401 N. Lindbergh Blvd.
St. Louis, MO 63141
Tel.: 314.993.1700, #546
Toll Free: 800.424.2841, #546
Fax: 800.708.1364
Cell: 314.283.1983
Send via email to: jbode@aaortho.org and cyoung@aaortho.org

AAO Foundation Final Report Form
(a/o 6/30/2020)

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?*

Please prepare a report that addresses the following:

Type of Award, Biomedical Research Award

Name(s) of Principal Investigator(s): Carlos Flores-Mir, Daniel Graf

Institution: University of Alberta

Title of Project: Etiology and consequences of nasal airway obstruction

Period of AAOF Support (e.g. 07-01-20 to 06-30-21): 07-01-18 to 06-30-20

Amount of Funding: 30,000 USD

Summary/Abstract:

Abstract

This 4-year project has two broad aims: to understand how changes in cartilage/bone remodeling lead to nasal airway obstruction and in consequence to pediatric obstructive sleep apnea (OSA), and to develop and validate novel, accessible diagnostics for OSA that can be used in clinical practice to screen for OSA.

Bringing together scientists and clinicians, this project will pursue three goals: 1) Characterization of the molecular and cellular processes regulated by Bmp7 that underlie cartilage/bone remodeling using *in vivo* and *in vitro* approaches. 2) Identification of systemic

metabolics changes suitable for use as biomarkers in the diagnosis of OSA. 3) Retrospective and prospective clinical cohort studies with children with OSA to test the metabolics markers for their suitability as novel, accessible diagnostics.

Pediatric OSA is a relatively common sleep breathing disorder often caused by physical obstruction of either nasal and/or pharyngeal airways and affects approximately 1-5 in 100 children. The reduced air intake leads to reduced tissue oxygen levels, which can cause a variety of systemic morbidities including cardiovascular, metabolic, neurocognitive as well as behavioral problems. Early diagnosis and timely surgical intervention have the potential to greatly alleviate or avoid these morbidities. However, such interventions can often not be realized in a timely manner, because of 1) inability to reliably predict future midfacial growth, which affects the precision of the diagnosis and 2) need for novel, accessible diagnostic tests that could complement or replace polysomnography in specialized sleep clinics, for which long waiting-times exist as well as significant associated cost.

We have recently found that mice with a deletion of Bone Morphogenetic Protein 7 (Bmp7) in neural crest cells (*Bmp7*^{ncko}) show similar alterations as some children diagnosed with OSA: displaced nasal septum and asymmetric nasal turbinates. We have found that this is caused in the mice by premature ossification of the nasal septum (perpendicular plate of ethmoid bone - PPE), which leads to nasal airway obstruction and reduced airflow intake. We thus hypothesize that Bmp7 regulates processes that control the ossification of the cartilaginous nasal septum as well as of the turbinates, and that these processes are disturbed in (a large proportion of) children with OSA. We furthermore hypothesize that these processes are also connected to midfacial hypoplasia and reduced bone mineral density seen in the mouse and children. We lastly hypothesize that changes to these processes lead to metabolics changes detectable by metabolomics, which would provide a novel way to diagnose airway obstruction/OSA. To understand the etiology of the PPE displacement, we will use already established methods: comparative analysis of mouse and human computer tomography dataset, histology, immunohistochemistry and gene expression analysis, cell culture to investigate mechanisms. The targeted metabolomics screen will be performed in collaboration with TMIC. Markers will be validated in the mouse and subsequently within a prospective observational clinical cohort study. One central objective of this part of the project is to translate findings from our re-clinical mouse model to the clinic (knowledge translation). Three groups will be followed: Children with signs and symptoms but negative to PSG-diagnosed OSA, and children without signs and symptoms and no PSG-diagnosed OSA.

In summary, by screening the *Bmp7* mutant, we will identify novel biomarkers suitable for diagnosis of consequences of airway obstruction. This will allow simple, accessible diagnosis of airway obstruction/OSA in children.

