Comparing the mandibles and eruption patterns of 2 osteocyte-specific Dicer deficient mouse models

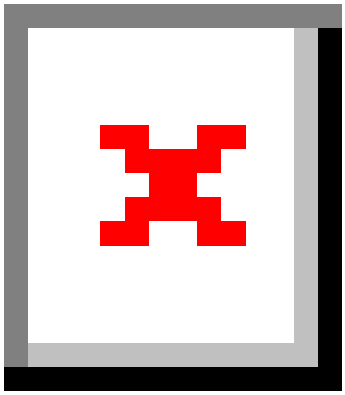
2022 Research Aid Awards (RAA)

Dr. Youngjae Sung

ysung6@uic.edu
O: 781-315-3394

FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

Comparing the mandibles and eruption patterns of 2 osteocyte-specific Dicer deficient mouse models

Award Type

Research Aid Award (RAA)

Period of AAOF Support
July 1, 2022 through June 30, 2023

Institution
University of Illinois Chicago

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Youngjae Sung (PI), Dr. Phimon Atsawasuwan (mentor, Co-I)

Amount of Funding
$5,000.00

Abstract
(add specific directions for each type here)

Osteocytes are bone-regulatory cells that originate from osteoblasts secreting bony matrix around themselves. As osteocytes mature and embed themselves in bone, specific markers have been identified at different maturation stages, which allow visualization of the formation process of the osteoblast prototype to the mature osteocyte. Dentin matrix protein-1 (DMP1) was found to be a key genetic marker in the differentiation of osteoblasts to early osteocytes, particularly during the mineralization and bone regulation process. As the osteocyte continues to mature, sclerostin (SOST) is shown to be highly expressed in mature osteocytic phenotypes. Osteocytes are reported to play a crucial role in bone homeostasis via controlling osteoclast activity. The in vivo ablation of osteocytes led to delayed tooth movement and defective bone resorption via reduced osteoclast numbers and their activities. Tooth movement and tooth eruption are complex biological processes and are dependent on osteoclastic activity, which implicates the potential roles of osteocytes in such processes. MicroRNAs (miRNAs) have been shown to take a central role in the regulation and remodeling of bone. The in vivo deficiency of Dicer, an enzyme essential for miRNA maturation and function, in osteoblasts or osteoclasts led to disruptive bone phenotypes. Until now, the effect of an in vivo deficiency of Dicer in osteocytes has not been elucidated. In this study, we propose to investigate the effect of Dicer deficiency in two models of transgenic mice; a conditional Dicer deficient under Dmp1 promotor (Dicer/Dmp1 KO) and a conditional Dicer deficient under Sost promotor (Dicer/Sost KO) on their mandible phenotypes and tooth eruption patterns.
To characterize and compare the mandible phenotypes and tooth eruption patterns of Dicer/Dmp1 KO mice, Dicer/Sost KO mice and their controls (Dicer flox, Dmp1cre, and Sostcre) mice, we will use a total of 80 mice (40 males and 40 females) consisting of 16 Dicer/Dmp1 KO, 16 Dicer/Sost KO, 16 Dicer flox, 16 Dmp-1cre, and 16 Sostcre mice. The animals will be sacrificed at 4 weeks and 10 weeks old. Mandibles will be harvested and stored at -80°C until use. One side of the hemimandibles will be used to evaluate the expression levels of the following osteocyte markers Podoplanin, Mepe, Phex, Dmp1, and Sost, osteoblast markers Runx2, Sp7, and Ocn and osteoclast differentiation factors Opg and Rankl using quantitative real-time PCR analysis. The other side of the hemimandibles will be fixed with 10% formalin, and subjected to microcomputed tomography (microCT) and histomorphological studies. After microCT scanning, the specimens will be subjected to demineralization and processed for H&E staining, to evaluate the distribution of cells, thickness of bone-matrix, and the eruption patterns of molars. The statistical analysis of the differences will be analyzed with a non-parametric statistical analysis at the significance level of 0.05.

