# Linking Tooth Brushing Behavior in Children and Oral Microbiota

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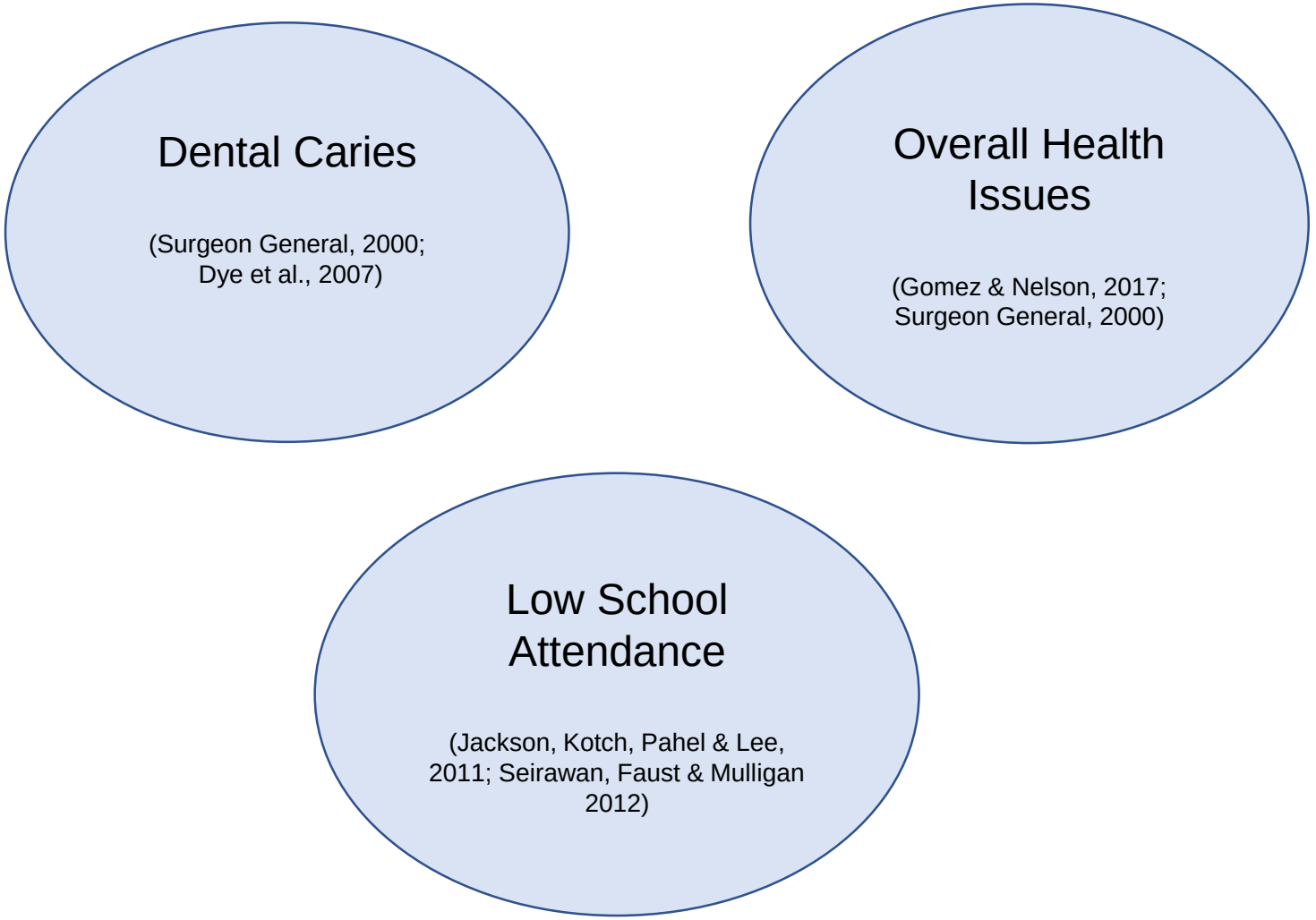
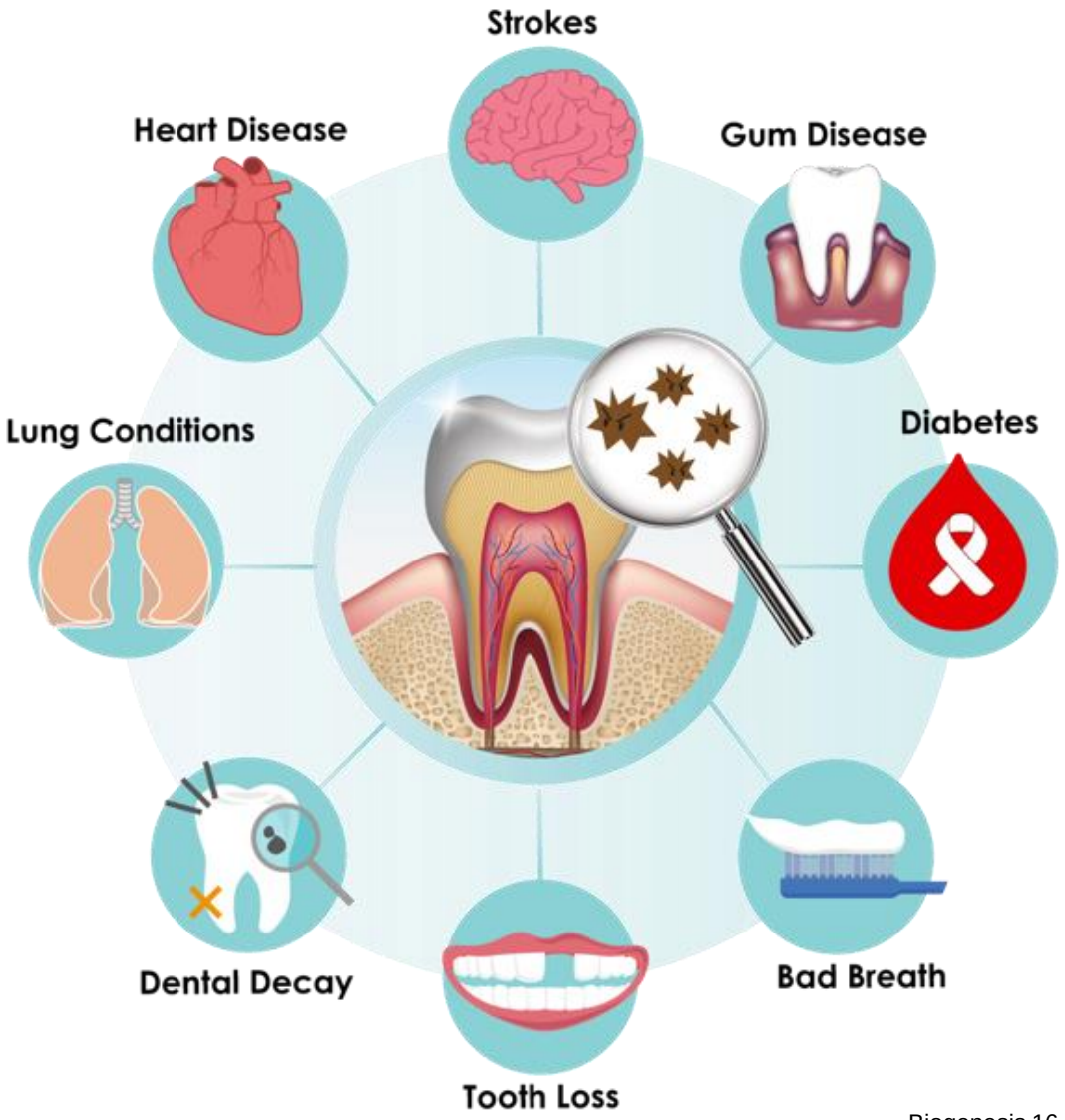
# Children in the United States face potential consequences of poor oral health.






