AAO Foundation Award Final Report

1. Type of Award: Biomedical Research Award

2. Name(s) of Principal Investigator(s): Sercan Akyalcin, DDS, MS, PhD, and Ariadne Letra DDS, MS, PhD

3. Institution: The University of Texas Health Science Center School of Dentistry at Houston

4. Title of Project: Finding the etiology of familial oligodontia

5. Period of AAOF support: 07-01-15 to 06-30-16

6. Amount of AAOF Funding: 30,000

Signature: _ _____ Date: 07.08.16

Abstract:

Objectives: to identify causative genes for familial oligodontia.

Sample population: a four-generation multiplex family of 24 individuals (11 affected, 13 unaffected) diagnosed with nonsyndromic oligodontia.

Material and Methods: Genomic DNA was obtained from peripheral blood samples of 12 family members and submitted for whole exome sequencing using the Agilent SureSelect 50MB Target Enrichment System (Agilent Technologies, Santa Clara, CA), and an Illumina HiSeq 2000 sequencing platform (Illumina Inc., San Diego, CA). Variants were annotated using dbNSFP database and prioritized for validation based on prediction of mutation function. Variants identified as potentially causal were verified using direct sequencing.

Results: A pathogenic heterozygous missense mutation in the *PAX9* gene (c.A271G; p. K91E) was found segregating with oligodontia in all affected individuals and is the likely cause of oligodontia in the family. A novel heterozygous missense mutation in the *B3GALTC6* gene (c. C515T; p. A172V) was also identified segregating with the condition, although predicted as benign and therefore unlikely to be contributing to the condition.

Conclusion: Our results support the involvement of *PAX9* in the etiology of familial oligodontia.

Keywords: oligodontia, tooth agenesis, gene, whole exome sequencing

Questions:

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

Yes. The specific aims of this proposal were:

(1) Identify genetic variation underlying familial oligodontia using whole exome sequencing (WES)

(2) Perform bioinformatic analysis to prioritize variants/genes and experimentally validate top variants.

We performed whole exome sequencing in twelve family members (seven affected and five unaffected) from a large multiplex family in which oligodontia was found segregating in autosomal dominant form through four generations. No evidence of ectodermal dysplasia or other structural abnormalities were found in any of the family members, supporting a nonsyndromic nature of the condition. Our study proband (IV.8) (see arrow in Figure) is missing 16 permanent teeth, while additional affected family members are missing between 6-12 teeth. WES was performed using the Agilent SureSelect 50MB Target Enrichment System (Agilent Technologies, Santa Clara, CA), and an Illumina HiSeq 2000 sequencing platform (Illumina Inc., San Diego, CA). Variants identified through WES as potentially causal were verified using direct sequencing.

A known heterozygous missense mutation in the *PAX9* gene (c.A271G; p. K91E) was found segregating with oligodontia in all affected individuals and is the likely cause of oligodontia in the family. This change results in a lysine to glutamine substitution in the protein structure and is pathogenic. A previous study has identified this mutation in families with autosomal dominant hypodontia (Das et al. Am J Med Genet 2003). However, this mutation was previously reported in association with oligodontia of posterior teeth. In this study, affected family members have a combination of anterior and posterior teeth missing, and this may represent some variable expressivity of the phenotype with regards to the *PAX9* genotype.

A novel heterozygous missense mutation in the *B3GALTC6* gene (c. C515T; p. A172V) was also identified in all affected individuals, while unaffecteds were all homozygous for the C allele. However, this mutation was predicted as benign by computational algorithms and therefore unlikely to be contributing to the condition.

