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
E-Mail: rhazel@aaortho.org

AAO Foundation Final Report Form
(a/o 5/31/2016)

Please prepare a report that addresses the following:

Type of Award: 2015 AAOF Orthodontic Faculty Development Fellowship Award

Name(s) of Principal Investigator(s): Shankar Rengasamy Venugopalan

Title of Project: Genomic Studies in Hemifacial Microsomia and Pierre Robin Patients

Period of AAOF Support: July 1, 2015 to December 31, 2017 (No cost extension end date)

Amount of Funding: $20000

Summary/Abstract of Completed Project (250 word maximum)

Objective: Despite the tremendous advances made in identifying the genetic basis of craniofacial anomalies, the underlying genetic cause of Hemi–Facial Microsomia (HFM) and Pierre–Robin Sequence (PRS) are poorly understood. This is due to the sporadic nature of occurrence and rarity of Mendelian inheritance patterns of HFM and PRS. Recent studies have shown that the *de novo* germline or somatic changes in the genome could be deleterious and contribute to major abnormalities. The objective of this study was to identify the *de novo* (germline or somatic) variants in HFM and PRS.

Methods: Children's Mercy Hospital (CMH) approved this study and request to rely obtained from University of Missouri Kansas City IRB for non CMH investigator. Informed assent/consent were obtained for all family members prior to any research activities. The study subjects were HFM and PRS probands and their unaffected parents. The buccal swab DNA from affected/unaffected sides or the tissues from corrective surgery and/or blood samples were collected from study probands. The unaffected parents' blood samples or buccal swab DNA were also collected for trio analysis. The collected samples were prepared for whole exome sequencing (WES) using the IDT Exome Research Panel (IDT, Coraville, IA) according to manufacturer's protocols. Sequencing was completed on an Illlumina HiSeq 2500 instrument with TruSeq v4 reagents, yielding paired end 125 nucleotide reads, with an average of 8.58GB of data resulting in a mean 84X coverage. Gapped alignment to reference sequences (GRCh37.p5)

was performed with BWA and the GATK and analysis completed using custom-developed software, RUNES and VIKING as previously reported.

Results and Conclusions: Preliminary analyses of the genomic data has identified *de novo* germline variants in *EFTUD2, ZCCHC14, AMIGO2* and *SZT2,* and a somatic variant in *NYAP1* genes. The identified variants in these genes are likely pathogenic and further functional studies are required to confirm their molecular role in causing craniofacial disorders.

<u>Response to the following questions</u>:

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

Yes, the original specific aims of this proposal was realized. In my 2015 OFDFA, I proposed didactic and practical experience components. Under the didactic component, I was fortunate to receive the AAO scholarship to attend the 2-phase training program offered by the *American Dental Education Association /Academy of Academic Leadership Institute for Teaching and Learning*, Aug. 20–22 and Oct. 22–24, 2015, in Atlanta, GA. During the funding period, I also attended the "*Lunchtime Learning*" (noon hours/monthly), "*Morning Messages*" (weekly presentations), and "*Faculty Workshops*" (twice/year). I learnt about "*ExamSoft® - An assessment tool*" and "*Clickers – A classroom response system*" through these training sessions and successfully implemented these technologies in the 2016 *D6436 Growth and Development Course*.

The following table describes the courses that I co-directed/guest lectured during the 2015-16 funding period.

Term	Course/Program	Participation
Fall 2015	D6436 Growth & Development (DDS Year 2)	Co–director, 5 lectures, 1 review session
Spring 2016	BISC5706 Growth & Development (Graduate)	2 lectures
Spring 2016	BISC5710 Genetics and Biochemistry of Craniofacial Biology (Graduate)	1 lecture
Spring 2016	D6526 Orthodontics I (DDS Year 2)	2 lectures
Spring 2016	D6526L Orthodontics Lab (DDS Year 2)	10 lab sessions
Summer 2016	BISC5707 Growth & Development (Graduate)	2 lectures
Fall 2016	BISC5760 Physiology of Mineralized Tissues (Graduate)	1 lecture
All terms	D6656 Orthodontic Clinic (DDS Years 3 & 4)	Alternating Mondays (1pm to 4pm) and every Wednesday (1pm to 4pm)

As a co-director of the *D6436 Growth and Development course*, the 2015 curriculum was restructured by focusing on the pre-natal development in the first half and the post-natal development in the second half of the course. Every lecture introduced students to a genetic clinical condition relevant to the lecture topic, for example *Treacher-Collins Syndrome* in the lecture on *Cranial Neural Crest Cells*. The lecture material was presented in light of the clinical genetic condition to integrate the basic and clinical sciences. In this course students were also

asked to write a *Scientific Review Paper* on a clinical condition and was evaluated using a structured rubric in *Turnitin® Software*.

