Type of Award: Postdoctoral Fellowship Award

Principal Investigator: Siddharth R Vora

Title of Project: **IDENTIFICATION AND CHARACTERIZATION OF NOVEL GENES INVOLVED IN MIDFACE HYPOPLASIA WITH CRANIOSYNOSTOSIS**

Period of AAOF Support: 6/1/2013 – 6/1/2015

Amount of Funding: $ 50,000/year

Summary/Abstract:

The pathogenesis of midface hypoplasia (MFH) remains largely understudied, despite its common occurrence and complex management. The co-presentation of MFH and craniosynostosis in patients with craniofacial syndromes (for eg. Apert, Crouzon, Pfeiffer, Muenke) suggests a linked genetic basis. Supportive of this notion, significant midface hypoplasia is also found in many mouse models of craniosynostosis syndromes. This study was aimed at enhancing our understanding of the pathogenesis of midface hypoplasia via the following two aims:

Aim 1: Detailed phenotypic characterization of new craniofacial mutant mouse strains exhibiting midface deficiency and identification of the underlying mutation. Phenotypic analysis identified two discrete types of mid-facial disorders within the investigated mutant strains: midface hypoplasia in the *snol*, *stn* and *shsn* strains and craniofacial microsomia (CFM) in the *sbse* and the *frg* lines. A significant finding in the *snol*, *shsn* and *sbse* strains is the premature fusion of one or more cranial base synchondroses accompanying/preceding the development of the associated midface phenotype. Chromosome region-specific sequencing of mapped regions has revealed the mutations in the *shsn* (*Dnajc25*) and *sbse* (*Plag1*) strains. Efforts to identify the mutations in the *snol*, stn and frg strains are ongoing. Potential candidates include *Gcm2, Hivep1* and *Jarid2* for *frg, Phc2* and *Rrp12* for *snol,* and *Thrap3, Cpeb3 and Hpse2* for *stn.*

Aim 2: Whole genome sequencing of patient exhibiting complex craniosynostosis with associated midface hypoplasia to identify novel causative mutations of midface hypoplasia. Data derived from exome sequencing of 20 trios and 15 diads, was subjected to standard bioinformatics pipelines designed to detect *de novo* SNPs, CNVs, SVs as well as small insertion/deletions. Application of customized and publicly available filtering and pathway analyses algorithms (Endeavour, Ingenuity) identified potential candidate genes which are being prioritized based on assessment of (dys)functional consequences. The work on this aim is ongoing as a preliminary list of targets is being scrutinized for potential causality

Response to the following questions:

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

The original aims of this project have been partially realized and work is ongoing, extending into a third year of post-doctoral research. Specifically, detailed phenotypic analysis is being performed on the *shsn* and *snol* strains to further elucidate the involvement of cranial base

growth defects in the striking midface phenotype in these animals. Data collection in Aim 2 will be extended for another year before final analysis is completed. Additionally, the work resulted in the perusal of related lines of investigation in two main areas. One, the designing of algorithm based automated methods of landmarking the mouse craniofacial skeleton in order to limit the variability introduced into data due to investigator errors and reduce the time/manpower spent on this labor intensive process. Two, conditional knockout strains targeting the Apert mutation has yielded interesting data supporting the findings from our novel mouse strains, enhancing our understating of the overall craniofacial consequences of growth defects in the cranial base. Pilot data, from these studies is currently being used in the preparation of an independent NIH grant application.

2). Were the results published? If not, are there plans to publish? If not, why not?

The following manuscripts (in various stages of publication) reflect the work performed in the past two years:

1) **Vora SR,** Camci ED, Borgeia S, LaCourse M, Cox TC. "Postnatal Ontogeny of the Cranial Base and Craniofacial Skeleton in C57BL6/J mice: A Reference for Quantitative Analysis". (In preparation)
2) **Vora SR,** Camci ED, Finkleman S, Borgeia S, Rolfe S, Cox TC. "Phenotypic Characterization of Novel Mouse Models of Midface Hypoplasia." (In preparation)
3) Aneja D, **Vora SR,** Camci ED, Shapiro L, Cox TC. "Automated Detection of 3D Landmarks for the Elimination of Investigator-introduced Variation for Shape Analyses." **IEEE Eng Med Biol Soc** (April 2015)
4) Cox TC, Camci ED, **Vora S,** Luquetti DV, Turner EE. "The Genetics of Auricular Development and Malformation: New Findings in Model Systems Driving Future Directions for Microtia Research". **Eur J Med Genet.** 2014 May 29.] PMID: 24880027

3). Have the results of this proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?

The results of this work have been presented at the following meetings:

1) Oral presentation: **IADR 2015** – Boston, Massachusetts "Novel *mouse models of midface hypoplasia display cranial base growth defects"*
2) Oral presentation: **Moyers Pre-symposium 2015** – Ann Arbor, Michigan *"From the cranial base to the mid-face"*
3) Oral presentation: **Oral Health Sciences Research Symposium 2014** – Seattle WA "Phenotyping *novel mouse models of midface hypoplasia"*
4) *Poster: 2014 Consortium for Orthodontic Advances in Science and Technology – Itasca IL*
5) *Poster: 2014 Society of Developmental Biology (73rd Annual Meeting)- Seattle WA*

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

The AAOF-PFA has been instrumental in facilitating my perusal of an academic career. In a financially volatile research environment, this award has afforded me the ability to follow an organized and mentored period of research development, which I believe will prove to be crucial to my future goals of obtaining independent research funding. Continued support from the

AAOF is not only encouraging but, more importantly, enabling. I thank the foundation and all of its champions and hope to continue a positive relationship with this organization.