OFDFA AWARD Final Report 6-30-2012

Principal Investigator: Lina M. Moreno Uribe

Award Type: OFDFA

Research Project Title: Searching for “Extreme Growth” Genes.

Project Year: 2008-2011. Note that a 1 year no cost extension (NCE) (6-13-2011-6-30-2012) was granted.

Institution: University of Iowa.

Summary/Abstract: My goal is to pursue a successful academic career in orthodontics with strong research, clinical, and teaching components. I have been in the faculty at the Orthodontics Department as a tenure track assistant professor at the University of Iowa since July 1st of 2008. My research area involves projects aimed at identifying genetic contributors to human craniofacial growth and utilizing this knowledge to improve the diagnosis and treatment of craniofacial anomalies and malocclusions. The monetary support from this award permitted the collection of preliminary data necessary for the submission of R01 level grants at NIH to continue with large scale projects aimed at the understanding of the etiology of malocclusion. I currently dedicate 30% of my time to clinical practice and it involves providing state-of-the-art orthodontic care to patients at the intramural faculty orthodontic practice, providing instruction in the clinic floor and being the staff orthodontist at the cleft lip and palate clinic at UI Hospitals and clinics. A strong teaching and mentoring commitment has been an exciting component of my academic career. So far I have taught courses and lectures on craniofacial growth and development with an emphasis on genetics and molecular biology and have been thesis director of 3 orthodontic master’s thesis projects and 3 summer research projects, results of which have been presented at the AADR and AAO meetings in the last 2 years. The AAOF award has given me the resources and support to fulfill my career goals as an academic orthodontist.

Were the original, specific aims of the proposal realized: The following specific aims were met during the 3-year support and 1 year NCE period and constitute the essence of the career development plan proposed:

1. To develop a research project entitle: Searching for “Extreme Growth” Genes.
2. To provide state of the art orthodontic care to patients at the intramural faculty orthodontic practice.
3. To acquire orthodontic teaching skills as well as student mentorship abilities in the development of master’s and doctoral thesis projects.
4. To design and teach courses on craniofacial growth and development with an emphasis on genetics and molecular biology.

As indicated by the above specific aims, the specific goal of this OFDFA award was to provide initial funding to carry out a plan for pursuing a successful academic career with research, clinic practice and teaching components within the Department of Orthodontics at the University of Iowa. My research area involves projects aimed at identifying genetic contributors to human craniofacial growth and utilizing this knowledge to improve the diagnosis and treatment of craniofacial anomalies and malocclusions. The support received from the AAOF for the last 3 years in addition to 1 year of NCE has enable me to start my own genetics lab with enough equipment to pursue projects aimed at the genetic characterization of human samples with malocclusion or craniofacial anomalies such as cleft lip and palate with the aim of understanding the genetic

etiology of these conditions. To date we have identified 760 individuals with moderate to severe malocclusion and have performed complete phenotypic characterization of their phenotype for 657 of them. Results obtained constituted an integral part of two Master's thesis in the Department of Orthodontics successfully defended in the spring of 2012 by Drs. Kaci Vela and Sara Howe. In addition, both manuscripts are in preparation for publication of these results the end of the summer of 2012. The following paragraphs described a summary of each of these manuscripts that we plan on submitting to the AJO at the end of the summer of 2012:

