Title: Anatomical distribution of mrgprd-expressing nonpeptidergic c-fibers in the mouse knee

Authors: Alia Obeidat, Rachel E. Miller, Richard J. Miller, and Anne-Marie Malfait

Purpose: The voltage-gated sodium channel, $Na_V1.8$, marks the majority of nociceptors, including more than 90% of C-fibers. We have used $Na_V1.8$ tdTomato reporter mice to describe the nociceptive innervation of the mouse knee. We reported that, 16 weeks after destabilization of medial meniscus (DMM), osteoarthritic (OA) joint damage is accompanied by extensive remodeling of nociceptors in the medial compartment of the knee, including increased $Na_V1.8$+ innervation in the medial synovium, in addition to the presence of $Na_V1.8$+ fibers in the medial meniscus and within subchondral bone channels. Distinct functional classes of C-fibers have been identified, where it has been proposed that TRPV1+ C-fibers mediate heat sensitivity and C-fibers that express the G protein-coupled receptor (GPCR), Mrgprd (The Mas-related G protein-coupled receptor D), mediate behavioral sensitivity to noxious mechanical stimuli. Mrgprd marks about 75% of all $IB4^+$ nonpeptidergic nociceptive neurons and are believed to innervate the skin, while IB4+ C-fibers have been reported to be absent in rat joints. Their potential role in mechanosensation makes Mrgprd expressing neurons an interesting subject in the context of OA pain, which is why we developed Mrgprd-EGFP reporter mice and investigated if they are present in the murine OA joint.

Methods: Mrgprd-EGFPf mice on a C57BL/6 background were used. DMM surgery was performed in the right knee of 10-week old male mice. Knees were harvested from 10-week old naïve mice (n=5), 26-week old naïve mice (n=3), and mice 16 weeks after DMM (n=3). Mice were perfused transcardially with paraformaldehyde. Knees were harvested, post fixed, decalcified and cryo-sectioned. Twenty-µm thick coronal sections were collected throughout the knee joint using established methods. Sections were then imaged using laser-scanning confocal microscope (Olympus IX70) and the fluorescence signal was quantified using image J by an observer blinded to the groups.

Results: Mrgprd+ signal was present in the knee joint of 10-week old naïve mice, specifically at the insertions of the cruciate ligaments and in bone marrow cavities. By the age of 26 weeks, Mrgprd+ innervation decreased significantly in the cruciate ligament insertions (Fig 1a), which is similar to the age-related decline seen in the cruciate ligaments of $Na_V1.8$-tdTomato mice. No signal was observed in the medial synovium of young or old naïve mice (Fig 1b). Sixteen weeks after DMM, OA knees showed further decrease in the Mrgprd+ innervation of the cruciate ligament insertions, compared to 26-week old naïve knees (Fig 1a). In contrast to the increase in the medial synovial $Na_V1.8$+ innervation we previously observed in $Na_V1.8$-tdTomato mice, no change was observed in the Mrgprd+ innervation of the medial synovium after DMM (Fig 1b). Interestingly, Mrgprd signal was present in channel like structures in the subchondral bone of the medial femoral condyle and tibial plateau of the OA knee (Fig 1c), similar to findings in $Na_V1.8$-tdTomato mice.

Conclusion: The intra-articular Mrgprd innervation changed markedly with aging and after DMM surgery. Interestingly, the innervation pattern of this mechanosensitive nociceptor subset was different than the pattern we previously observed for $Na_V1.8$, which marks all nociceptors.

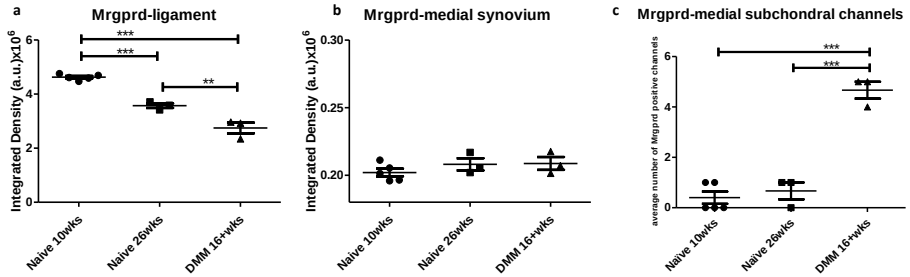

Figure 1 : Quantification of MRGPRD+ signal in knees of 10- and 26-week old naïve mice and 16 weeks after surgery, in (a) ligament; (b) medial synovium; (c) within subchondral channels.** p<0.01 , *** p < 0.001; mean±SEM