AAO BRA Final Report

1. Type of Award: Biomedical Research Award
2. Principal Investigador: Lina M. Moreno Uribe.
3. Institution: University of Iowa.
4. Period of AAOF Support: (7-1-12 to 6-30-2013)
5. Amount of AAOF funding: $25,000
6. Signature:  Date: 7-1-2013

**Original Specific Aims:**

The overall goal of this project is to generate candidate gene genotyping data on a previously collected sample of 250 patients with Class II and Class III moderate to severe malocclusion, to study the genetic factors that confer risk to malocclusion phenotypes. These data will establish the foundation for future comprehensive approaches to identifying causal factors and building a better understanding of the biology of malocclusion. We are proposing the following Aim:

1. Perform genotyping and association analyses of selected craniofacial candidate genes.
    a. Genotype SNPs within selected candidate genes/loci for malocclusion.
    b. Test the association of these genes/loci with the phenotypes defined in previous work by the PI.

We will implement this Specific Aim in a sample of 250 patients from the Orthodontics Department at the University of Iowa and private orthodontic practices in the state of Iowa. The phenotypic measures have been previously collected by the PI with funds provided in part from an AAOF faculty development award (2008 -2012). We have conducted preliminary studies described below that highlight the strengths of our phenotyping approach (Phenotypic diversity in class III adults, Moreno et al., 2013 Forthcoming: Am. J. Orthod. Dentofac Orthop 2013;144: 32-42 and Phenotypic diversity in class II adults, Moreno et al., 2013 currently in review at the AJO). We have access to a fully equipped research laboratory and to the University of Iowa DNA core facility to utilize multiplex genotyping platforms for the genotypic experiments planned in this proposal. This study provides important advantages over all previous studies by recruiting a large sample with moderate to severe malocclusion on both ends of the phenotypic spectrum, employing more comprehensive phenotyping and investigating several key candidate genes.

**Interim Results:**

At the end of the first 6 months of the funding period for this project and interim report was submitted which included the analyses of a subset of our sample with 115 individuals and 23 single nucleotide polymorphisms (SNPs) within 16 top priority craniofacial candidate genes that have shown expression on the face, association with mandibular prognathism in previous studies or that are important for facial phenotypes including facial asymmetry. These preliminary data was presented as a podium presentation at the American Association of Physical Anthropology in April of 2013. This abstract is attached below.

Abstract: Miller et al., 2013Potential Genetic Determinants of Dental Arch Form: Human dental arch shape is an important aspect of cranio-dental variation with implications in evolutionary biology, comparative anatomy, functional morphology, and clinical treatment. Most studies have focused on characterizing the morphological variation in dental arches, however the genetic determinants underlying such variation are largely unknown, particularly in individuals with severe malocclusion. To address this knowledge gap, a largely Euro-American adult sample (n=276) of dental casts (100 males, 179 females) presenting moderate to severe distal and mesial malocclusion (i.e. Class 2 and Class 3 malocclusion) were digitized in occlusion and landmarked with 58 landmarks (figure 1 below) along the gingival margins of the maxillary and mandibular arches. 3D Coordinate data were analyzed in MorphoJ using relative warps analysis (RWA) to extract symmetric and asymmetric arch shape phenotypes. For a subsample of 115 individuals, phenotypes were regressed (adjusting for sex) against genotypes of 23 single nucleotide polymorphisms (SNPs) within 16 candidate genes (*PAX7, EPB41, ABCA4-ARGHAP29, IRF6, LEFTY1, LEFTY2, MSX1,* 4p16*, PiTX2, GHR, ISL1, 8q24, FOXE1, MAFB, SNAI1*) implicated in human midfacial and mandibular phenotypes, symmetry, and dental variation. Significant ($p<0.05$) correlations for symmetric shape components representing variation in anterior-posterior (AP) (figure 2 below) and transverse arch relations were found with *GHR* and *LEFTY2* respectively. Also, significant correlations were found for asymmetric shape components demonstrating right to left arch rotations and *ABCA4-ARGHAP29, IRF6, LEFTY1, PAX7* and *TGFB3.* Finally, centroid size was correlated with *LEFTY1* and 4p16 and fluctuating asymmetry was correlated with *PITX2*. Results provide insights into the craniofacial genetic pathways that are also important in determining arch shape.