Manuscript # 1 Summary. Phenotypic characterization of the class III malocclusion. Vela K., Kummet C, Dawson D, Moreno LM. Am. J. of Orthodontics (Manuscript in Preparation):
Severe malocclusion resulting in distorted facial appearance significantly reduces patients' quality of life, limits occlusal function, increases the risk of dental trauma, and diminishes the individual's value for oral health (Feu et al., 2010; Proff, 2010; Stenvik et al., 2011). Identifying the causes of malocclusion will provide opportunities for improving treatment and prevention and for decreasing the burden of these conditions on affected individuals. Class III malocclusion affects 1% of the US population and is characterized by a composite of skeletal patterns leading to the forward positioning of the lower teeth in relation to the upper teeth (edge to edge incisor relation or reverse overjet) and a concave profile. The purpose of this study is to identify the phenotypic variation present in a moderate to severe class III malocclusion sample. Pre-treatment cephalometric records of 292 Caucasian adult class III patients (126 male, 166 female; age range 16-57 years) who met our eligibility criteria for moderate to severe Class III malocclusion were submitted to a cephalometric analyses containing 63 clinically relevant ceph variables. The original sample included 311 individuals; eighteen of non-Caucasian race were excluded due to lack of power and one additional subject was found to be ineligible based on inclusion criteria. Measurements were standardized by age and gender and were submitted to multivariate data reduction methods such as Principal Component Analysis (PCA) and Cluster Analysis (CA) to obtain clinically meaningful phenotypes that could be utilized in genetic and environmental studies of class III malocclusion. Results of the PCA analyses (Figure 1) revealed that six principal components accounted for 81.17% of the total variance in the data and represent (in order from PC1 to PC6); the antero-posterior position of the mandible in relationship to the cranial base, maxillo-mandibular horizontal and vertical size discrepancies, the position and inclination of the lower incisor and its effect on lower lip protrusion, the lower incisor angulation, facial taper and variation in maxillo-mandibular discrepancies, variation in the upper incisor and the maxillary horizontal position and finally variation in the cranial base. For each of these components of variation each research subject has a principal component score that defines each subject's position on a particular axis of variation. Such scores can be used as quantitative phenotypes and therefore be correlated to each individual's genetic and environmental data. The cluster analysis resulted in the identification of five sub-phenotypes within Class III subjects (Figure 2). Clusters 1 and 2 represent borderline CIII phenotypes with a combination of mild maxillary retrognathism and mandibular prognathism. Cluster 3 corresponds with the vertical CIII phenotype, while Cluster 4 and Cluster 5 represent the severely mandibular prognathic and severely maxillary retrognathic phenotypes, respectively. These results described the phenotypic variation found in a large moderate to severe class III sample. These phenotypes will be the basis for studies of the genetic and environmental causes of class III malocclusion. The phenotypic modeling derived from this sample can also be utilized for the classification of additional class III individuals in particular phenotypic clusters and therefore their data can be pooled with the current sample until we obtain sufficient power for large genome-wide level studies of genetic and environmental susceptibility to malocclusion.

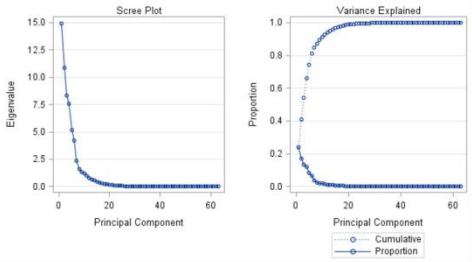
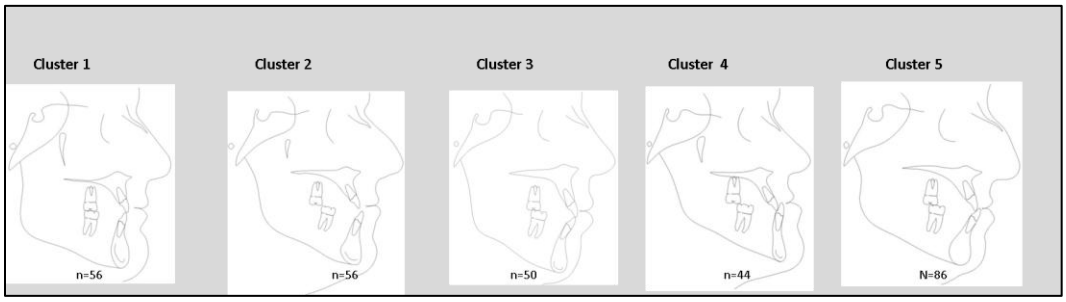


