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DESTABILIZATION OF THE MEDIAL MENISCUS AS A MODEL FOR THE STUDY OF PAIN PATHWAYS ASSOCIATED WITH DEVELOPMENT OF MURINE OSTEOARTHRITIS

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Purpose: Pain is the major symptom in osteoarthritis (OA) and one of the leading causes of impaired mobility in the elderly. There is no simple correlation between pathological changes in the joint and pain severity. No animal models adequately describe the correlation between joint structure and symptoms, and very few have properly examined mechanisms of OA pain generation. We recently reported that, following DMM in the right knee, C57BL/6 mice develop rapid-onset progressive mechanical allodynia in the ipsilateral hindpaw only, as early as 2 weeks and developing over 8 weeks post DMM surgery. *Adamts5* knockout (KO) mice, which do not develop OA-like structural changes after DMM, did not develop allodynia. Our long-term goal is to quantitatively measure pain and dissect molecular pathways involved in pain generation in a mouse model of OA. We chose destabilization of the medial meniscus (DMM) in C57BL/6 mice because, unlike other rodent OA models, the joint pathology in this model is slowly progressive over 16 weeks and thus optimally suited for studying pain at different stages of disease.
Methods: DMM surgery was performed in the right knee of 10-week old male C57BL/6 mice. At different time points after surgery (2, 4, 8, 12 and 16 weeks), pain was assessed in DMM operated mice, in sham controls and in age-matched naïve controls. Pain-dependent measures included 1) von Frey analysis to measure mechanical allodynia; and 2) behavioral monitoring with Laboras™ equipment, which quantifies activity using pattern recognition software, including total distance traveled over a specified time period. Concurrently, innervating dorsal root ganglia (DRG) L2-L5 were harvested from both the operated and the non-operated side at different time points after surgery for RT-PCR analysis. Knee joints were collected for histopathology or India ink staining.
Results: In the current studies, we found that the unilateral mechanical allodynia subsided 8-16 weeks after DMM surgery. Total distance traveled over a specified period was relatively constant over the first 8 weeks after surgery, but decreased from 12 weeks after surgery. RT-PCR analysis of innervating DRG at different time points (4, 8, 16 weeks) following DMM revealed significantly increased levels of mRNA (compared to naïve age-matched controls) for the neurotrophic factors, NGF (nerve growth factor) and GDNF (glial cell-derived neurotrophic factor), and for the chemokine, MCP-1 at the 8-week time point. mRNA levels were elevated in both the ipsi- and in the contralateral DRG (but more so in the ipsilateral DRG). mRNA levels for substance P or for the NGF receptor, TrkA, were not elevated (Figure 1). mRNA levels for the genes studied were not elevated in *Adamts5* KO mice.

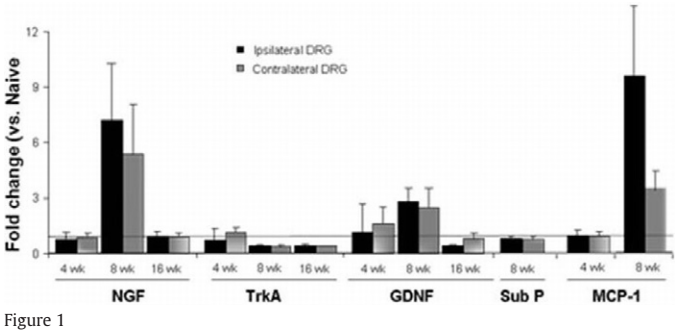

Figure 1

Conclusions: Time-course experiments in the murine DMM model of OA enable us to quantify pain at different stages of disease. Pain-related outcome measures in the DMM model can essentially be divided into an early stage (wk 2-8, allodynia only) and a late stage (wk 8-16, resolution of allodynia and appearance of reduced locomotion). Increased mRNA levels for neurotrophic factors and for MCP-1 in the innervating DRG were detectable 8 weeks after surgery. These observations suggest that the DMM model is suitable for studying mechanisms of chronic OA pain generation.

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RUNT-RELATED TRANSCRIPTION FACTOR 2 ALLELES AND KNEE CARTILAGE LESIONS ARE ASSOCIATED WITH INCREASES IN KNEE PAIN OVER 5 YEARS IN OLD ADULTS

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Purpose: Runt-related transcription factor 2 (RUNX-2) is the pivotal transcription factor for chondrocyte hypertrophy. This study aimed to determine if RUNX-2 and knee cartilage lesions were associated with change in knee pain over 5 years in older adults.
Methods: A total of 755 randomly selected subjects (mean 62 years, range 51-81, 50% female) were studied at baseline, 2.9 and 5 years later. Knee pain (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) at baseline and 5 years was assessed using WOMAC. RUNX-2 alleles (BB, Bb and bb) were genotyped at baseline. Fat-suppressed MRI of the right knee was performed to determine knee cartilage volume and defects at baseline and 2.9 years.
Results: In multivariable analysis, RUNX-2 b allele (Bb 11.8% and bb 1.4%) was associated with a borderline increase in total knee pain score ($\geq$1) (OR 1.89, P=0.06), an increase in knee pain at night (OR 2.61, P=0.012) and an increase in sitting/lying knee pain (2.51, P=0.028) over 5 years. Increase in total knee pain score over 5 years was associated with female sex (OR 2.2, P=0.03), body mass index (OR 1.07 per kg/m^2, P=0.004), knee radiographic osteoarthritis (OR 2.03 per grade, P=0.006), medial tibiofemoral cartilage defects (OR 1.46 per grade, P=0.006), lateral tibiofemoral cartilage defects (OR 1.38 per grade, P=0.025), change in medial cartilage defects over 2.9 years (OR 1.68 per grade, P=0.004) and loss of medial tibial cartilage volume over 2.9 years (OR 1.07 per percent loss, P=0.03).
Conclusions: Multiple factors contribute to an increase in knee pain over 5 years. This is the first study to report that the RUNX-2 b allele predicts increased knee pain. Cartilage lesions are related to knee pain, independently of potential confounders.

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THE CATHEPSIN K INHIBITOR AZ12606133 REDUCES ARTICULAR CARTILAGE BREAKDOWN AND JOINT PAIN IN OSTEOARTHRITIC KNEES

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Purpose: The aim of the present study was to evaluate the chondroprotective and pain modulating effects of a novel cathepsin K inhibitor AZ12606133 in the Dunkin-Hartley guinea pig model of spontaneous osteoarthritis (OA).
Methods: Nineteen mature (9 month old) Dunkin Hartley guinea pigs were chronically implanted with an Alzet osmotic pump (2.5μl/hr; total volume = 2ml) which was set to deliver either the cathepsin K inhibitor AZ12606133 (0.5μg/kg/day, n=10) or vehicle (n=9). On day 28 of treatment, urine was collected over a 6 hour period by placing guinea pigs in metabolism cages. The urine was stored at -20[[Unsupported Character - ]]C until later analysed for evidence of cartilage degradation using the cross-linked C-telopeptides of type II (CTXII) collagen assay. Animals were then prepared for electrophysiological recording of knee joint primary afferent nerves. Recordings from mechanosensory nerves were made at rest (spontaneous activity) and in response to rotation of the knee either in the normal working range (40mNm) or the noxious range (60mNm).
Results: AZ12606133 caused a 63% reduction in urinary CTXII levels compared to vehicle treated guinea pigs (Figure A). Spontaneous afferent firing rate in vehicle-treated animals was 52.9±14 action potentials/min (n = 26