The abstract described above utilized data from scanned pre-treatment dental models in occlusion (Figure 1). The most significant finding was for a SNP in the GHR gene with AP variation in which more copies of the rare allele for this SNP shifts the variation towards a more Class II relation.

Figure 1. Landmark data set of 58 landmarks along the gingival margins to study dental arch shape.

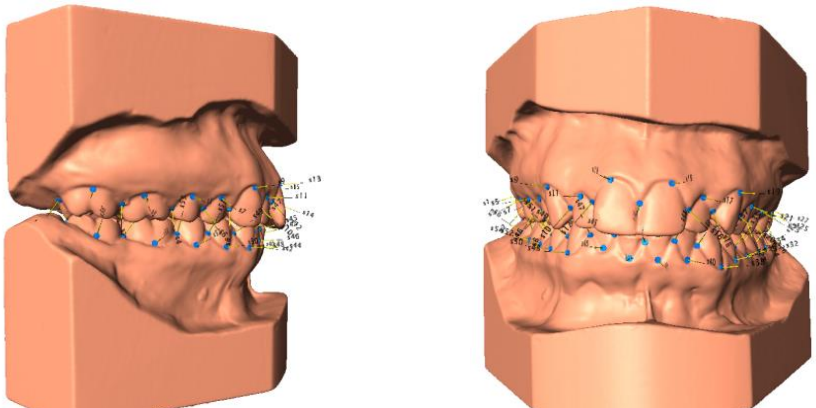
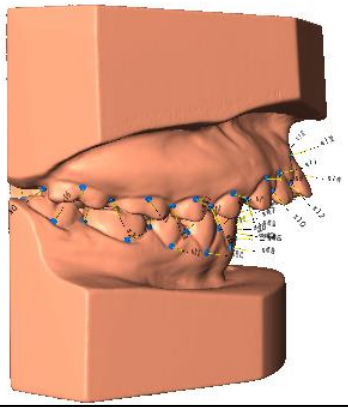
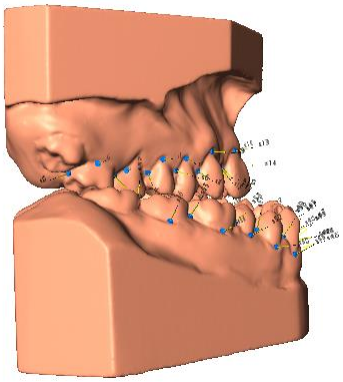


Figure 2. Results from the 1st principal component which represents variation in the anterior-posterior dimension. Dental models represented here depict individuals with the most negative (left, class II relation) and most positive (right, class III relation) principal component scores (PCs) for the 1st principal component of arch shape variation. Regression analyses results indicated that SNP rs6180 within the GHR gene was associated (p=0.018) with this anterior-posterior variation and thus more copies of the rare allele for this SNP shifts the variation towards a more Class II relation.



Most negative PC scores indicate a Class II tendency. More copies of the rare allele of the SNP rs6180 in the GHR gene shifts the variation towards a more class II relation



Most positive principal component scores indicate a Class III tendency.

**Progress and Final Results:**

As described in the specific aim above, we originally proposed to genotype a sample of 250 individuals with moderate to severe malocclusion. Currently, we have successfully recruited 326 adults with malocclusion. With our multiplex platform (Fluidigm 96x96) we can simultaneously genotype 96 SNPS in one run of 96 individuals. We currently use 6 controls (three non-template and three known DNA samples) and therefore we were able to perform genotyping experiments for 3 full plates for a total of 270 individuals with malocclusion. A total of 216 single nucleotide polymorphisms (192 Fluidigm and 24 Taqman assays) located within 75 craniofacial genes and loci were attempted. We obtained excellent genotyping results for 198/216 SNPs. Preliminary analyses in the form of phenotype-genotype correlations will be presented in detail below. Recruiting efforts are progressing well and with additional funds from the PI, we hope to recruit

34 more individuals to complete a 4th plate for a total of 360 individuals with malocclusion in the next 6 months to be included in a future manuscript for submission in early 2014.