Figure 1. Results from PCA indicating the % of variation explained by each of the components



Manuscript # 2 Summary. Phenotypic characterization of the class II malocclusion. Howe S., Kummet C, Dawson D.V., Vela K., Moreno L.M. Am. J. of Orthodontics (Manuscript in Preparation):
Severe malocclusion resulting in distorted facial appearance significantly reduces patients' quality of life, limits occlusal function, increases the risk of dental trauma, and diminishes the individual's value for oral health. Identifying the causes of malocclusion will provide opportunities for improving treatment and prevention and for decreasing the burden of these conditions on affected individuals. Class II malocclusion is present in 15% of the US population and is characterized by a deficient mandible and a convex profile. The purpose of this study is to describe the skeletal and dental variation present in Class II malocclusion into distinct phenotypes to reduce heterogeneity and empower studies aimed at identifying the etiology of malocclusion. This will specifically be done using cephalometric radiographic landmarks and statistical data reduction methods to find the most common phenotypic groupings of Class II patients. The sample consisted of 309 healthy Caucasian subjects (227 male, 82 female; age range: 16-60 years) who met specific inclusion criteria. 2-D pre-treatment records were used from the University of Iowa College of Dentistry and Hospital Dentistry at the University of Iowa. All had a full set of pre-orthodontic treatment records including lateral cephalographs, intra and extra-oral photos, and models. The original sample included 346 individuals; 37 of non-Caucasian race were excluded. Data on the remaining 309 subjects were used for these analyses. Measurements were standardized by age and gender and were submitted to multivariate data reduction methods such as Principal Component Analysis (PCA) and Cluster Analysis (CA) to obtain clinically meaningful phenotypes that could be utilized in genetic and environmental studies of class II malocclusion. Results from PCA analyses (Figure 3) indicated that 7 principal components account for 80.95% of the total variance in the data and represent (in order from PC1 to PC7), the vertical dimension in regards to the inclination of the mandibular plane, the maxillary incisor angulation, the mandibular horizontal and vertical lengths as well as the posterior facial height, the antero-posterior position of the maxilla, especially in regards to the maxillary incisor angulation, the mandibular incisor position relative to the mandibular plane and the degree of facial taper, the angulation of the cranial base and finally the amount of variation in overjet. For each of these components of variation, we obtained a principal component score for each research subject that defines each subject's position on a particular axis of variation or principal component. Such scores can be used as quantitative phenotypes and therefore be correlated to each subject's genetic and environmental data. Results from cluster analyses indicate that the class II data is best represented by a 5 cluster model. Cluster 1 depicts a mild skeletal Class II with a normal vertical component, a mildly retrusive maxilla and a normal size mandible yet posteriorly positioned in regards to the cranial base. Cluster 2 represents a moderate Class II patient with a normal maxilla, a moderately retrusive mandible, normal vertical dimension and abnormal inclination of incisors (i.e. dental compensations present). Cluster 3 represents subjects with a protrusive maxilla and a retrusive mandible leading to an increase in profile convexity and worsening of the Class II malocclusion. Cluster 4 is defined by the steepness of the mandibular plane due to the shortness of the ramus, which creates an open-bite tendency and a more severe maxillo-mandibular discrepancy implicating both jaws and resulting in a very convex profile. Finally, Cluster 5 is characterized by a deficient vertical dimension causing lip redundancy, a protrusive maxilla and also protrusive maxillary incisors with an increased overjet. These results described the phenotypic variation found in a large moderate to severe class II sample and this work constitutes the largest and most detailed phenotypic characterization performed for the class II malocclusion so far. These phenotypes will be the basis for studies of the genetic and environmental causes of class II malocclusion. The phenotypic modeling derived from this sample can also be utilized for the classification of additional class II individuals in particular phenotypic clusters and therefore their data can be pooled with the current sample until we obtain sufficient power for large genome-wide level studies of genetic and environmental susceptibility to malocclusion.

