AWARD NUMBER: W81XWH-19-1-0186

TITLE: Targeting Piezo Ion Channels for Mitigation of Osteoarthritis Pain and Disease Progression

PRINCIPAL INVESTIGATOR: Tatsuya Kobayashi

CONTRACTING ORGANIZATION: Massachusetts General Hospital, Boston, MA

REPORT DATE: October 2021

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2021	**Annual report**	30Sep2020-29Sep2021

4. TITLE AND SUBTITLE

Targeting Piezo Ion Channels for Mitigation of Osteoarthritis Pain and Disease Progression

5a. CONTRACT NUMBER
W81XWH-19-1-0186

5b. GRANT NUMBER
PR181712

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Tatsuya Kobayashi

E-Mail: tkobayashi1@mgh.harvard.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)

MASSACHUSETTS GENERAL
HOSPITAL
DAVID WALDRON
55 FRUIT ST BOSTON MA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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 second year is generation of genetic mouse modles and validation, and creation and analysis of OA in these models for Aim 1 (neuron specific Piezo KO) and Aim 2 (cartilage-specific Piezo KO).
Major findings: The entire project is unfortunately pushed back due to colony cutdown and the facility shutdown during the pandemic, and therefore we have not been able to complete.

We have successfully created surgry-induced mouse OA in cartilage-specific Piezo1/2 KO mice and we began to collect tissue samples and pain-assessment data. A part of these samples are analyzed.
For Aim 1, due to unforeseen breeding issues, we have just created a neuron-specific KO mouse colony and are starting creating OA in these mice.
For Aim 2. We found that joint cartilage-specific Piezo1/2 doubly null mutat mice [Gdf5-Cre:Piezo1(fl/fl):Piezo2(fl/fl)] show normal joint cartilage development and homeostasis at the age of 6 month. Thus far, we have obtained histological data of two cartilage-specific Piezo1/2 KO and one control littermate that were surgically induced with OA. The data suggest that Piezo1/2 KO mice show milder degree of OA compared with the control. We will test whether Piezo1/2 deletion lessens OA progression after analyzing more samples. As for pain-assessment, based on analysis of using incapacitance meter, OA induced pain both in control and cartilage-speicific Piezo1/2 KO mice at 1 through 3 months after OA-inducing DMM surgery. Due to the small sample number (3 each) as of now, we are unable to reach a firm conclusion, but so far, presence or absence of Piezo1/2 in cartilage does not seem to significantly influence pain.

The significance of these finding thus far, Piezo1 and 2 are not required for normal articular and growth plate chondrocytes *in vivo* (at least up to 6 months), suggesting mechanical signaling via Piezo1 in cartilage plays a limited role in normal cartilage physiology. It is too premature to conclude, but Piezo1/2 KO cartilage may show lesser degree of OA change upon DMM surgery compared with control. Therefore, it is suggested that inhibition of Piezo1/2 in joint cartilage is safe and possibly beneficial for OA.

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

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
				9	USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

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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 spent 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. After resumption of animal breeding, we encountered breeding issues, which again delayed colony establishment. Now we have started getting mice with desired genotypes, and are growting them to 3 months of age when we plan to perform DMM surgery to induce OA.

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. We are currently growing mice to the age for OA induction.

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. Meanwhile, we have tested two pain assessment systems, the incapacitance meter test and the gait analysis method that MGH's orthopaedic research laboratory developed. We found that the incapacitance meter can detect weight-baring imbalance better than gait analysis. DMM surgery in control mice decreases the weight baring of the surgery side by 20-30% compared to the contralateral side.

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 now finishing Subtask 1. Subtask 2 will be done with samples obtained in Subtask 3. Subtask 3 has started.

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: We found that deletion of Piezo1 and 2 in joint cartilage using Gdf5-Cre transgenic mice [Gdf5-Cre:Piezo1(fl/fl):Piezo2(fl/fl)] causes no overt abnormalities at 3 and 6 months of age. This result suggests that Piezo suppression itself has no negative effects in cartilage development or homeostasis.

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 started inducing OA in cartilage-specific Piezo1/2 KO mice by DMM surgery at 3 months of age. Pain is analyzed by the incapacitance test, and histology is analyzed at 6 months of age (3 months after OA induction). We have started obtaining data. We harvested and processed two KO and one control samples. As shown in Fig. 1. DMM surgery induced OA in two Piezo1/2 KO with the OARSI score of 3 at the posterior side of the tibial plateau, and in the control with the OARSI score of 5.

