

## Role of Piezo2 in Osteoarthritis Pain Development in Mice of Both Sexes

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**INTRODUCTION:** Osteoarthritis (OA) and its associated pain is one of the leading causes of disability in the world<sup>1,2</sup>. Mechanical loading triggers OA pain, leading to avoidance of activities of daily living. Hence, targeting mechanosensation may prove fruitful in reducing the symptoms of OA. Piezo2 is a mechanosensitive ion channel expressed by sensory neurons. We have previously shown that male nociceptor-specific Piezo2 conditional knock out mice (Nav1.8-Cre;Piezo2<sup>fl/fl</sup>; Piezo2cko) were protected from both evoked and mechanically mediated pain in experimental OA, as well as protected from pain and swelling in a model of NGF-induced joint pain<sup>3</sup>. Recent work has shown that styryl dyes, FM1-43 or its derivative FM4-64, which can block several types of mechanically activated currents in sensory neurons, are dependent on Piezo2 activity for uptake of the dye by sensory nerve endings *in vivo*<sup>4,5</sup>. A toxin, GsMTx-4, has also been shown to non-selectively block mechanosensitive channels. The goal of the current study was two-fold. First, to test the effects of male and female Piezo2cko mice in OA models, and secondly, to test the effectiveness of mechanosensitive ion channel blocking using FM dyes or GsMTx-4.

**METHODS:** All animal procedures were approved by an IACUC committee. *Experiment one:* At 12 weeks of age, male and female Piezo2cko and C57BL/6 WT mice underwent sham or partial meniscectomy (PMX) surgery of the right knee. Knee hyperalgesia (pressure application measurement, Ugo Basile) was assessed at baseline, 4, 7, and 12 weeks post-surgery. After confirming the presence of knee hyperalgesia at 7 weeks post op, mice were intraarticularly (i.a.) injected under isoflurane anesthesia with FM dye (5nmol in 2.5µL) or saline (2.5µL) in the right knee and assessed for knee hyperalgesia once more 90 minutes post injection<sup>4,6</sup>. The following week mice were retested for knee hyperalgesia and then injected with GsMTx-4 i.a. (75µM in 5µL). Weight bearing (static incapacitance meter, Bioseb) was assessed pre, 4, 8, and 12 weeks post-surgery. The asymmetry index was calculated by averaging the difference of the force recorded on the right hind limb from the left hind limb over three trials. *Experiment two:* Aged 2-year-old male Piezo2cko and WT control mice were tested for knee hyperalgesia as in experiment one. Following baseline testing, mice were injected with saline(5µL) i.a. to determine if a vehicle injection would elicit a positive or negative response to knee hyperalgesia and no effect was noted. Three days later, aged mice were then injected with FM dye (5nmol in 2.5µL) and were tested for knee hyperalgesia 90 min post injection. Knee histology is ongoing. All behaviors were assessed by a blinded observer.

