

Myeloid-specific AMPK α 1 and myeloid-specific ACLY knockout mice exhibit differential pain-related behavior during progression of post-traumatic osteoarthritis

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INTRODUCTION: AMP-activated protein kinase (AMPK) is a master regulator of cellular energy metabolism with anti-inflammatory function. ATP citrate lyase (ACLY), a metabolic enzyme responsible for producing citrate-derived acetyl-coenzyme A (CoA), plays a critical role in supporting a proinflammatory response. Inhibition of ACLY can lead to activation of AMPK. AMPK activators have been shown to alleviate pain in a broad variety of preclinical pain models. We previously demonstrated that metformin and berberine, both are AMPK activators, not only limit joint structural damage but also reduce pain in a post-traumatic OA model in mice. Since macrophages play important role in modulating OA pain, in the study, we examined and compared post-traumatic OA pain-related behavior in mice with deficiency of AMPK α 1 or ACLY specifically in myeloid lineage.

METHODS: AMPK α 1^{fllox/fllox} or ACLY^{fllox/fllox} mice were crossed with LysMCre mice to generate AMPK α 1^{fl/fl}LysMCre+ or ACLY^{fl/fl}LysMCre+ mice, which are myeloid-specific AMPK α 1 knockout (mAMPK α 1KO) or mACLYKO mice, respectively. AMPK α 1^{fl/fl}LysMCre- or ACLY^{fl/fl}LysMCre- mice were used as wild type (WT) control. WT mice (male n=7, female n=6), mAMPK α 1KO (male n=8, female n=7) and mACLYKO (male n=8, female n=7) at ~4 months of age were subjected to the destabilization of medial meniscus (DMM) surgery on the right knee. At 1 week before (baseline), 2-, 4-, 6-, and 8-weeks post DMM surgery, pain-related behavior was evaluated by SMALGO, Von Frey, static weight bearing tests and to assess pressure-based hyperalgesia (primary), mechanical stimulus evoked hyperalgesia (secondary) and pain-induced weight distribution asymmetry.

RESULTS: Compared to WT mice, pressure force (SMALGO), paw withdraw threshold (Von Frey) and ipsilateral (DMM) to contralateral ratio were greatly increased in both male and female mACLYKO mice at almost all time points starting from 2-weeks post DMM surgery (Figure A-C), indicating reduction in primary and secondary hyperalgesia and weight distribution asymmetry induced by pain. In contrast, all 3 parameters of pain-related behavior were decreased in male mAMPK α 1KO compared to WT mice with statistical significant difference seen in paw withdraw threshold and ipsilateral to contralateral ratio at 4- and 6- but not 8-weeks post DMM surgery (Figure A,B), suggesting transient aggravating secondary hyperalgesia and weight distribution asymmetry induced by pain. Female mAMPK α 1KO mice, compared to female WT mice, showed slight but not significant decrease in all 3 parameters of pain-related behavior at most of time points studied (Figure D-F).

DISCUSSION: As with WT mice, mice with myeloid-specific deficiency of AMPK α 1 exhibited pain-related behavior after joint injury. Especially, male mAMPK α 1KO mice appeared to transiently aggravate it. In contrast, mice with myeloid-specific deficiency of ACLY greatly reduce pain-related behavior in mice after joint injury without sex bias. These data suggest targeted inhibition of ACLY in myeloid lineage could relieve pain during progression of post-traumatic OA. The underlying mechanism of myeloid-specific ACLY deficiency in relieving pain remains to be determined.

SIGNIFICANCE/CLINICAL RELEVANCE: ACLY is emerging as a drug target for long-term use in metabolic disorders. Our previous studies have demonstrated the role of inhibition of ACLY in maintaining cartilage matrix homeostasis. Pharmacological inhibition of ACLY has potential to be used as an OA disease-modifying therapy.

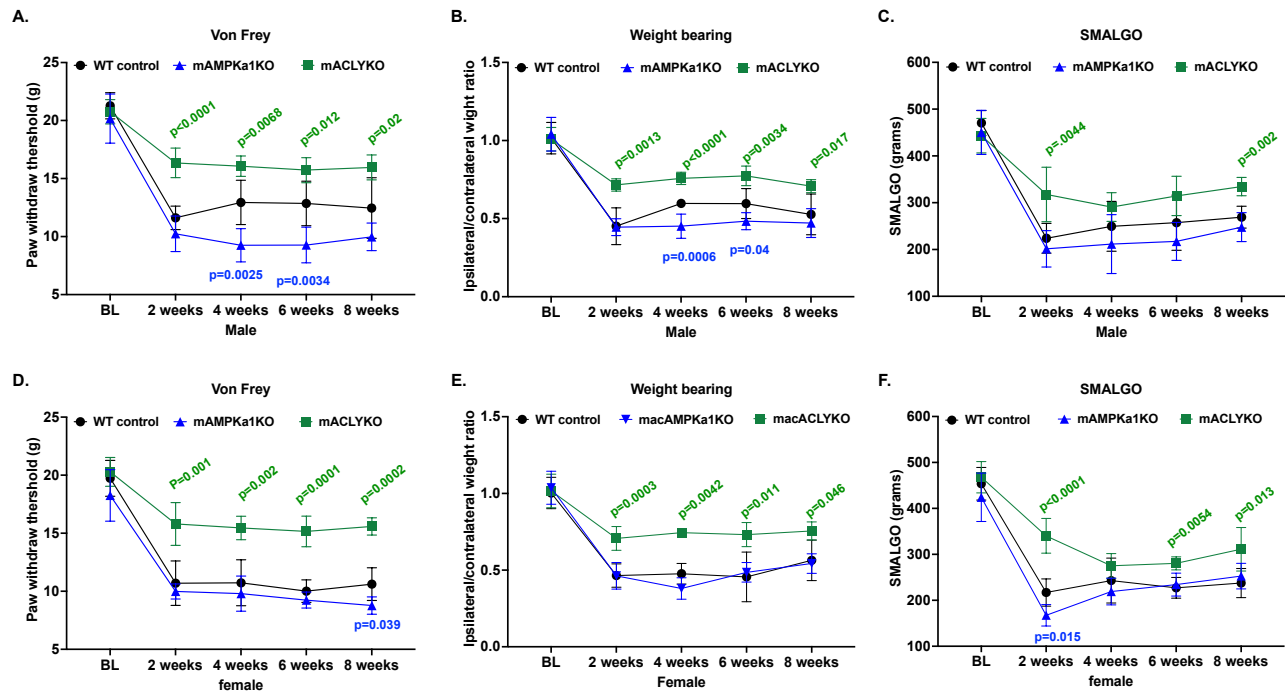


Figure legend. Pain-related behavior during progression of post-traumatic OA. Both male and female WT, mAMPK α 1KO and mACLYKO mice were subjected to the DMM surgery to induce post-traumatic OA. At 1 week before (baseline) and 2-, 4-, 6-, and 8-weeks post-DMM surgery, pain-related behavior was evaluated by Von Frey (A,D), static weight bearing (B,E) and SMALGO (C,F) tests for assessment of pressure-based hyperalgesia (primary), mechanical stimulus evoked hyperalgesia (secondary) and pain-induced weight distribution asymmetry. Data shown at each time point represents mean \pm SEM. GraphPad Prism 10 software was used for the statistical analysis by comparing mAMPK α 1KO to WT or mACLYKO to WT using the mixed effects (repeated measure) model.