In the funding period (2015-16), under the *Independent Study Course,* I diagnosed, treatment planned, and successfully started 10 orthodontic patients. I treat these patients in preparation for the *Initial Certificate Examination (ICE)* for the *American Board of Orthodontics (ABO)*. I met with the full time faculty members (*Drs. J. Osborne, Clinical Director; J. Nickel, Program Director; and L. Iwasaki, Chair*) periodically to review and discuss the progress of these cases. I have finished two of the ABO cases and anticipate that I will be able to finish rest in a year's time to take the *ABO ICE*. I also successfully passed the *National Board Dental Examination* and the *Western Regional Examining Board Dental Examination* as proposed. Furthermore, I participated as one of the examiners in the *Comprehensive Clinical Examinations* conducted in June, August, and December of 2015. During these exams, I evaluated the in-progress and finished orthodontic cases presented by the residents. I completed formal evaluation of the presenting residents using a structured rubric. I closely worked with the year 3 and 4 DDS students in one-on-one basis to help them treatment plan their orthodontic cases. In the pre-doctoral orthodontic clinic, I supervised, alternating Mondays (1pm to 4pm) and every Wednesday (1pm to 4pm), the DDS students rendering orthodontic care during the funding period.

The Research Plan for my 2015 OFDFA is a collaborative endeavor with *Drs. Emily Farrow* (Children's Mercy Hospital Center for Pediatric Genomic Medicine), *Dr. Michael Lypka* (Children's Mercy Hospital Plastic and Craniofacial Surgery Department), and *Dr. Pedro Sanchez* (Los Angeles Children's Hospital). This collaborative endeavor has resulted in multiple presentations/conference proceedings in national meetings and a manuscript. We are currently analyzing the genomics data of HFM and PRS subjects, which we anticipate result in a second manuscript.

2. Were the results published?

a.) If so, was AAOF support acknowledged?

Yes, the results from preliminary analyses of the genomic data has been published. The funding from 2015 AAOF OFDFA has been acknowledged in all the presentations and published manuscript and we will be acknowledging AAOF in the future manuscripts as well.

Manuscript: **S.Rengasamy Venugopalan,** E.G. Farrow, M. Lypka. Whole–Exome Sequencing Identified a Variant in EFTUD2 Gene in Establishing a Genetic Diagnosis. Orthodontics and Craniofacial Research. Orthodontics and Craniofacial Research. 2017; 20(Suppl 1): 50-56

b.) If not, are there plans to publish? If not, why not and will AAOF support be acknowledged?

We have completed sequencing all the subjects recruited for this study. Currently, we are analyzing the remaining genomics data of HFM and PRS subjects, which we anticipate will result in a second manuscript. AAOF will be acknowledged in all the future presentations and mauscripts.

3. **Have the results of this proposal been presented?**

a.) If so, when and where? Was AAOF support acknowledged?

1. Oral Presentation: ***S. Rengasamy Venugopalan**, E.G. Farrow, M. Lypka. Genetics of Mandibular Hypoplasias, COAST Workshop 2016, West Palm Beach, FL
2. Oral Presentation: ***S. Rengasamy Venugopalan**, E.G. Farrow, M. Lypka, S. Jiang. Next Generation Sequencing Analysis in Hemi-Facial Microsomia Trio (Abstract #0382), Craniofacial Genetics, IADR/AADR/CADR General Session and Exhibition 2017, San Francisco, CA

3. Oral Presentation: ***S. Rengasamy Venugopalan**, E.G. Farrow, M. Lypka. Resolving a Genetic Odyssey of Mandibulofacial Dysostosis Using Next-Generation Sequencing (Abstract #1478), ACPA 74th Annual Meeting 2017, Colorado Springs, CO

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

I would like to thank the members of PARC and the AAOF for funding the OFDFA titled *"Genomic Studies in Hemifacial Microsomia and Pierre Robin Patients"*. This award has been tremendously helpful in developing my teaching and clinical skills as well as generate preliminary data for extra-mural grant application.

Accounting for Project: No left over funds.

Please return to AAOF via email attachment to aaofevp@aaortho.org