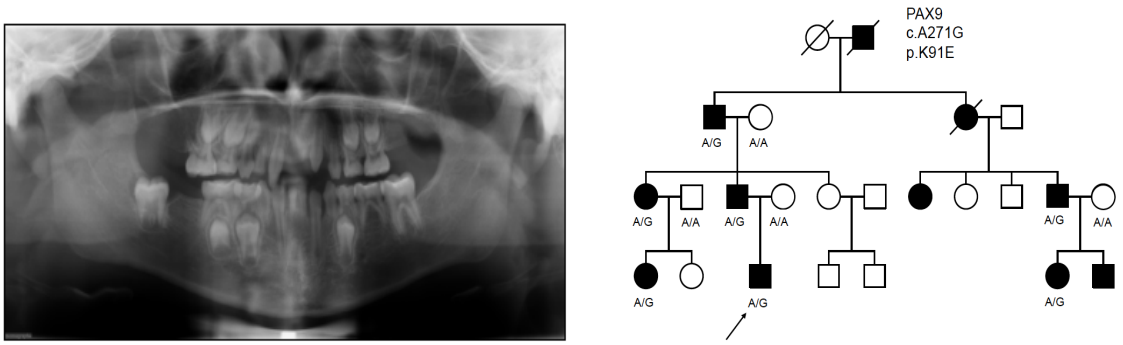

Figure 1. A, Panoramic radiograph of study proband. B, Pedigree of study family. Individuals with oligodontia are denoted by filled symbols. Proband is denoted by arrow. *PAX9* genotypes are presented.

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

These results have not yet been published or presented in full as data analysis was completed just recently. Whole exome sequencing is still a novel technology and takes approximately 6 weeks to obtain individual results. WES also yields an insurmountable amount of data that needs to be carefully analyzed. Over 100,000 variants were identified collectively in this study and so data analysis was a long process. Although no individual publication with these results have yet been possible, we have included these results in our broader studies of human tooth agenesis as described below. We plan to submit a manuscript for publication by the end of the current year.

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 study have been included in local, national and international scientific presentations, some of which won prestigious awards as follows:

Williams MW, Dinckan N, Maheswhari K, Silva R, Akyalcin S, Vieira AR, Fakhouri W, Letra A. Association between colorectal cancer polymorphic variants and isolated tooth agenesis. **1st place award. Junior category**. IADR Hatton Competition. Seoul, South Korea. June 20, 2016.

Dinckan N, Du R, Petty LE, Akdemir ZC, Jhangiani SN, Muzny DM, Kayserili H, Akyalcin A, Gibbs RA, Boerwinkle E, Lupski JR, Below JE, Uyguner ZO, Letra A. Identification of isolated tooth agenesis genes using whole exome sequencing and linkage analysis. The Rolanette and Berdon Lawrence Bone Disease Program of Texas. 2016 Scientific Retreat. Houston, TX, May 6, 2016.

Williams MW, Dinckan N, Maheswhari K, Silva R, Akyalcin S, Letra A. Association between colorectal cancer polymorphic variants and isolated tooth agenesis.

2nd place award. Junior category. 2016 IADR Annual Meeting Hatton competition. March 16-19, 2016, Los Angeles, CA.

Williams MW, Dinckan N, Maheswhari K, Silva R, Akyalcin S, Letra A. Association between colorectal cancer polymorphic variants and isolated tooth agenesis. Hinman Student Research Symposium. Memphis, TN. Oct. 30-Nov. 1, 2015.
Most Outstanding Presentation in Clinical Research Award.

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

The present AAOF award has allowed us to undertake a new and sophisticated technology in our genetic studies of human tooth agenesis. It allowed us to learn 'big data' analysis from colleagues at the School of Public Health, and prompted us to start new collaborative endeavors within UTHealth. We now have preliminary data and proof of principle that our research approach is effective in identifying disease variants, which will highly benefit us in future grant applications.

Additionally, this award has allowed us to share our research findings in important scientific meetings, elevating the name of our lab, school, and the AAOF to the scientific community. We were successful in obtaining multiple awards and make important connections with other clinicians and investigators.

On the clinical side, this award allowed us to identify the cause of oligodontia in our study family, and provided them with important information to be used in future genetic counseling for family members. The family is very grateful for our findings and has been instrumental in disseminating the quality of the clinical treatment and the research being performed in the field of craniofacial genetics at UTSD, and the support of the AAO Foundation.

Respectfully submitted,

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