We hypothesize that there will be a difference in the expression level of osteocyte markers, bone thickness, bone volume, and tooth eruption patterns among the tested animals. The results will provide more insights into the roles of functional microRNAs in mature osteocytes, leading to abnormal phenotypes of mandibles and eruption patterns of the teeth of the Dicer/Dmp1 KO and Dicer/Sost KO mice compared to their control mice. I plan to present these study findings at the annual session of the American Association of Orthodontists (AAO), and the annual meeting of the American Association of Dental, Oral, and Craniofacial Research (AADOCR). This type of research is one that I continue to have much interest in. In the future, I hope to continue my role in research as a part-time educator at an orthodontic program, so that I can contribute to the knowledge of the future generation of orthodontists.
Aims and Objectives
To examine the effect of functional miRNA deficiency in osteocytes at different stages of maturation on mandible phenotypes and tooth eruption patterns in vivo, the Dicer deficiency mouse models driven by Dmp1 and Sost-cre promotor will be evaluated. Shedding light on how miRNAs can affect osteocytes at different stages of maturation (i.e. Dmp1 vs. Sost gene targets) can be an important step in elucidating the origin of bone-regulatory diseases and roles of miRNAs in osteocyte during the tooth eruption. Understanding the role of osteocytes in bone remodeling/modeling for tooth eruption is clinically important in craniofacial orthodontics, and examining the differences in how these mechanisms happen in pathological forms of bone, can expand the therapeutic scope of orthodontics in patients with craniofacial disorders.
Hypothesis:
(A) There are no differences in mandible phenotypes and the tooth eruption patterns between the two animal transgenic mouse models due to their Dicer deficiency at different stages of osteocytes, as well as in bone cortical thickness, bone mineral density and volume in both trabecular and cortical bones in the two animal models.
(B) There are no differences in thickness of cortical bone and cell distribution of the mandibles at the histological level between both transgenic animals.

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

Sung_E_Poster.pdf
Due to time and sample constraint (i.e. there were not enough sample mice in the listed genotype/sex groups to do a comprehensive analysis), the project was changed to the following:

Calvarial characterization in Novel Dicer-deficient Mouse Models Specific to Osteocytes

This project has a similar trajectory to the previous approved proposal, but with a focus on quantifying and analyzing the differences in cranial vault and calvarial shape development rather than mandibular phenotypes.

A poster has been uploaded, with results and analysis.

Were the original, specific aims of the proposal realized?*

Unfortunately due to time and constraints related to mouse sample availability, the original aims had to be altered to take account for the given circumstances. We believe that the new project does have clinical merit, and we hope that with time, the original proposal will be realized and adequate data and analysis will be done to complete its specific aims.

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*

AAOF funding has been instrumental for this project's completion. With the aid of AAOF, we were able to obtain and utilize supplies and software to better understand how various bone-regulatory cells can affect various processes and phenotypes relating to craniofacial development in mice, which we believe can lead us to better understand how bone-related pathologies (specifically osteocytes) can affect the skull and how it develops.

I believe that with this knowledge, it can allow us to elucidate how craniofacial disease can affect skull development, which is an area of research that is of particular importance to the field of orthodontics. With increased data of how bone-regulatory cells work in skull development, it will allow orthodontists to intervene and treat affected patients with better knowledge and deeper understanding.

I want to use the information and data from this project, which would not have been possible to complete without AAOF funding, to inform my future decisions as a clinician and member of AAO. AAOF has provided the opportunity to me and my colleagues to explore areas of research that are not just of interest to us, but can benefit the field of orthodontics as a whole. This whole process was one that I am grateful for, and I am excited to see our potential as clinicians and researchers grow.

Comment: *The results of this study, although you needed to pivot the project goals are laudable.*

Accounting: Were there any leftover funds?

$0.00

Not Published

Are there plans to publish? If not, why not?*

There are plans to publish the results once there is additional data for the specific group (male Sost-cre transgenic mice), allowing for comprehensive analysis of the differences in the listed phenotypes above. The calibration of a specific area of histological sections to confirm the findings of the microCT studies need to be done to avoid the bias from the location selection. In addition, the in-depth analysis of GPA among genotypes in each sex group of mice needs to be performed to examine the severity of shape differences among each genotype in male and female groups. Once these tasks are complete, the data should be ready to publish.

Comment: *I approve this report but also encourage you to submit results in a Word or PDF format if possible. I commend you on the project and look forward to your future successes in publishing and future grants.*

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

New title: Calvarial characterization in Novel Dicer-deficient Mouse Models Specific to Osteocytes

Authors:

Youngjae Sung, University of Illinois at Chicago, 2023
Dr. Phimon Atsawasuwan, University of Illinois at Chicago
Dr. Christina Nicholas, University of Illinois at Chicago
Dr. Maysaa Oubaidin, University of Illinois at Chicago
Ms. Grace Viana, University of Illinois at Chicago
Dr. David Reed, University of Illinois at Chicago
Dr. Anne George, University of Illinois at Chicago

Was AAOF support acknowledged?