For the phenotypic analyses we utilized pre-treatment lateral cephs of 270 Class II, Class I and class III total individuals in approximately equal numbers from each malocclusion group. A cephalometric landmark data set including 29 commonly used landmarks was utilized for these analyses. Twelve individuals with missing landmarks had to be excluded from the analysis and 258 individuals remained. Two-dimensional coordinates of the 29 cephalometric landmarks were exported for geomorphometric analysis with Morpho J software. The coordinate landmark data set was submitted to a Procrustes fit and subsequently the Procrustes residuals were utilized in a principal component analysis. Results showed that 4 principal components explained 60% of the variation in this data set (Table 1, Figure 2).

| Table 1. Principal Component Analysis | | |
|---|---|---|
| Component | % Variance | Cumulative % |
| PC1 | 24.801 | 24.801 |
| PC2 | 21.485 | 46.286 |
| PC3 | 7.690 | 53.976 |
| PC4 | 5.904 | 59.879 |

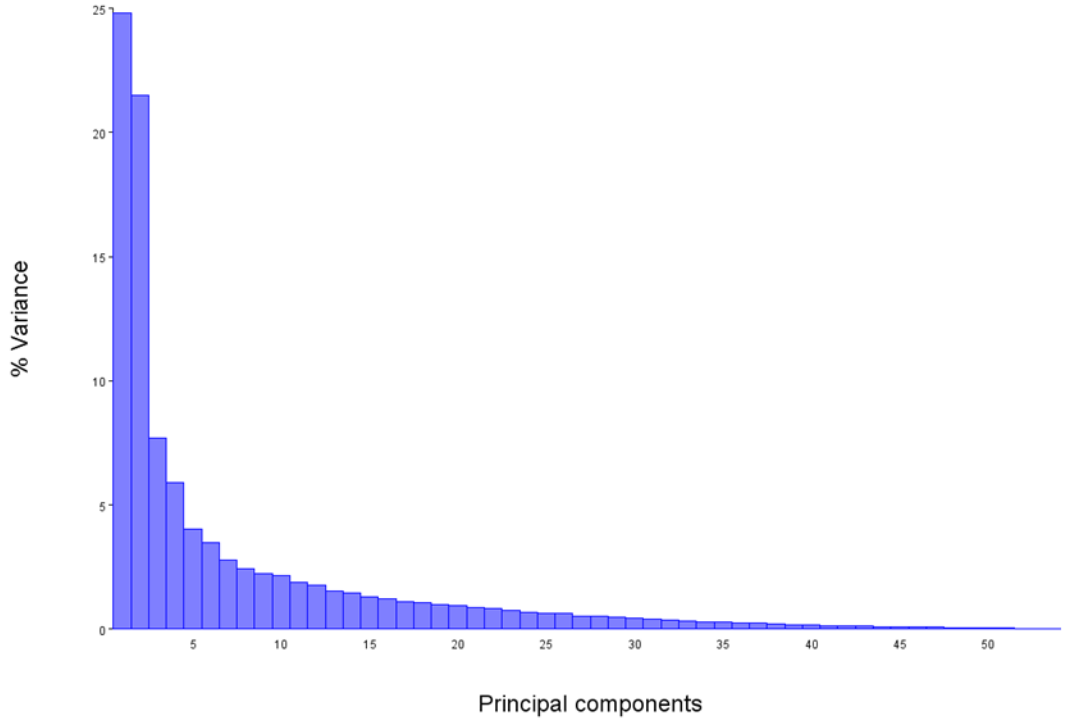



Figure 2. Scree plot of the variation explained by each of the principal components

Regression analysis was utilized to investigate the correlations between the principal component scores (PCs) of the first 4 principal components with the 198 SNPs successfully genotyped. Genotypes were recoded as 0, 1, 2 depending on the number of copies of the rare allele present in an individual's genotype. Results showed that PC1 which explains 25 % of the variation in the data set is correlated ($p<0.01$) with a SNP in the *PAX5* gene. PC2 which

