Development and progression of mechanical allodynia after DMM surgery to the same extent as in WT mice. In contrast, unlike WT mice, PCSK6 null mice did not develop locomotor changes that are indicative of movement-provoked pain. This was associated with the absence of microglia activation in the dorsal horn of the spinal cord. These results further support earlier findings that PACE4 may play a role in pain and justify further studies to analyze central mechanisms.

Conclusions: In summary, by 16 weeks after DMM surgery, PCSK6 null mice developed OA pathology to the same extent as WT mice. In addition, knee joint pathology was associated with mechanical allodynia after DMM surgery to the same extent as in WT mice. In contrast, unlike WT mice, PCSK6 null mice did not develop locomotor changes that are indicative of movement-provoked pain. This was associated with the absence of microglia activation in the dorsal horn of the spinal cord. These results further support earlier findings that PACE4 may play a role in pain and justify further studies to analyze central mechanisms.

752 PAIN PATHWAY ACTIVATION IN DORSAL ROOT GANGLIA AND DORSAL HORN IN A MURINE SURGICAL MODEL OF OSTEOARTHRITIS
P.B. Tran, R.E. Miller, R.J. Miller, A.-M. Malfait. Rush Univ. Med. Ctr., Chicago, IL, United States; Northwestern Univ., Chicago, IL, United States

Purpose: In nerve injury models, maintenance of chronic pain involves microglial activation in the dorsal horn (DH) of the spinal cord. This is a dynamic process, which depends on signaling molecules, such as the chemokine fractalkine (CX3CL1), which are produced in dorsal root ganglia (DRG) neurons and transported to the DH, resulting in activation of DH microglia. We monitor pain and associated pathways over a period of 16 weeks post destabilization of the medial meniscus (DMM) surgery in the mouse. This model of slowly progressive knee osteoarthritis is associated with changing pain-related behaviors and concurrent molecular changes in the innervating DRG over 16 weeks post surgery. Specifically, mice develop progressive mechanical allodynia over the first 4 weeks, while locomotive changes indicative of chronic pain first appear 8 weeks post DMM. The purpose of the current study was to investigate fractalkine expression in DRG neurons as well as microglial activation in the DH over 16 weeks following DMM surgery.

Methods: DMM or sham surgery was performed in the right knees of 10-week old male C57BL/6 mice. Four, 8, and 16 weeks post DMM and sham surgeries, L3-L5 DRG were harvested and cells were cultured for 4 days; supernatants were collected for fractalkine ELISA. For immunohistochemistry, mice were perfused transcardially with paraformaldehyde, and the spinal column was decalcified prior to cryosectioning. To assess microglial activation in the DH, spinal cord sections were immunostained with anti-Iba1 and quantification of the number and morphology of Iba1 immunoreactive microglia was performed according to established methods. Microglia in which process length was more than double soma diameter were classified as effector (i.e., activated) microglia while microglia in which process length was more than double soma diameter were classified as surveyor microglia.

Results: At 4 weeks post DMM surgery, cultured DRG cells released similar amounts of fractalkine compared to age-matched naive cells. At 8 weeks post surgery, DRG cells released elevated levels of fractalkine protein compared to sham and age-matched naive DRG cells ($p < 0.01$), and by 16 weeks post surgery levels were still somewhat elevated in DMM cells ($p < 0.01$), but less so than at 8 weeks. The dorsal horn showed increased Iba1 expression at 8 and 16 weeks but not at 4 weeks post DMM compared to sham and age-matched naive mice.
At 8 and 16 weeks post DMM surgery, there was more effector microglia expression in the DH, particularly at the L4 level of the spinal cord (Fig 1).

**Conclusions:** The temporal correlation of fractalkine release with microglial activation suggests that fractalkine may contribute to microglial activation in the DH of the spinal cord. Microglial activation in the DH has been reported in murine mono-iodoacetate (MIA) arthritis as well as nerve injury models. Results from this study will help elucidate how pain signals propagate from the peripheral to the central nervous system in osteoarthritis.

**Figure 1. Trend for individual repeats of each QST measure at the Sternum**

**Figure 2. Group differences in Mechanical local and distant QST measures**

**Conclusions:** These results suggest that three not five repeats are required for testing the QST modalities of heat pain threshold, mechanical pain threshold and mechanical pain sensitivity. For warm detect threshold the first measure should be omitted before calculating an average measure. Mechanical pain sensitivity was able to distinguish painful ROA positive knees from pain-free ROA positive knees. The fact that sensitivity at the sternum, as well as the knee, was able to predict concordant pain and structural status supports previous work showing that centrally mediated widespread pain sensitisation is present and highlights it potential use in future clinical research.