If so, please describe:

Yes, it was acknowledged in the presentation and poster.

Internal Review

Reviewer comments

[Unanswered]

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- Sung_E_Poster.pdf

Calvarial characterization in Novel Dicer-deficient Mouse Models Specific to Osteocytes

Youngjae Sung, Grace Viana, Maysaa Oubaidin, David Reed, Phimon Atsawasuwan

April 22, 2023

Background and literature review

- Proper skull development and ossification requires precise regulation of genes and signaling pathways at the **suture-bone** interface (Opperman et al, **2005**)
- Craniosynostosis:
  - Syndromic– part of a constellation of severity and symptoms (often genetic)
  - Simple– one suture fusing
- Osteocytes: originates from osteoblasts that secrete bony matrix around themselves
  - Can control bone response to mechanical stimuli
- Genetic markers are expressed at different stages of osteocyte maturation (Matsumoto et al, **2013**)
  - Dentin matrix protein 1 (DMP1)– highly expressed in early-forming osteocytes (Yang et al, **2005**)
  - **Sclerostin (Sost):** highly expressed in **fully mature osteocytes** (Dallas et al, **2013**)

Background and literature review

- MicroRNAs have been an increasingly important field of interest in gene regulation and cellular mechanisms
  - Embryo development (Yang et al, **2005**)
  - Progression in breast cancer (Khoshnaw et al, **2012**)
  - Formation of proper chromosome structure (Fukagawa et al, **2004**)
- **Dicer-1:** endoribonuclease that is crucial part of miRNA processing
  - **Inactivation of Dicer:** a wide range of changes in regulatory mechanisms and phenotypes
- Pairing a Dicer floxxed gene with a Cre recombinase utilized under a specific promotor will allow to knock out Dicer in that specific gene

Objectives

- To examine the effect of an *in vivo* deficiency of Dicer in the cellular regulation of osteocytes.
- To evaluate the regulatory role of mature miRNAs in osteocytes, and how calvarial bone development can be subsequently affected.
- To characterize and quantify the differences in sutural development in a sclerostin (*Sost*) promotor driven Dicer deficient mouse model.

Hypothesis

- Based on our objectives, we have formulated the following null hypotheses:

H_0: There is no difference in calvarial bone and sutural phenotypes and morphologies in Sclerostin promotor-driven Dicer-deficient mice and control mice.

H_0: There is no discernable effect of the absence of functional miRNA in osteocytes on calvarial bone and sutural development at a histomorphological level.

Materials and Methods

To evaluate the calvarial phenotypes of Dicer-deficient under an osteocyte-rich promotor (Sost promotor) (UIC ACC no 22-197), both male and female mice (N=18) of the below genotypes were bred:

- ***Dicer-deficient under Sost promotor mice*** (Sost/Dicer KO; Dicerfl/fl/Sost+) (n=10; 5 males and 5 females)

- ***Dicer floxxed mice*** (Dicer flox; Dicerfl/fl/Sost-) (n=8; 4 males and 4 females)

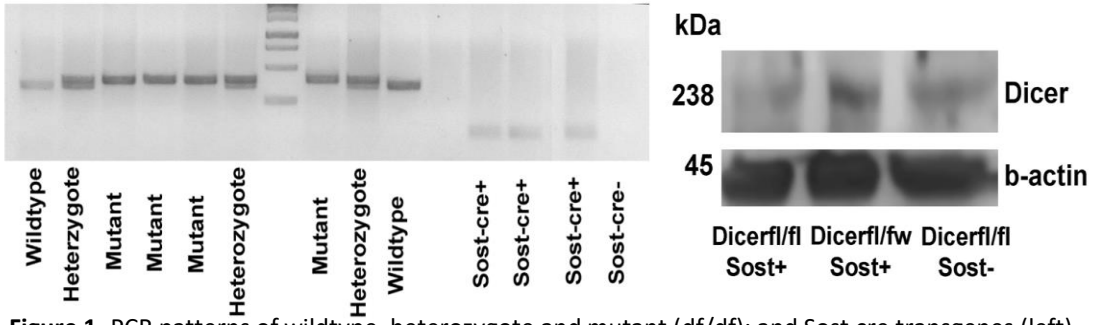


Figure 1. PCR patterns of wildtype, heterozygote and mutant (df/df); and Sost cre transgenes (left) and Western blot analysis confirms less expression of Dicer in dicer deficient mouse (Dicerfl/fl/Sost+ compared to its control (Dicerfl/fl/Sost-). b-actin was used to normalized the Dicer protein.(right)

Animals' genotypes were verified with PCR tail genotyping and Western blot analysis of bone lysates against anti-Dicer antibody.

