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TITLE: Targeting Piezo ion channels for mitigation of osteoarthritis pain and disease progression

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**14. ABSTRACT**

**The purpose** of this project is to determine the roles of Piezo mechano-sensing channels in osteoarthritis (OA) and pain associated with OA using mouse genetic models. Two major aims are proposed.1) assessment of the effect of cartilage-specific loss of Piezo1 and Piezo2 in OA progression in surgically created OA in mice, and 2) assessment of OA-associated pain in Piezo2 haplo-insufficient mice.

**The scope** of this project for the first year is generation of mouse modles and validation, optimization of assay system, and surgical procedure for Aim 1 (neuron specific Piezo KO) and Aim 2 (cartilage-specific Piezo KO) to establish and collect essential tools and information for the second year experiments using mice induced with OA by DMM surgery to assess the effect of Piezo deletion in OA and OA-associated pain.

**Major findings**: The progress of the project is unfortunately significantly slowed down due to colony cutdown and the facility shutdown during the pandemic, and therefore we have collected limited amounts of data.

We have bred floxed Piezo1 and floxed Piezo2 and Cre transgenic mice to generate breeding mice with cartilage-specific and neuron specific Piezo deletion. For Specific Aim 1, we collaborate with the orthopaedic research laboratory at MGH to start testing the feasibility of assessing pain with incapacitance test as well as their gait assessment system using mice received DMM surgery. Unlike reported in a small number of literature, our preliminary data suggest that assessment of mouse OA-associated pain is technically challenging because the changes detected by these assays modest compared with the assay variability. For Specific Aim2, during the first year, we have mainly worked on Major Task3 generating and validating mouse models with cartilage-specific Piezo KO. We have generated and have analyzed the basal phenotype of cartilage-specific Piezo1 knockout mice. Histological analysis suggests that Piezo1 is not essential for normal development nor homeostasis of the knee synovial joint. Generation and analysis of the basal phenotype of Piezo1/2 double knockouts are in progress.

Thus, **the significance** of the result in the first year is that Piezo1 is not required for normal articular and growth plate chondrocytes *in vivo*, unlike bone, suggesting mechanical signaling via Piezo1 in cartilage plays a limited role in normal cartilage physiology. The absence of negative impact of Piezo1 loss is important for ultimately developing Piezo-targeted treatment for OA. In the coming term, we will generate OA in mice with conditional Piezo deletion to evaluate whether suppression of Piezo channels is protective against OA and pain sensation.

**15. SUBJECT TERMS**

Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research

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## 1. Introduction

Osteoarthritis (OA), associated with joint injuries and aging, is a prevalent condition. One of the theories for OA development is culumative mechanical stress. It is known that in response to mechanical stress chondrocytes, a major cell population of joint cartilage, produces diverse molecules to affect the microenvironment including cytocyines and matix proteases. We hypothesize that suppressing response chondrocytes to mechanical stress inhibits progression of mechanical-stress-induced OA. Piezo mechano-sensing channels mediates mechanical stress in chondrocytes and mediates sensation in sensory nerves including nociception. This project aims to determine the role of Piezo mechano-sensing channels in OA progression and chronic pain using genetic mouse models. OA is surgically induced in mice with cartilage-specific and neuron-specific Piezo channel deletion to assess OA and pain. The purpose is to provide scientific basis whether Piezo channels can be therapeutic targets for OA.

## 2. Keywords

Osteoarthritis, mouse, genetic models, mechano-sensing ion channel, Piezo1, Piezo2, pain, joint, synovial.

## 3. Accomplishments

**Major goals**

The following tasks are proposed in SOW during the entire research period:

Major Task 1 (Aim1) : Establish animal model and system validation (neuron-specific Piezo2 and Piezo1 ablation) (original plan 6-12 months)

Major Task 2 (Aim1) : Evaluation of Piezo deletion effects on OA (neuron-specific Piezo2 and Piezo1 ablation). (original plan 6 – 18 months)

Major Task 3 (Aim2) : Establish animal model and system validation (cartilage-specific Piezo deletion) (original plan 6 – 12 months)

Major Task 4 (Aim2) : Evaluation of Piezo deletion effects on OA (cartilage-specific Piezo deletion). (original plan 6 – 18 months)

**Accomplishments under major goals**

Major Task 1 (Aim1) : Establish animal model and system validation (neuron-specific Piezo2 and Piezo1 ablation)

1) Major activities: The reporting period was spend on the subtask1 establish mouse colonies.