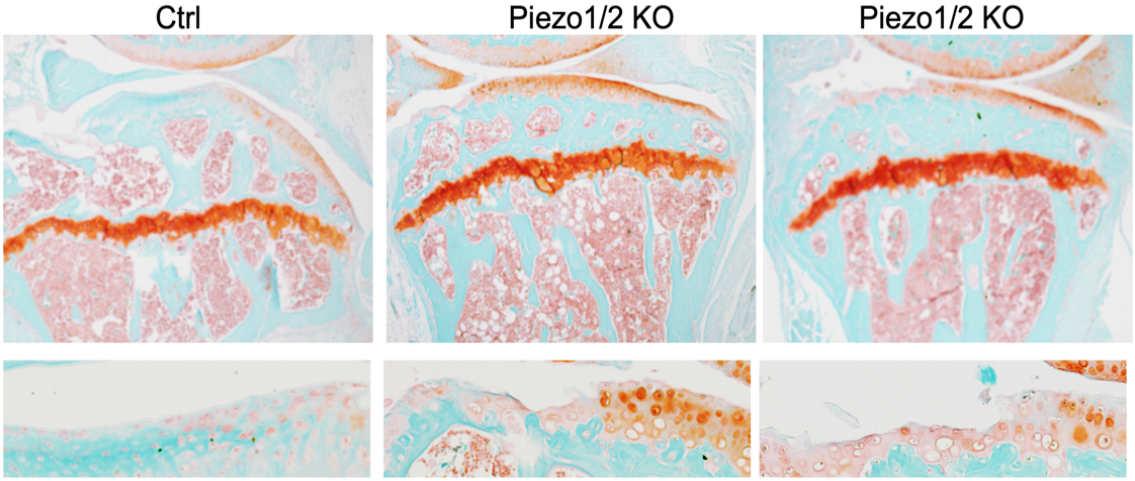

Figure 1. Safranin-O-stained sections of 6-month old male mice with Gdf-5-Cre:Piezo1/2 cKO mice that received DMM surgery at the age of 3 months. OA was induced in the posterior side of the tibial plateau. OARSI OA score; Ctrl 5, Piezo1/2 KO 3.

For pain assessment, we have not observed

beneficial effects of Piezo1/2 deletion in cartilage, although the sample number as of now is too small to reach a firm conclusion (Fig.2).

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: Piezo1/2 KO might show less severe OA damage (to be confirmed by analyzing a greater number of samples).

4) Other achievements: Nothing to report

Opportunities for training and professional development

This project provided learning opportunities for two US college graduates (BS) and a Chinese MD, PhD student. Unfortunately, he had to go back to China due to the pandemic and the anti-Chinese policy of the former administration. This project and its progress are 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 using the no cost extention period.

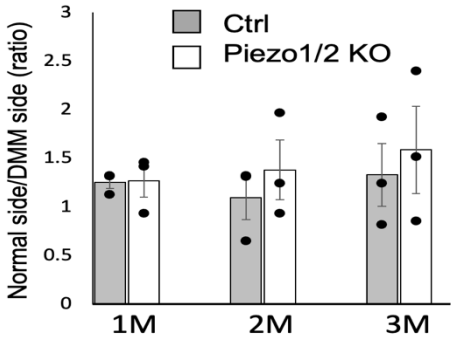

Figure 2. Pain assessment using the incapacitance test at the indicated periods after DMM surgery. Weight baring of the limb with surgery is 20-30% less than the contra-lateral side. There are no signficiant difference between Ctrl mice and cartilage-specific Piezo1/2 KO mice, although the sample size is small (n = 3 each).

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 Piezo1/2 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 the incapacitance meter test was the best pain assessment method available to us. However, the values obtained from this analysis are still highly variable. This is caused by combination of biological and technical variations. This can be resolved as the sample number increases. We will analyze the degree of pain assessed by this method and the extent of cartilage damage assessed by histology. This analysis might reveal the relationship between OA and pain.
Mouse breeding and growing animals to build experimental mouse colonies are the most time consuming part. We increased breeding pairs to accelerate this process, but impact of the facility shutdown last year still lingers. No solution other than increasing the magnitude of mouse breeding.

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 swithced to use articular cartilage-specific Gdf5-Cre as a Cre driver to delete Piezo1 and 2 specifically in joint chondrocytes. This decision delayed building up the mouse conlony. Fortunately, Gdf5-Cre-mediated deletion of Piezo1 and 2 show no significant deleterious effects on cartilage development or homeostasis, and we hope to get interpretable results.

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:	*4*
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 (Year 1 only)*
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:	6
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:	9
Contribution to Project:	*Mouse management, surgery, data analysis*
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