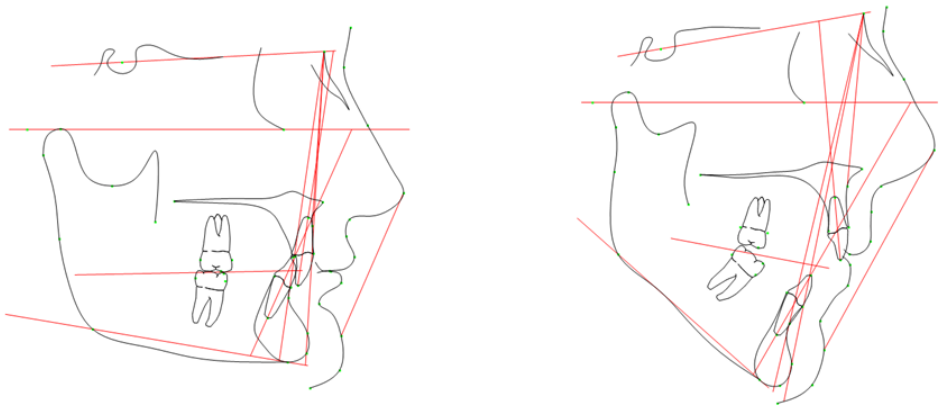


Figure 4. Represents individuals with the most negative (severe deep bite) and most positive (severe open bite) principal component scores for PC1 which explains 25% of the variation. Variation in PC1 is correlated ($p<0.01$) with a SNP in the *PAX5* gene.

explains 21.5% of the variation is correlated ($p <0.01$) with SNPs in *MYO1H* and *SNAI3*, PC3 which explains 7.7% of the variation is correlated with SNPs in *RUNX2, PAX5*, *NRIP2* and loci 12q13.13. Finally PC4 which explains 6% of the variation is correlated with SNPs in *IRX1* and *TWIST1* (Figures 4-7).

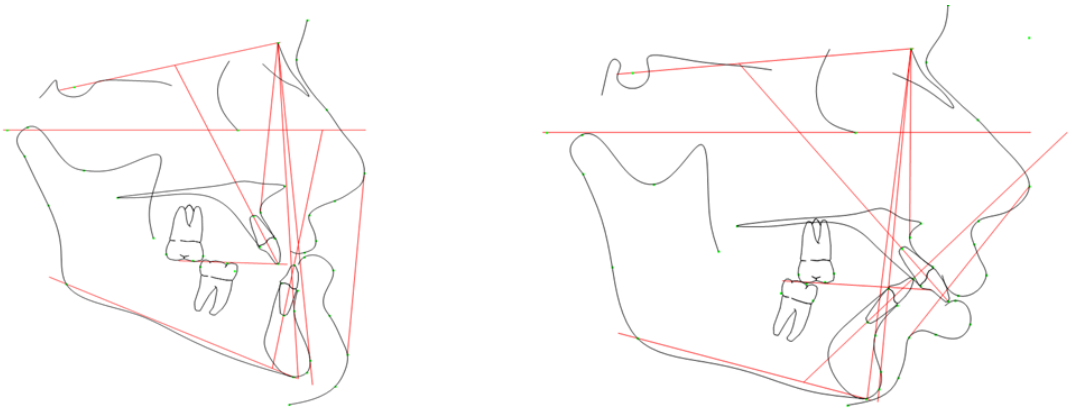


Figure 5. Represents individuals with the most negative (maxillary retrusion) and most positive (maxillary protrusion) principal component scores for PC2 which explains 21.5% of the variation. Variation in PC2 is correlated ($p<0.01$) with SNPs in in *MYO1H* and *SNAI3*.