Detailed results and inferences:

1. If the work has been published, please attach a pdf of manuscript OR
2. 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 be included.

Respond to the following questions:

1. Were the original, specific aims of the proposal realized?

The project is a 4-year project, and specific aims for each year were illustrated in the form of a GANTT chart in the application. For simplicity, the GANTT chart is shown again below. With funding from AAOF have now completed two years of this study and will report below on progress with the project.

Task	***Month***							
	6	**12**	**18**	**24**	**30**	**36**	**42**	**48**
Bmp7 expression in one/cartilage								
In vivo bone/cartilage phenotype analysis								
In vitro bone/cartilage culture analysis								
Additional/extended physiologic assessments								
Mouse sample collection (urine/serum)								
Testing of published diagnostic markers								
Metabolomics screen (primary and validation)								
Retrospective cohort analysis								
Prospective cohort recruitment								
Prospective cohort – clinical assessment								
Prospective cohort – metabolomics marker validation								

At the end of June 2020 (2-year period, end of AAOF funding) the project status is

A. determining BMP7 expression in bone and cartilage
   -> completed
B. In vivo bone/cartilage phenotype analysis
   -> 2x manuscripts are currently under review (Journal of Anatomy, Journal of Physiology), 1x manuscript is in preparation, 4x abstracts submitted (BMP conference, IADR, Gordon Research Conference on Craniofacial Development and Regeneration)
C. In vitro bone/cartilage culture analysis
   -> using ATDC5 chondrocyte progenitor cells and ex vivo chondrocyte pellet cultures, we have identified molecular and cellular consequences of BMP7 on cartilage formation.
D. Extended physiological assessment
   -> morphometric analysis to map craniofacial growth and breathing analysis by plethysmography were completed (manuscript submitted for publication)
E. Mouse sample collection (urine/serum) (completed)
   -> We collected approx. 30 serum samples from mice 2-4 weeks of age for metabolomics analysis.
F. Testing of published diagnostic markers
   -> was not initiated (for explanation, see below)
G. Metabolomics screen (primary and validation) (50% completion)
   -> A primary screen has been performed focusing on markers for bone metabolism, a potential marker has been identified
H. Retrospective cohort analysis
   -> completed, manuscript accepted for publication by AJODO
I. Prospective cohort recruitment (to be initiated)
   -> study concept discussed, ethics approval in preparation

Specific details:

1) Mouse studies (covering aims A-C) established that Bmp7 plays a key role in cartilage differentiation. We identified a hitherto unknown 'lineage choice' between hyaline and elastic cartilage. Bmp7 is required to prevent differentiation of chondrocyte progenitor cells towards elastic cartilage. Deletion of Bmp7 in facial cartilages results in misspecification of cartilage type. Instead of hyaline cartilage, the nasal septum now shows characteristics of elastic cartilage, which is the likely reason for the observed nasal septum deviation. Although the deviation is not observed before 3-4 weeks of age, molecular differences are already noted at birth and become most prominent at 2 weeks of age. A quantitative proteomics analysis was performed and we identified key differences between normal and Bmp7-deficient nasal septum cartilage. These differences go beyond above mentioned changes to extracellular matrix components. They include differences in cell metabolism, epigenetic and proteasome regulation. Latter are all cell-intrinsic differences, supporting the concept that the different cartilage types are the result of cell lineage choices presumably at the progenitor level. This work was presented at the IADR General Session, Vancouver 2019 and the Gordon Research Conference on Craniofacial Development and Regeneration, Il Ciocco, Italy, February 2020. A manuscript describing the role of Bmp7 in determining cartilage type is currently in preparation.
2) To better understand the etiology of the craniofacial defects in Bmp7-mutant mice, we performed a detailed morphometric analysis. This was followed by a breathing analysis using plethysmographs to show that mutant mice invariably develop airway obstruction and apneas very similar to what is observed in children with midfacial hypoplasia and pOSA. Results of this study have just been submitted to the Journal of Physiology.
3) The study described under 2) above prompted us to perform a detailed analysis of histological and cellular characteristics of the mouse nasal septum over time. We found that the nasal septum cartilage is a much more dynamic structure than anticipated. Although generally described as hyaline cartilage, it presents features that are very different from better studied hyaline cartilages in the growth zones of limbs or articular surfaces of joints. This study is currently under review by the Journal of Anatomy.
4) A retrospective cohort (covering aim G) study was performed that illustrates the difficulty to predict airway obstruction and resulting disordered breathing from CBCT scans commonly taken in Orthodontics clinics. Results of this study have been presented at the IADR General Session, Vancouver 2019, and the study was recently accepted for publication by AJODO.