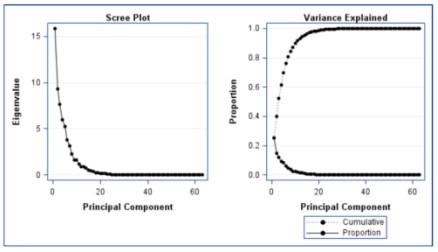


Figure 3. Class II PCA results and % of the variation explained

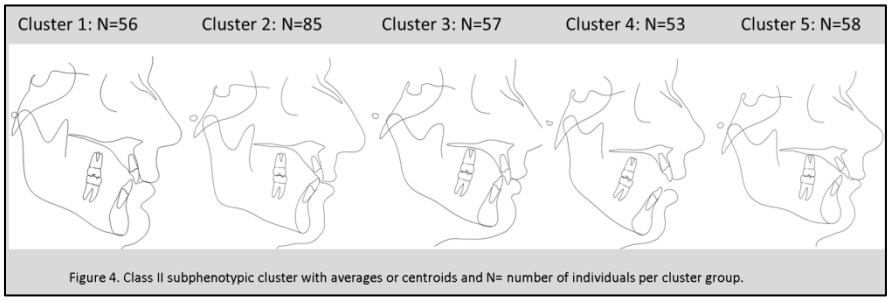


Figure 4. Class II subphenotypic cluster with averages or centroids and N= number of individuals per cluster group.

In addition, we have collected and processed DNA samples and environmental data on 308 individuals so far. The genotyping of 250 of these individuals for craniofacial development candidate genes will be performed with additional funds that were obtained this year from the AAOF foundation in the form of a BRA award. My clinical practice goals involved providing state-of-the-art orthodontic care to patients at the intramural faculty orthodontic practice and the cleft lip and palate clinic. My clinical practice has increased to 30% of my time, distributed between my faculty practice (10%), being an instructor in the clinic floor with the orthodontic residents (10%) and also as the staff orthodontist at U. of Iowa Hospitals and Clinics for the cleft lip and palate clinic (10%). My immediate teaching goals are to acquire orthodontic teaching skills and mentorship abilities. So far, I have continued to teach the Facial Growth and Development graduate course by contributing to lectures on the topics of factors that control and influence facial growth, approaches to prediction of facial growth, clinical applications of growth prediction and treatment timing. Also, I became course director for the Introduction to Cephalometrics course directed to both Orthodontic and Pediatric Dentistry residents. As far as mentorship, I have been the thesis director of 3 Master's thesis projects and 3 summer research projects. Results of these projects have been presented at different AADR meetings and also AAO meetings in the last 2 years. In summary, this award not only has provided me with funds for collecting the preliminary research data to compete for NIH funding and become an independent scientist but has also has giving me support to obtain further excellence and succeed in my career as an orthodontic professor.

Were the results published? If not, are there plans to publish? If not, why not?: Results of the 2 manuscripts described above, will be submitted to the AJO by the end of the summer of 2012.

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 following is a list of abstracts that have been submitted to National meetings in the last 2 years:

•K. Vela., C. Kummet, D. Dawson, M. Hoppens L. Moreno. Phenotypic Characterization of CIII Malocclusion using Principal Component and Cluster Analysis. Iowa City, Iowa. Poster presented AAO Chicago, 2011.

•D. Defay, C. Kummet, D. Dawson, D. Blanchette, S. Weinberg, L. Moreno. Midfacial Plane Selection in Parents of Individuals with and without Orofacial clefting. Iowa City, IA. Poster presented AAO Chicago, 2011.

•S. Miller, D. Defay, S. Weinberg, C. Kummet, J. C. Murray, ML. Marazita, G L. Wehby, L.M. Moreno. Candidate Gene Effects on Human Craniofacial Variation in Males and Females. Submitted to ASGH meeting at Montreal 2011.