Materials and Methods

All animals were subject to the following experiments:

Microcomputed tomography (µCT)
To evaluate calvarial bone phenotypes and posterior frontal suture patency, the Scanco µCT 40 system (Scanco Medical) with 10µm resolution was utilized. Calvarial anatomy and bone quality were assessed and compared among the animals.

Histology: Hematoxylin and eosin (H&E) staining
Fixed calvaria from each genotype were subjected to demineralization, microtomed, paraffinated and stained with H&E staining for histological evaluation at 20x magnification.

General Procrustes Analysis (GPA)
Each calvarial µCT DICOM file was converted to STL file. GPA was used to analyze raw coordinate landmark data and to compare specific calvarial shape across genotypes.

Statistical analysis: The data distribution was tested with Shapiro-Wilk test. Student t-test statistical analysis and ANOVA with Post-hoc Bonferroni multiple comparisons was performed. Kruskal-Wallis test with Post-hoc Dunn multiple comparisons were performed in GPA. The statistical significance was 0.05.

Results

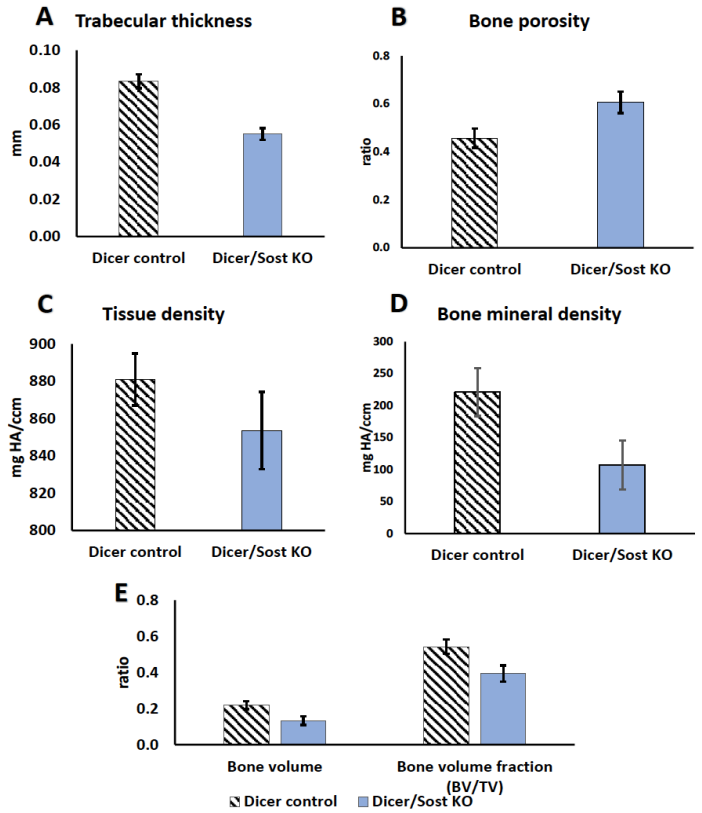


Figure 2. In males, significant differences were found in 6 parameters; namely, bone volume, bone volume fraction, trabecular thickness, bone mineral density, tissue density and bone porosity. Note that all parameters except bone porosity, the Dicer/Sost KO exhibited less values than the controls. P<0.05.

