

SPECIFIC AIMS

Rationale: In the US, Early Childhood Caries is the most prevalent chronic disease in children, occurring 5 times as frequently as asthma, and when left untreated costing \$1.6 billion in emergency dental care in 2012 alone. Nationwide, caries rates of Hispanic, Native American, and Native Hawaiian and Other Pacific Islander children, are almost double those of white children. Approximately 52% of pre-school aged Hispanic children (in Yuma County, Arizona) and 50% of NH/OPI children (on Hawai'i Island) have untreated caries. Traditional socioeconomic factors (poverty and access to dental care) explain only a portion of the elevated ECC incidence rates in ethnic minorities, with biological components likely having a strong impact. Infection by bacteria from mutans streptococci group (*S. mutans* and *S. sobrinus*), have been implicated as the cause of ECC. When established in the oral cavity, these bacteria metabolize carbohydrates and produce highly acidic byproducts that change the pH of the oral cavity, leading to demineralization of tooth enamel. *The impact of the genomics, virulence (acid production), and bacterial load of mutans streptococci in the oral cavity on observed ethnic ECC incidence disparities are unknown but are likely significant. Similarly unknown is the role that preschool classrooms perform in selecting for highly acidic strains of mutans streptococci.*

We have proposed to address these knowledge gaps in our existing project titled: “*Defining Microbiological Drivers of Early Childhood Caries in Preschoolers in Southern Arizona*”. However, while the parent study is well-poised to explore the biological component of ECC in Hispanic and, to a lesser extent, Native American children, Native Hawaiians and Pacific Islanders are insufficiently represented in Arizona (and elsewhere in the continental US) to draw statistically significant conclusions. With this administrative supplement we propose to focus on disambiguating the biological drivers of dental decay in NH/OPI children. At 33.9%, the proposed catchment area for this supplement (Hawai'i County, HI), has one of the highest concentrations of Native Hawaiians, per capita, in the country, making it an ideal option to disambiguate NH/OPI from other ethnicities.

Specific Aim #1: Quantify the impact *S. mutans* and *S. sobrinus* genomic diversity (phylogenetic clade membership), virulence, and bacterial load on caries progression and outcomes in Native Hawaiian and Other Pacific Islander children.

Rationale: The impact of genomic differences in caries-causing strains on ECC incidence disparities in NH/OPI populations is, at best, unclear. By surveying the genomic diversity and function of mutans streptococci strains we can determine if phylogenetic differences in NH/OPI, when compared to strains from other populations, drive the observed ethnic health disparities. At the same time, we will evaluate the predictive potential of mutans streptococci genotype and bacterial load at the beginning of academic year on ECC outcomes at the end of the academic year. Results may guide the implementation and need for preventive interventions. **Hypotheses:** (1) There will be observable and statistically significant genomic differences in *S. mutans* and *S. sobrinus* strains circulating in different ethnic subpopulations (NH/OPI vs Hispanic vs Asian vs NHW); and (2) *S. mutans* and *S. sobrinus* strain virulence and bacterial load at first collection event serve as a statistically significant predictors for the speed and extent of caries progression later in the academic year. **Approach:** We will collect and analyze oral microbiota (saliva and plaque) samples from up to 700 pre-school children (1-5 years old), with up to 200 linked sample sets across 2 sampling events (start and end of academic year), focusing on *S. mutans* and *S. sobrinus* strain genotype.

Specific Aim #2: Quantify the impact of classroom-based oral microbiome and *S. mutans* / *S. sobrinus* transmission on caries rates in Native Hawaiian and Other Pacific Islander children.

Rationale: The initial transmission of *mutans streptococci* strains to an affected child has been strongly tied to parental (typically primary caregiver) oral cavity microbiome. At the same time, close quarters interactions common in preschool settings, are likely to facilitate classroom-based microbiome convergence and mutans streptococci transmission. We intend to quantify the change, if any, in child's oral microbiome and *S. mutans* and *S. sobrinus* genotype upon joining a new preschool setting. **Hypothesis:** Microbiome composition and mutans streptococci genotypes from the classroom will out-compete the familial microbiome and genotype once the child enters preschool. **Approach:** We will identify a subset of children who have recently started pre-school or joined a particular classroom, and collect oral microbiota samples from both the child and the primary guardians (as in Aim 1). Oral microbiomes of the children and their guardians will be sequenced using traditional 16S RNA methods, with ECC-causing bacterial strains genotyped as in Aim 1. Microbiome and mutans streptococci genotype change over time will be assessed via Bayesian and ANCOVA techniques.

Impacts / Outcomes: This supplement is the first large scale effort to explicitly understand the biological factors driving caries formation, both at home and at the school (or daycare), in NH/OPI children. Existing continental US-based studies typically do not have sufficient populations (and thus statistical power) to disaggregate NH/OPI children from the Asian population.