally invasive biomarkers in a mouse model of joint disease. Gait, motor coordination, and pain sensitivity were evaluated in 12 month-old Col9a1 knockout (KO) and wild-type (WT) mice to evaluate differences in functional and symptomatic measures.

Methods: Gait data were acquired from high-speed videos (Phantom V4.2, 250 fps) of 10 KO and 10 WT mice freely exploring a gait arena (unprompted) or responding to a gentle brush (prompted). Nose, tail, and paw pixel coordinates were used to calculate velocity, stride length, step width, and percentage of a stride a given limb is in ground contact. Rotarod (Med-Associates) tests were conducted to test motor coordination. Mice were first placed on an accelerating rotorod (4-40 rpm/5-min); the next day, mice were tested at steady speed (16 rpm for 5-min). Four trials separated by 30 min were given each day. Latency to fall was recorded. Von Frey filaments (Stoelting, sizes 2.83-4.56) calibrated to known forces were applied to the hind-paw plantar surfaces to test mechanical allodynia. Each filament was applied to the left then right paw, and presence of a withdrawal response was recorded (4 trials). Force at 50% likelihood of paw withdrawal was determined by fitting a sigmoid function to the withdrawal frequency versus filament force. To test thermal sensitivity, mice were placed on a 52.0 ± 0.20°C hot-plate (Columbus Instruments) and latency to paw flick was recorded. From the hot-plate, mice were placed in a tail-flick apparatus (Columbus Instruments) and the mid-portion tail was exposed to a radiant light source. The latency for tail withdrawal was scored. Both tests were repeated at 0, 15, 30, 60, 90, 120, and 240 mins.

Results: Gait studies revealed Col9a1 KO mice employ lower unprompted velocities, but when prompted, achieve velocities comparable to WT controls (Figure 1). KO mice also have wider hind-limb step widths, take shorter strides, and their hind-paws remain in ground contact for a larger percentage of the stride in unprompted trials; no genotype differences were observed on prompted trials. Force at 50% likelihood of paw withdrawal was determined by fitting a sigmoid function to the withdrawal frequency versus filament force. To test thermal sensitivity, mice were placed on a 52.0 ± 0.20°C hot-plate (Columbus Instruments) and latency to paw flick was recorded. From the hot-plate, mice were placed in a tail-flick apparatus (Columbus Instruments) and the mid-portion tail was exposed to a radiant light source. The latency for tail withdrawal was scored. Both tests were repeated at 0, 15, 30, 60, 90, 120, and 240 mins.

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Conclusions: These data for gait behaviors and mechanical nociception indicate that Col9a1 KO mice self-select slower, less strenuous gaits, perhaps due to increased mechanical sensitivity. When challenged to perform at higher activity levels, KO mice can do so in short sustained bursts (prompted gait), but may not have sufficient endurance to engage in more strenuous activities (rotarod). Alterations in response to mechanical, but not thermal, stimuli suggest these changes may be driven by specific central mechanisms. Additional studies are required to determine whether the joint or spine pathologies contribute to the observed gait and pain changes.

4

NAPROXEN AND A MATRIX METALLOPROTEINASE INHIBITOR BOTH REDUCE WEIGHT BEARING ASYMMETRY, INDICATIVE OF BEHAVIOURAL PAIN, IN THE RAT MENISCAL TRANSECTION MODEL OF OSTEOARTHRITIS

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Purpose: It has been postulated that the development of chondroprotective agents will be of little consequence to patients unless they manifest improvements in quality of life measures. The rat meniscal transection (MT) model demonstrates cartilage erosion, consistent with some facets of human pathology and this joint destruction correlates with weight bearing asymmetry, indicative of behavioural pain. The aim of this study was to in-
investigate whether oral administration of the NSAID, naproxen, or a matrix metalloproteinase (MMP) inhibitor would alter cartilage erosion and/or weight bearing asymmetry in the rat MT model of osteoarthritis.

Methods: Pathology was induced in male Lewis rats, (270g, n=10/group) by surgically transecting the medial collateral ligament and a full thickness cut through the meniscus of the left knee, day 0. Sham animals underwent the same procedure with omission of the meniscus transection. MT animals were dosed orally bid with vehicle (HPMC/tween, 1% DMSO), naproxen (10mg/kg) or an equipotent MMP 2, 8, 9, 13 inhibitor, (0.125, 0.5, 2.5 mg/kg), from day -1. Changes in weight bearing asymmetry were assessed at -2, 7, 14, 21, 28, and 35 days post surgery, using an incapacitance meter. Rats were sacrificed 35 days post surgery and the leg removed for histological assessment. Toluidine blue stained coronal step sections (10µm) across the entire knee joint were scored for cartilaginous changes and lesion size measured by image analysis, using in house software. Treatment effect was determined using ANOVA (Dunnet's post hoc analysis), for weight bearing (AUC) and lesion size, whereby P <0.05 was considered statistically significant.