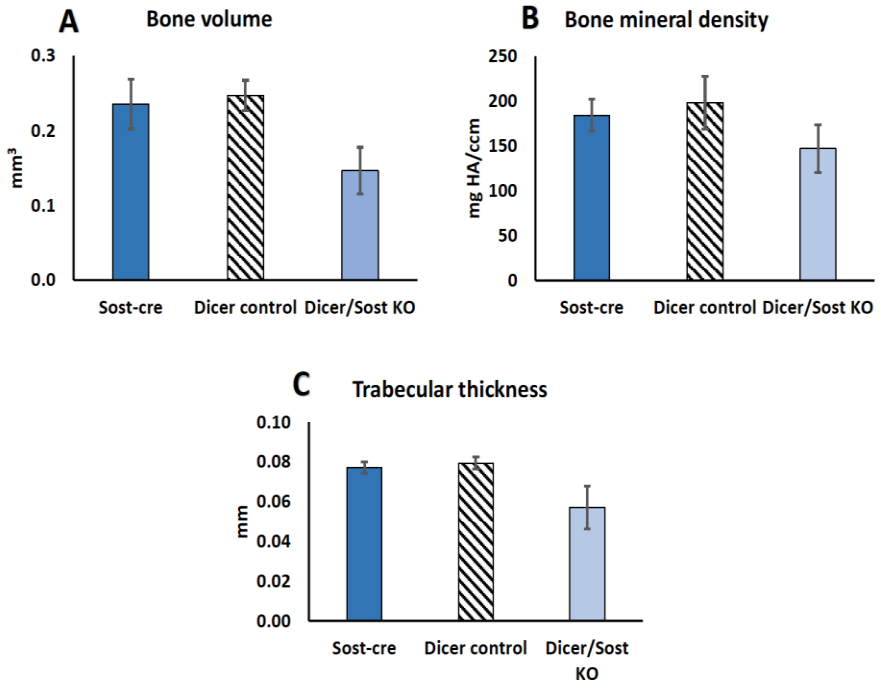


Figure 3. In females, significant differences were found in 3 parameters; namely, bone volume, trabecular thickness, and bone mineral density. Note that all parameters, the Dicer/Sost KO exhibited less values than the controls. P<0.05.

Results

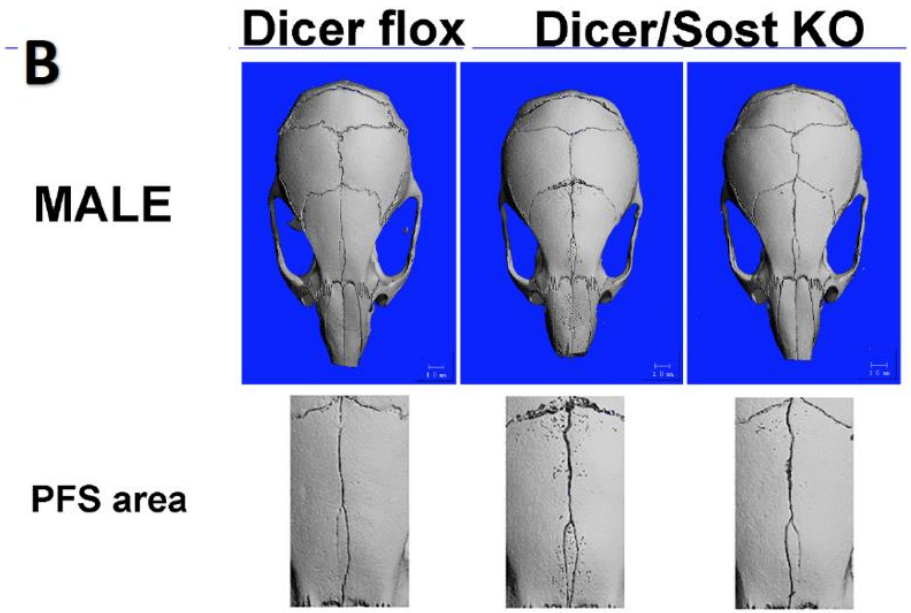


Figure 4. In males, 3D rendered photos of Dicer flox (control) and Dicer/Sost KO skulls (upper panels) confirmed the findings from bone parameters. The magnification of PFS area exhibited more patency of suture and poorer bone quality in the Dicer/Sost KO mice compared to the control.