<u>Summative Progress Report</u>

Results from this project have led to date to one publication in press, two publications under review, and one publication in preparation (excepted submission date August/September 2020) as well as several oral and poster presentations at International conferences (IADR, GRC, BMP conference)

The detailed physiological analysis of this Bmp7-mutant mouse as well as the careful molecular and immunohistochemical characterization revealed that cellular changes to nasal chondrocytes lead to reduced growth and altered cartilage properties predisposing to nasal septum deviation. This change in cartilage properties were unexpected, and it will be important to test in future whether similar changes are also observed in children (we plan to do this through analysis of tissue biopsies from corrective septoplasties).

This Bmp7-mutant mouse ($Bmp7^{ncko}$) is to our knowledge the first in detailed characterized model that recapitulates the craniofacial growth deficiencies observed in children with midfacial hypoplasia and shows disordered breathing similar to children. We are planning to use this mouse model in future (second part of this project) to better understand physiologic consequences of disordered breathing as well as for the identification of novel diagnostic markers. We hope that new insights and novel diagnostic tools will help to identify and better manage children at risk of pOSA.

2. Were the results published?
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers

        - Pranidhi Baddam, Claudine Thereza-Bussolaro, Carlos Flores-Mir, Daniel Graf. Nasal cavity structural anomalies among children at high risk of sleep-disordered breathing: an exploratory cone-beam computed tomography study, AJODO (accepted for publication) (AAOF funding acknowledged)
        - Pranidhi Baddam, Tiffany Kung, Adetola Adesida, Daniel Graf. Histological and Molecular Characterization of the Growing Nasal Septum in Mice. Journal of Anatomy, under review (study was realized with leveraged funding)
        - Pranidhi Baddam, Daniela M Roth, Vivian Biancardi, Farah Eaton, Rupasri Mandal, David S Wishart, Amy Barr, Joanna McLean, Carlos Flores-Mir, Silvia Pagliardini, Daniel Graf. Neural crest-specific deletion of Bmp7 leads to midfacial hypoplasia, nasal septum deviation, and disordered breathing. Journal of Physiology, under review (AAOF funding, leveraged funding acknowledged)

    b. Was AAOF support acknowledged? Acknowledged where appropriate (manuscript 1 and 3)
    c. If not, are there plans to publish? If not, why not? An additional publication is in preparation.

3. Have the results of this proposal been presented?
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
        - International BMP conference, Tokyo, October 2018 (Poster)
        - IADR General Session, Vancouver, June 2019 (Oral)
        - Gordon Research Conference on Craniofacial Development and Regeneration, Il Ciocco, Tuscany, Italy, February 2020 (Poster)
    b. Was AAOF support acknowledged? Yes
    c. If not, are there plans to do so? If not, why not?

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

AAOF funding was critical for initiating the career of Pranidhi Baddam and to advance Claudine Bussolaro's. AAOF funding has helped to leverage funding:

- Women and Children Health Institute (WCHRI), University of Alberta CAD$ 50,000

- G & R Sperber Fund in Craniofacial Research, University of Alberta CAD$ 30,000
- NSERC PhD Studentship (Pranidhi Baddam) CAD$ 63,000

Accounting for Project: i.e., any leftover funds, etc.

All funds have been spent.