per cell (um2) (P < 0.01) compared to the control group (6 months old mice).

**Conclusion:** These results support the hypothesis that autophagy is decreased with aging and that compromised autophagy represents a novel mechanism in the development of OA.

51 CARTILAGE INJURY REGULATES NERVE GROWTH FACTOR (NGF) IN VITRO AND IN VIVO AND DRIVES PAINFUL BEHAVIOR IN MURINE OA

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**Purpose:** Pain is the foremost clinical symptom of osteoarthritis (OA), however the processes that lead to pain in the joint are poorly understood. We have previously shown that nerve growth factor (NGF) is regulated in the joints of mice at the time they get OA-induced pain and NGF neutralisation is an effective analgesia (McNamee et al. 2010). In pharmaceutical trials of monoclonal anti-NGF antibodies, clinical efficacy in the treatment of OA pain has been demonstrated (Lane et al. 2010) but a small minority of patients developed accelerated disease, raising the possibility that analgesic effects of anti-NGF specifically contribute to direct progression of pain. This might occur by removing the mechanical protection afforded by painful behaviour, or be due to a direct disease modifying effect of the antibody. The purpose of this study was to investigate the regulation of NGF in painful murine OA joints, specifically focusing on where it is regulated and its induction following mechanical injury. The dependence of NGF on FGF2, a cytokine upon NGF specifically, was measured to determine how and upon FGF2 knockout cartilage, a cytokine released upon cartilage injury, was also examined. Finally, we modelled the effects of chronic NGF neutralisation or indomethacin (a non-steroidal anti-inflammatory agent) on disease progression in murine OA.

**Methods:** Surgical joint destabilisation, a validated model of OA, was performed on 10 week old male or female mice and painful behaviour (a combination of Von Frey, cold plate sensitivity, algometry and Linton incapacitance) assessed weekly. RNA was isolated from whole knee joints or micro-dissected tissues (articular cartilage, meniscus and epiphysis). For cartilage injury responses in vitro, RNA was isolated from hip epiphyses of 5 week old wild type or FGF2 knockout mice. Additional injury studies were performed on porcine cartilage that had been pre-incubated with the FGF receptor inhibitor SB402451. Anti NGF, indomethacin or relevant controls were administered to wild type OA mice from the time of pain onset for a further 6 weeks. The analgesic response to anti-NGF was assessed over this period of study.

**Conclusions:** NGF is a validated pain target in murine OA and is regulated largely within the articular cartilage in vivo and upon mechanical injury in vitro. Cartilage injury-induced NGF in vitro was strongly FGF2 dependent, but this did not seem to be the case in vivo. Indeed, FGF2 knockout mice developed earlier pain, apparently correlating with accelerated disease seen in this strain. We were unable to demonstrate any differences in disease progression between mice chronically dosed with anaesthetics and those with pain over this period of study.

52 THERAPEUTIC EFFICACY OF ANTI-ADAMTS5 ANTIBODY IN THE DMM MODEL

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**Purpose:** Adamts-5 null mice are protected from cartilage degeneration and accompanying mechanical alldynia in experimental osteoarthritis induced by destabilization of the medial meniscus (DMM). A highly selective and potent monoclonal antibody (Ab) that neutralizes ADAMTS-5 was able to mimic these findings when administered prophylactically through 8 weeks post DMM surgery. The goal of the current study was to test whether this Ab can provide similar beneficial effects when administered in a therapeutic setting, i.e., after onset of joint pathological changes and mechanical alldynia. The therapeutic effects of anti-ADAMTS-5 on progression of joint damage, mechanical alldynia in the hindpaw, and concomitant molecular changes in the nociceptive pathway were analyzed.

**Methods:** DMM surgery was performed in the right knees of 10-week old C57Bl/6 mice. Four weeks later, mice were administered weekly injections of anti-ADAMTS-5 or IgG isotype control Ab (i.p. 10 mg/kg). Untreated mice served as a control group. For the next 12 weeks, mice were monitored bi-weekly for mechanical alldynia in the ipsilateral hind paw using von Frey fibers and the up-down staircase technique. Sixteen weeks post DMM, mice were taken down (n=11-12), and knees were collected for histopathology according to OARSI recommendations. Total joint score is a sum of cartilage degeneration, osteophyte formation on both the lateral and medial sides of the joint, with a maximum possible score for cartilage degeneration = 60, and osteophyte formation = 6 (max total joint score = 66). Additionally, subchondral bone sclerosis in the medial compartment was assessed, using a bone score with a maximum score of 5. In addition, a set of n=4 mice/treatment group were taken down 8 weeks after surgery. Ipsilateral knee innervating dorsal root ganglia (DRG) L3-L5 were harvested, and sensory neurons were isolated and cultured for 4 days. Cell culture supernatants were analyzed for the pro-algesic chemokines, MCP-1 and fractalkine, via ELISA.

**Results:** Histopathology of the knee joint 16 weeks post surgery revealed that untreated mice developed moderate levels of cartilage degeneration and osteophyte formation (total joint score = 19.3±3.1) and subchondral bone sclerosis (bone score = 2.5±0.7). When anti-ADAMTS5 was administered beginning at 4 weeks post DMM, mice were protected against cartilage degeneration and osteophyte formation (total joint score = 9.5±2.0) (p<0.02), but not against subchondral bone changes (bone score = 2.6±0.3) (p=0.92) by 16 weeks post surgery. IgG isotype control Ab afforded some protection against change in the total joint score, but this was not statistically significant compared to untreated DMM mice (total joint score = 13.1±3.0) (p<0.2). Four weeks after DMM surgery, mice presented with robust mechanical alldynia in the ipsilateral hind paw, an indicator of sensitization in the pain pathway. In untreated mice and in mice receiving IgG isotype control Ab, the alldynia was maintained through 16 weeks post surgery (p<0.001). In mice treated with anti-ADAMTS-5, the alldynia resolved and this protection lasted through 12 weeks post surgery. By 16 weeks post surgery, mechanical alldynia had returned (p<0.055) (Fig 1). Eight weeks after surgery, innervating DRG neurons from untreated DMM mice produced elevated levels of MCP-1 and fractalkine protein compared to naive and sham mice. When DRG neurons were cultured from mice treated with anti-ADAMTS5 between weeks 4 and 8 post DMM, the levels of MCP-1 and fractalkine were significantly downregulated compared to untreated mice (p<0.001). This downregulation reflects decreased activation of sensory neurons and correlates with decreased mechanical alldynia after treatment with anti-ADAMTS-5.

**Conclusions:** This study demonstrates therapeutic efficacy of a potent and selective anti-ADAMTS-5 Ab in the DMM model, when administered in early stages of disease. Therapeutic effect on cartilage degeneration, mechanical alldynia as an indicator of pain and concomitant molecular changes in sensory neurons further supports the idea that structural joint damage and development of pain are linked. These studies will help elucidate whether slowing progression of joint damage can prevent or slow the development of pain. Ongoing experiments are evaluating therapeutic effect of ADAMTS-5 blockade in late stage experimental osteoarthritis.