•Moreno LM, Miller SF, Defay D, Weinberg S, Marazita ML, Murray JC, Wehby GL. Genetic Effects on the Craniofacial Morphology of Unaffected Parents of Children with Oral Clefts. Submitted to ASGH meeting at Montreal 2011.

•Blanchette, Dawson, Kummet, Wang, Defay, Miller, Moreno LM. Impact of superimposition on 3D stereophotogrammetric facial landmark reliability. Submitted to AADR meeting 2012.

•Brian J. Smith, Lina M. Moreno, Steven F. Miller, George L. Wehby, Martine Dunnwald . Digital Image Analysis for Reliable Characterization of Cleft Wound Phenotypes. Submitted to AADR meeting 2012.

•Emily Wang, Derek Blanchette, Colleen M. Kummet, Steve Miller, Lina Moreno, Deborah V. Dawson. Three-dimensional Soft Tissue Asymmetry in Unaffected Relatives of NSCL/P Individuals. Submitted to AADR meeting 2012.

•Colleen M. Kummet, Derek Blanchette, Deborah V. Dawson, David Defay, Steven Miller, Lina Moreno. Impact of Midline Rotational Standardization on Estimates of Facial Asymmetry. Submitted to AADR meeting 2012.

•Geoffrey Skinner*, Richard Burton, Colleen Kummet, Deborah Dawson, Teresa Morgan, Kaci Vela, Lina Moreno, Andrew C. Lidral. Maxillary Distraction Osteogenesis Treatment Changes in Patients with Orofacial Clefts. Submitted to AADR meeting 2012.

•Hoppens CME, Miller SF, Marazita ML, Wehby GL, Moreno LM. Microesthetic Dental Analysis in Parents of Children with Oral Clefts. Submitted to AADR meeting 2012.

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

Currently my research focuses on the understanding of the genetic factors underlying the abnormal dento-facial phenotypic variation present in patients with craniofacial anomalies such as nonsyndromic cleft lip and palate, moderate to severe malocclusion and most recently Ectodermal dysplasia conditions. I have used the AAOF funding to support the collection of preliminary data for my research projects. These data will be submitted as proof of principle to NIH applications to obtain funding for large scale projects aimed at understanding the etiology of common craniofacial anomalies. AAOF support has further my academic career greatly by being able to start my own genetics lab and allowing me to process and performed initial phenotyping and genetic characterization in samples collected so far. With support from the AAOF and additional funds from the University of Iowa, I have been working on the implementation of methods for deriving complex multivariate dento-facial phenotypes via shape analyses such as geometric morphometrics and data reduction methods applied to both 2D and 3D facial hard and soft tissue records such as lateral cephs, CBCTs, dental models and facial surface images of patients with moderate to severe malocclusion. Once generated, these multivariate phenotypes will be correlated with genetic and environmental information that has been collected on these individuals. My AAOF-OFDA provided important seed funds for the initial phenotypic, genetic and environmental data collection which at this moment consists of 698 untreated adults with moderate to severe malocclusion. Results of this phenotypic characterization will be submitted for publication during the summer and fall of 2012. In addition, we have DNA and environmental data collected on 308 of these individuals and recruitment procedures are currently ongoing at a rate of 5 individuals per week. Recently I was able to secured funds by means of an AAOF 2012 BRA proposal with which I plan to perform a large candidate gene study of 250 individuals with moderate to severe malocclusion. For this analysis I will use all the extensive phenotypic data that I have analyzed so far in combination with genotypic data that will be generated in my lab for 147 candidate genes for craniofacial development and malocclusion. This work will serve as preliminary and as proof of principal for future R01 level funding applications tailored to perform whole genome genotyping and sequencing projects in the future to continue the search for genetic factors responsible for moderate to severe malocclusion. To this end, the continued financial support from the AAOF has been essential for establishing the necessary infrastructure to perform large genetic and environmental studies of malocclusion. Results of these studies will contribute to a better understanding of these conditions which will likely result in improved treatments and ultimately prevention of these disorders.