2) Specific objectives: Subtask 1) To generate sensory nerve specific Piezo2 (heterozygous) with Piezo1 (homozygous) deletion in mice. Subtask 2) confirmation of gene deletion and the absence of an overt basal phenotype. Subtask 3) confirmation of the absence of basal cartilage phenotype.

3) Significant results

During this period, we bred mice to generate experimental animals. Unfortunately mouse breeding is significantly delayed primarily due to the colony reduction and the inablility to perfom animal breeding caused by the facility shutdown during the pandemic. As or writing, we have established breeder colonies to generate

experimental animals [Avil-CreER:Piezo1(fl/fl) males and Piezo1(fl/fl):Piezo2(fl/fl) females]. Subtask2 and 3 require generation of experimental animals, which we plan to peform in the next term.

4) Other achievements. Nothing to report.

Major Task 2 (Aim1) : Evaluation of Piezo deletion effects on OA (neuron-specific Piezo2 and Piezo1 ablation).

1) Major activities: This Task requires generation of mutant mice with DMM surgery. Unfortunately, we were unable to generate mutant mice for the reason stated above. Meanwhile, we have been establish experimental systems for pain evaluation and optimizing surgical technique.

2) Specific objectives: Subtask 1) To evaluate pain associated with DMM-induced OA in sensory nerve specific Piezo KO mice. Subtask 2) To evaluate the cartilage of sensory nerve specific Piezo KO mice with OA.

3) Significant results

The objectives of this Task were not achieved during the reporting period due to the delay in mouse breeding. We have optimized the surgical technique and using these mice, we tested the pain assessment systems available to us. Unlike chemically induced acute arthritis, chronic joint pain induced by DMM surgery causes very mild behavioral changes. The incapacitance test may not be sensitive enough to evaluate pain. Now we are assessing other gait assessment methods at the the orthopaedic research laboratory at MGH.

4) Other achievements. Nothing to report.

Major Task 3 (Aim2) : Establish animal model and validation (cartilage-specific Piezo2 and Piezo1 ablation).

1) Major activities: We are still in the middle of Subtask 1 to mouse breeding to generate breeder colonies.

2) Specific objectives: Subtask 1) To generate cartilage-specific Piezo KO mice. Subtask 2) To validate the anmal model. Subtask 3) Assessment of basal cartilage phenoytype.

3) Significant results: Although we have not obtained Piezo1 and 2 double conditional KO mice, we have generated cartilage-specific Piezo1 conditional KO as part of this aim, and started analyzing the basal phenotype. Upon histological analysis, we found that there were no overt abnormalities in knee joints in 3-month old animals (Figure). This result suggests that Piezo suppression itself has no negative effects in cartilage development or homeostasis. As of writing, we have established breeding colonies and the first Piezo 1 and 2 double conditional KO mice are almost ready for basal phenotyping. During the reporting period, we started evaluating the technical consistency in creating OA using DMM (destabilization of medial meniscus) surgery.

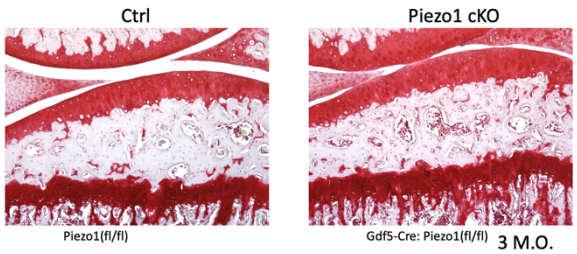

Fig. Safranin-O-stained sections of 3-month old male mice with Piezo1 cKO. No reduction in the proteoglycan content. Cartiage structure is normal. No OA-like changes.

4) Other achievements. Nothing to report.

Major Task 4 (Aim2) : Evaluation of Piezo deletion effects on OA (cartilage-specific Piezo2 and Piezo1 ablation).

1) Major activities: We are still in the middle of generating experimental mice. None of this major Task has been initiated in the first year.

2) Specific objectives: Subtask 1) To evaluate pain in cartilage-specific Piezo KO mice with OA. Subtask 2) To evaluate the cartilage phenotype of cartilage specific Piezo KO mice with OA.

3) Significant results: Nothing to report

4) Other achievements: Nothing to report

**Opportunities for training and professional development**

This project provided leaning opportunities for two US college graduates (BS) and a Chinese MD, PhD student. Unfortunately, he had to go back to China 3 months of after joining due to the pandemic and the anti-Chinese policy of the former administration. The project and its progress were also presented at departmental meetings.