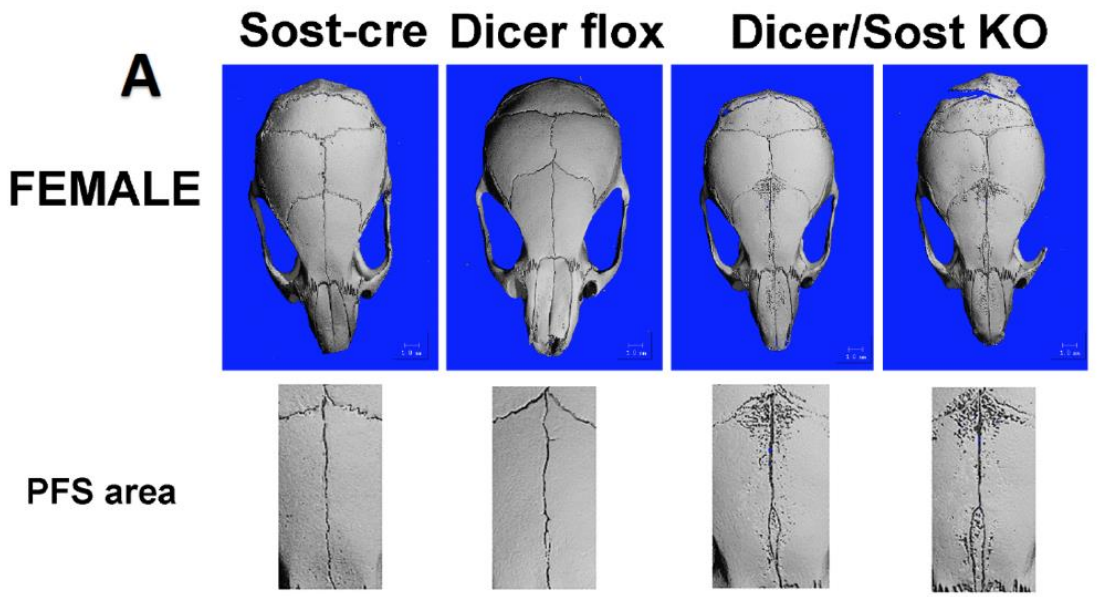


Figure 5. In females, 3D rendered photos of Dicer flox (control) and Dicer/Sost KO skulls (upper panels) confirmed the findings from bone parameters. The magnification of PFS area exhibited more patency of suture and poorer bone quality in the Dicer/Sost KO mice compared to the control.

Results

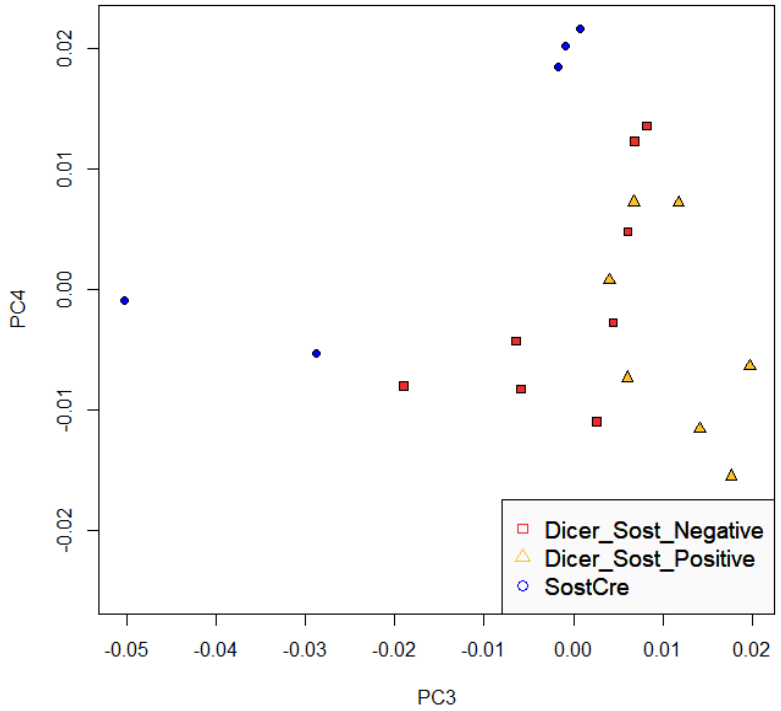


Figure 6. Principal component analysis of the landmark data from GPA. Note that the data point distribution of the Dicer flox group (in red) overlaps the Dicer/Sost KO group and does not show as distinct of a difference compared to the control group.