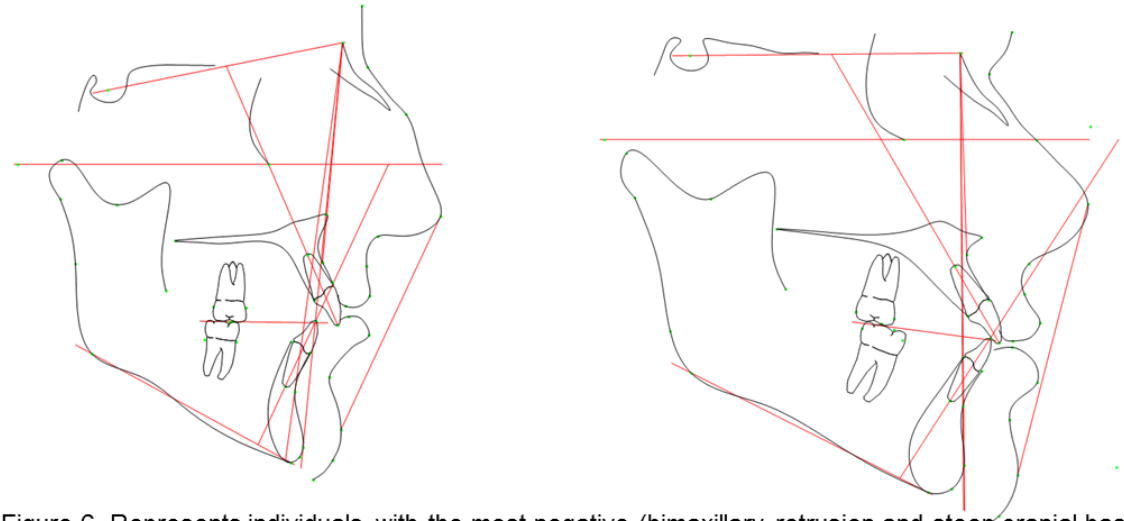


Figure 6. Represents individuals with the most negative (bimaxillary retrusion and steep cranial base) and most positive (bi maxillary protrusion and flat cranial base) principal component scores for PC3 which explains 7.7% of the variation. Variation in PC3 is correlated ($p<0.01$) with SNPs in *RUNX2, PAX5*, *NRIP2* and loci 12q13.13

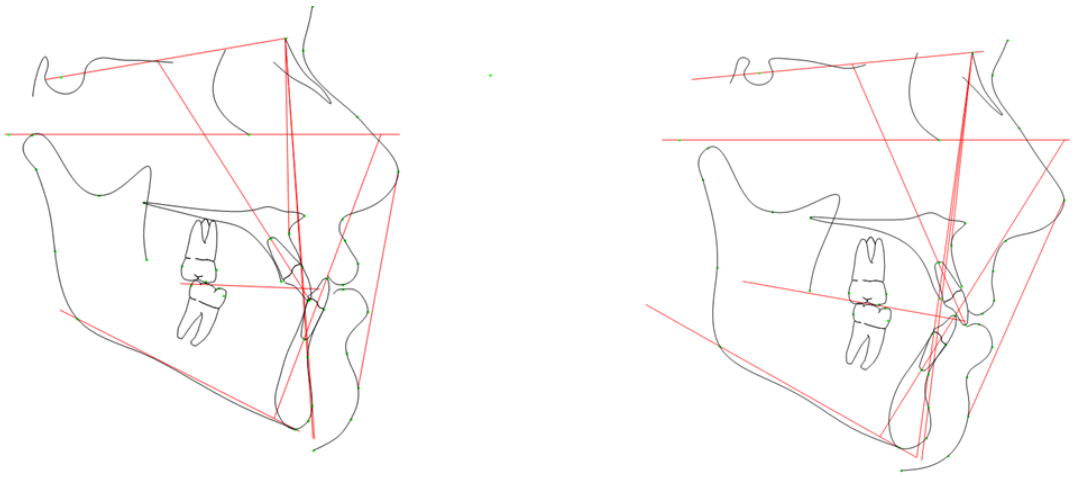


Figure 7. Represents individuals with the most negative (mandibular prognathism) and most positive (normal mandible) principal component scores for PC4 which explains 6%% of the variation. Variation in PC4 is correlated ($p<0.01$) with SNPs in *IRX1* and *TWIST1*.

Results from these preliminary analyses are very encouraging as they indicate the genetic pathways that will become high priority in future analyses. Of note are the SNPs in 12q13.13 and *MYOH1* both previously reported in studies of maxillary retrusion (Frazier Bowers et al., 2009 and mandibular prognathism (Tassoupoulou-Fishell et al., 2012).

**Future Directions:**

Our lab will continue to recruit individuals to genotype a 4th plate before manuscript submission. In addition we plan to utilize this preliminary data for seeking future funding to continue with our research in the etiology of malocclusion.

**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 most recent AAOF 2012 BRA grant allowed me to complete the candidate gene study of 270 individuals with moderate to severe malocclusion with 198 SNPs within 75 genes. 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.