**Results dissemination**

Nothing to report

**Plan to do during the next reporting period**

We plan to complete the remaining tasks. Based on the preliminary pain assessment experiments, pain assessment of mouse OA model would require a more sensitive methods. We plan to allocate time to establish a pain evaluation method in collaboration with the orthopaedic research lab first before expanding sensory-neuron specific Piezo KO mice. Accordingly, we will prioritize Task 3 and 4 to acquire data on cartilage-specific Piezo deletion.

## 4. Impact

**Impact on the development of the principal discipline of the project:**
Since the project is still in the tool generation and system setup/validation stage, currently we have not obtained conclusive results. The finding that Piezo1 deletion has no deleterious effects on cartilage development or maintaince is significant from the point of view of translational research targeging Piezo as a therapeutic strategy.

**Impact on other deciplines:** Nothing to report.

**Impact on technology transfer:** Nothing to report.

**Impact on society byond science and technology:** The finding obtained so far has little impact socially, but if the final result is positive, it may lead to a novel therapy for OA for which there is no medical cure.

## 5. Changes/Problems

**Actual or anticipated problems and plans to resolve them:**
1) In this reporting period, we found that assessment of pain associated with mouse OA was challenging as OA in mice apparently causes very mild behavial changes. We are testing other potentially more sensitive gait assessment methods that the orthopaedic research lab at MGH has in comparison with incapacitance weight bearing test.

2) After initiation of this project, several papers were published reporting that Piezo1 deletion in osteoblasts (bone) causes severe osteoporosis [PMIDs: 33180358 (2021), 32186512 (2020), 31941964 (2020), 31290742(2019)]. In the original proposal, we proposed to use Acan-CreER to delete Piezo 1 and 2 in chondrocytes. However Acan-CreER is also expressed in osteoblast progenitors [PMID: 25419849 (2014)], Acan-CreER will likely delete Piezo 1and 2 in a significant portion of ostelblasts over the course of experimental period. It is known that the subchondral bone integrity or bone shape (possibly altered by pathological fractures) changes mechanical stress to the joint and influeces OA development. For this concern, we plan to use articular cartilage-specific Gdf5-Cre as a Cre driver to delete Piezo1 and 2 specifically in joint chondrocytes.

**Significant changes in animal experiments:**
As described in 2) above, we will use Gdf5-Cre instead of Acan-CreER to delete Piezo 1 and 2 in cartilage in fear of compromising bone integrity. IACUC amendment has been submitted. ACURO was contacted.

## 6. Products

**Journal and other publications, meeting presentision, Web sites**
Nothing to report

**Technologies technique**
Nothing to report

**Inventions, patent applications, licenses**
Nothing to report

**Other products**
Nothing to report

## 7. Participants & Other Collaborating Organizations

| | |
|---|---|
| Name: | *Tatsuya Kobayashi* |
| Project Role: | *PI* |
| Researcher Identifier (e.g. ORCID ID): | 0000-0003-4264-5117 |
| Nearest person month worked: | *6* |
| Contribution to Project: | *Mouse management and analysis* |
| Funding Support: | *NIH and current project* |

| | |
|---|---|
| Name: | *Duo Xu* |
| Project Role: | *Visiting scholar (MD)* |
| Researcher Identifier (e.g. ORCID ID): | N/A |
| Nearest person month worked: | *3* |
| Contribution to Project: | *Mouse management system setup* |
| Funding Support: | Harbin Medical University Scholarship |

| | |
|---|---|
| Name: | *Melissa Caffrey* |
| Project Role: | *Research technician* |
| Researcher Identifier (e.g. ORCID ID): | N/A |
| Nearest person month worked: | *3* |
| Contribution to Project: | *Mouse management and genotyping* |
| Funding Support: | *NIH and current project* |

| | |
|---|---|
| Name: | *Cameron Young* |
| Project Role: | *Research technician* |
| Researcher Identifier (e.g. ORCID ID): | N/A |
| Nearest person month worked: | *2* |

| | |
|---|---|
| Contribution to Project: | *Mouse management and genotyping* |
| Funding Support: | *NIH and current project* |

**Changes in the active other support of the PD/PI(s) or senior/key personnel**

Nothing to report

**What other organizations**

Nothing to report

**8. Special Reporting Requirements**

Nothing to report

**9. Appendices**

Nothing to report