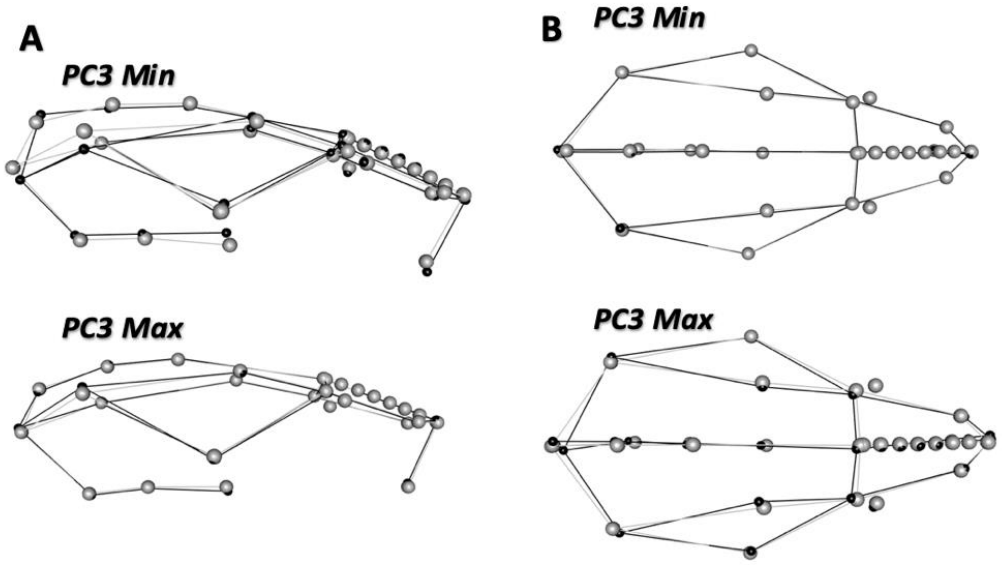


Figure 7. The minimum and maximum extremes of variations along PC1. (A) The sagittal view. (B) The axial view. Control mice appear to have more convex nasal bridges and more globular crania, with larger dimensions transversely and anteroposteriorly in the calvaria.

Results

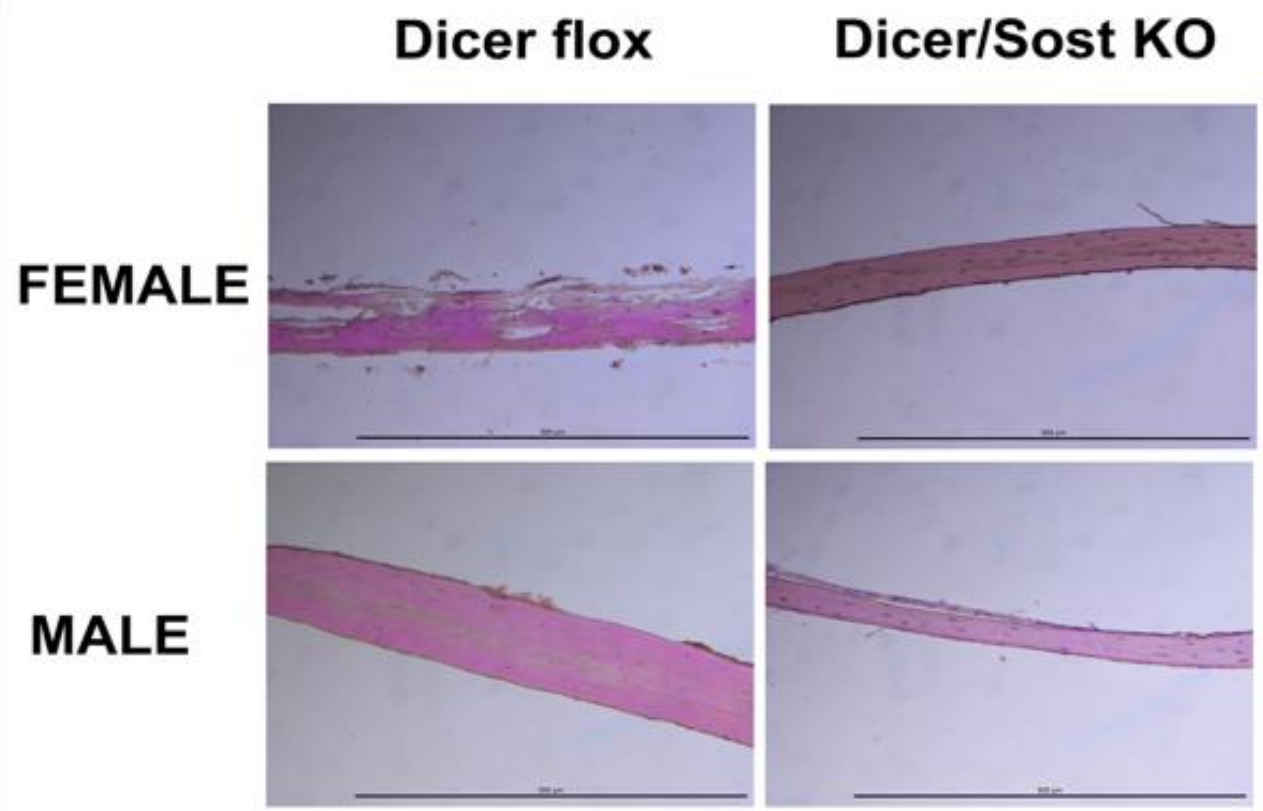


Figure 6. H&E images at 20x magnification of calvarial bone sections of female and male mice by genotype. There is markedly thicker bone in the calvarial vault of the Dicer flox control mice compared to Dicer/Sost KO mice. Note that the thickness of calvaria vault seems to be thinner in male Dicer/Sost Ko compared to the one in female Dicer/Sost KO.