Results: Osteoarthritic-like lesions developed in the vehicle treated MT animals on the medial side of the tibial plateau of the operated leg, resulting in loss of proteoglycan, fracturing, and erosion of cartilage. Sham operated animals had histologically normal cartilage. Treatment of MT animals with naproxen had no effect on pathology scores, whereas the MMP inhibitor treatment reduced cartilage fracture score (P <0.05 all doses) and cartilage lesion size by 52-65% (P <0.05 at 0.5, 2.5 mg/kg bid). There was a clear division in weight bearing asymmetry profiles of sham and vehicle treated MT animals from 14 days onwards. Sham animals gradually returned to symmetrical weight bearing by day 28 whilst vehicle treated MT animals maintained a 40 to 50g asymmetry between operated and contralateral legs (P <0.001). The weight bearing asymmetry was significantly ameliorated by naproxen (P <0.001) and the MMP inhibitor (P <0.01), evident from day 14 onwards.

Conclusions: Asymmetry was ameliorated with naproxen treatment, thus demonstrating the utility of the animal model to investigate novel treatments for reducing joint pain. Notably, treatment with an MMP inhibitor showed chondro-protection and reduced weight-bearing asymmetry. These data suggest that chondro-protective agents could improve functional joint mobility. Further work is required to understand the analgesic mechanism.

5
REVERSIBLE MRI FEATURES AND KNEE PAIN FLUCTUATION: THE MOST STUDY
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Purpose: While pain in knee osteoarthritis (OA) has long been considered a chronic condition, the symptoms experienced by OA patients are neither constant nor stable, but rather fluctuate. These observations suggest that factors that vary over time within a person are responsible for pain fluctuation. Few studies have been conducted to examine the effect of time-varying factors on knee pain fluctuation. We examined whether changes in MRI features (i.e., synovitis, effusion, and bone marrow lesions (BML)) are associated with change in knee pain among the subjects of Multicenter Osteoarthritis Study (MOST).

Methods: MOST is a longitudinal study of risk factors for knee structural changes and occurrence of pain. Subjects included in this analysis were asked about frequent knee pain in the past month (pain, aching and stiffness on most days) by interview and had knee MRIs performed at the baseline visit and at one or two follow-up visits. All subjects had a 30-month follow-up while a subset of subjects with new knee pain and a random sample of controls without knee pain had a 15-month visit. MRIs were scored using the WORMS scale by two radiologists (range of interobserver ICC: 0.68-0.96) blinded to knee pain status. Synovitis was scored 0-3 in two sites (infrapatella and intercondylar), effusion was scored 0-3 for each knee, and BMLs were scored 0-3 in each of 5 sub-regions in the medial and lateral and 4 subregions in the patellofemoral compartments. We summarized synovitis and BMLs within each knee by totaling the scores across sites or sub-regions. We defined a visit as a case-visit if a knee had pain on most days of the past month prior to that clinic visit, and as a control-visit if the same knee did not have pain on most days of the past month prior to that clinic visit(s). The visits with no knee pain served as the controls for the case-visits of the same knee. We evaluated the relation of summary score of synovitis, effusion, and BML to the knee pain fluctuation using a conditional logistic regression model.

Results: 250 knees (232 subjects) that had knee pain in at least one, but not all, clinic visits (255 case-visits and 261 control-visits) were analyzed. Of them, 25 knees had pain and MRI assessed at 3 visits and 225 knees assessed at 2 visits. Over the follow-up period, 7.4%, 9%, and 21% of the knees had total synovitis, effusion and BML scores improve, respectively; whereas 8.2%, 5.4%, and 25% of the knees had synovitis, effusion and BML scores worsen. Changes in synovitis, effusion and BML scores were strongly associated with knee pain fluctuation. The odds ratios (OR) of knee pain occurrence were 1.0, 4.4, 8.1 and 23.9 when synovitis score increased from 0 to 1, 2, 3-13 (p for trend <0.001); the ORs of knee pain were 1.9 and 9.2 respectively for an increase in effusion score to 1 or 2-3 compared with an effusion score of 0 (p for trend <0.001); the ORs of knee pain occurrence increased from 1.0 to 2.1, 2.7, 4.6, 11.3, 12.9 and 16.5 when the BML score increased from 0 to 1, 2, 3, 4, 5 and 6-13 (p for trend <0.001), respectively.

Conclusions: We found that changes of synovitis, effusion, and BML were strongly associated with the fluctuation of knee pain. Since these MRI features are reversible, clinical intervention on these features, if possible, may help to reduce knee pain symptoms.