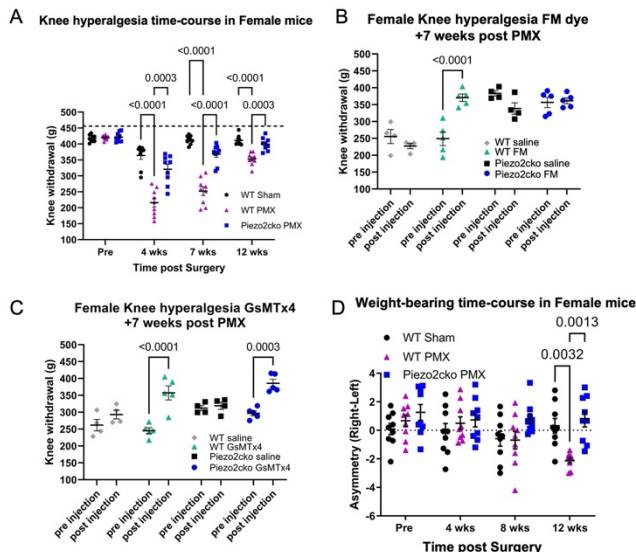
**RESULTS:** *Experiment one:* WT female PMX mice (n=9) developed knee hyperalgesia to a greater extent than WT female sham mice (n=9) at all time points tested post surgery (p<0.0001) (Fig 1A). Female Piezo2cko PMX mice had significantly less knee hyperalgesia in comparison to WT PMX mice at 4, 7, and 12 weeks post operatively (p=0.0003, p<0.0001, p=0.0003, 2-way ANOVA) (Fig 1A). Similarly, male Piezo2cko PMX mice (n=5) had less knee hyperalgesia than male WT PMX mice (n=5) 4- and 7-weeks post-surgery (p=0.01, p=0.02, 2-way ANOVA) (Fig 2A). Pharmacological testing of FM dye revealed a reversal in knee hyperalgesia in WT female PMX (p<0.0001) and WT male PMX mice (p=0.005) with no significant effect on Piezo2cko mice (Fig 1B and 2B), consistent with previous work suggesting a Piezo2 dependence of this dye<sup>5</sup>. In contrast, GsMTx-4 had an effect in both WT PMX (p<0.0001) and Piezo2cko PMX (p=0.0003) female mice in reversing knee hyperalgesia (Fig 1C). Finally, female WT PMX mice developed weight bearing asymmetry by 12 weeks post op in comparison to WT sham mice (p=0.0032) while Piezo2cko PMX mice continued to bear weight similarly in both limbs at this time point (vs. sham: p=0.81) (Fig 1D); male data is currently pending. *Experiment 2:* Aged male naïve WT mice (n=4) developed clear knee hyperalgesia by the age of 2 years, as we have seen before<sup>7</sup>, but aged Piezo2cko mice (n=6) were protected (p<0.0001, unpaired two-tailed T-test). Injection of FM dye in aged WT male mice significantly reversed knee hyperalgesia (p=0.0021 2-way ANOVA) and again no effect was seen in male Piezo2cko mice (p=0.63) (not shown).

**DISCUSSION:** Data from the current study supports previous work suggesting selective nociceptor deletion of Piezo2 is protective against OA pain development. Here, we expanded on previous work by comparing male and female surgical mice and providing evidence that Piezo2cko is effective in reducing evoked and spontaneous pain in both sexes. Further, we demonstrated that pharmacological targeting of Piezo2 may be effective in reducing mechanical sensitization in OA. Future work will examine the effects on the knee joint and will molecularly characterize changes in the DRG.

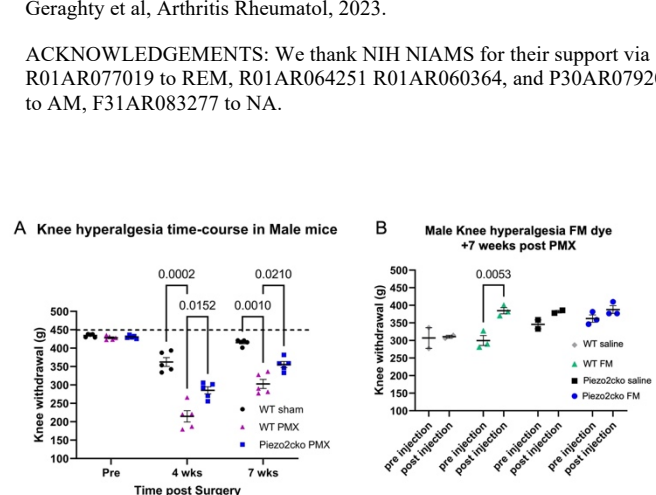
**SIGNIFICANCE:** The study provides more evidence that mechanical signaling through Piezo2 is important for OA pain development in both sexes. Targeting mechanosensitive ion channels pharmacologically has the potential to reduce pain, as suggested by the findings with FM drugs and with GsMTx-4. This may translate into the development of mechanical sensitive ion channel blockers for targeting OA pain.

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**Figure 1.** Female Piezo2cko mice develop less knee hyperalgesia and weight-bearing asymmetry compared to WT mice after PMX surgery. Mechanosensitive ion channel blockers can reverse knee hyperalgesia in WT PMX mice. A) Knee hyperalgesia; B) knee hyperalgesia after i.a. saline or FM dye; C) knee hyperalgesia after i.a. saline or GsMTx4; D) weight-bearing asymmetry. mean±SEM



**Figure 2.** Male Piezo2cko mice develop less knee hyperalgesia compared to WT mice after PMX surgery, and mechanosensitive ion channel blockers inhibit hyperalgesia in WT mice. A) Knee hyperalgesia; B) knee hyperalgesia after i.a. saline or FM dye. mean±SEM.