Conclusion

. Dicer deficiency in osteocytes led to a **dysmorphic** phenotype of the calvaria and skull shape.

. Dicer deficiency in osteocytes led to more patency of posterior frontal suture compared to the ones on the controls

. Dicer deficiency in osteocytes led to poorer calvarial bone quality and less thickness overall compared to the controls at both macroscopic and microscopic levels. In addition, the sexual dimorphism may play a role in the severity of calvarial bone phenotypes in Dicer/Sost KO mice.

. Mechanistic studies are needed to gain more insights into how functional miRNAs in osteocytes control the craniofacial bone phenotypes.

References

1. Fearon, J. A. Evidence-Based Medicine: Craniosynostosis. Plastic and Reconstructive Surgery 2014, 133 (5), 1261–1275. https://doi.org/10.1097/PRS.0000000000000093.

2. Rice, D. P. Clinical Features of Syndromic Craniosynostosis. Front Oral Biol 2008, 12, 91–106. https://doi.org/10.1159/000115034.

3. Carinci, P.; Becchetti, E.; Bodo, M. Role of the Extracellular Matrix and Growth Factors in Skull Morphogenesis and in the Pathogenesis of Craniosynostosis. Int J Dev Biol 2000, 44 (6), 715–723.

4. Yates, L. A.; Norbury, C. J.; Gilbert, R. J. The Long and Short of MicroRNA. Cell 2013, 153 (3), 516–519. https://doi.org/10.1016/j.cell.2013.04.003.

5. Bernstein, E.; Kim, S. Y.; Carmell, M. A.; et al. Dicer Is Essential for Mouse Development. Nat Genet 2003, 35 (3), 215–217. https://doi.org/10.1038/ng1253.

6. Lian, J. B.; Stein, G. S.; van Wijnen, A. J.; et al. MicroRNA Control of Bone Formation and Homeostasis. Nat Rev Endocrinol 2012, 8 (4), 212–227. https://doi.org/10.1038/nrendo.2011.234.

7. Weivoda, M.W., Youssef, S.J., Oursler, M.J. Sclerostin Expression and Functions Beyond the Osteocyte. Bone, 2017, 96 (3), 45-50.

8. Xiong, J.; Piemontese, M.; Onal, M.; et al. Osteocytes, not Osteoblasts or Lining Cells, are the Main Source of the RANKL Required for Osteoclast Formation in Remodeling bone. PLoS ONE. 2015. 10(9): e0138189. doi:10.1371/journal.pone.0138189.

9. Gaur, T.; Hussain, S.; Mudhasani, R.; et al. Dicer Inactivation in Osteoprogenitor Cells Compromises Fetal Survival and Bone Formation, While Excision in Differentiated Osteoblasts Increases Bone Mass in the Adult Mouse. Dev Biol 2010, 340 (1), 10–21.

10. Bendre, A.; Moritz, N.; Vaananen, V.; Maatta, J.A. Dicer1 Ablation in Osterix Positive Bone Forming Cells Affects Cortical Bone Homeostasis. Bone 2018, 106, 139-147.

11. Atsawasuwan P., Ouibaidin M., Dalal B., Khan H., Mohammed A. Calvarial bone development and suture closure in Dicer-deficient mice. Orthod Craniofac Res. 2017 Jun;20 Suppl 1(Suppl 1):26-31. doi: 10.1111/ocr.12169.

12. Mizoguchi, F.; Izu, Y.; Hayata T et al. Osteoclast-specific dicer Gene Deficiency Suppresses Osteoclastic Bone Resorption. J Cell Biochem 2010 109 (5): 866-875.

Acknowledgements

I would like to thank my mentor, Dr. Phimon Atsawasuwan, for all of his help and guidance.

Thank you to all co-authors and collaborators that made this all possible.

This study was partially funded by American Association of Orthodontists Foundation Research Aid Award and Brodie Research Award Department of Orthodontic UIC.