Biogenesis 16, n.d.

**Scientific Significance and Impact:**
Toothbrushing play important role in reducing the abundance of the caries-related bacteria and benefit certain bacterial species to improve oral health.

Recently, associations have been postulated between oral health, the oral microbiota and systematic diseases.

# Our purpose is to explore the association between the oral microbiota and oral health behaviors, such as tooth brushing, in children.

**Hypothesis:** **There is the influence of tooth brushing on oral microbiota across the six mouth sites.**

# Methods

## Design & Sample

- Cross sectional correlational design. A convenience sample of 16 children school age 7-12 years attending a community dental clinic in the Midwest.

## Data Collection

- Survey data included: 1) Demographics, 2) Oral health behaviors, and 3) Dental records

## Bio-specimens for Microbiome Analysis

- Oral swabs collected from six different sites

# Analysis

**Bio-specimen Analysis**

- PCR amplification and sequencing of 16S rRNA gene using Fluidigm and MiSeq

**Statistical Analysis**

- OTUs were identified from sequencing data and the abundance of identified Phyla were compared by tooth brushing frequency using Student's t tests.
- Alpha and Beta diversity statistics were calculated using R Studio.
- Beta diversity measures were compared across tooth brushing groups using Permanova and Anosim tests.

# Sample characteristics (N=16)

- Most participants were Hispanic (28.6%), followed by Caucasian and African American (21.4%, each), and Asian and Bi-racial (14.3%, each). Two participants opted out from reporting race.

- More than half of the participants were male (62.5%). Average age was 9 years old (range: 7-12 years), half of them in 2nd and 4th grade (25% each).

- Results from the sequencing were analyzed with DADA2 Phyloseq in R Studio on 32 samples collected from teeth (upper & lower).

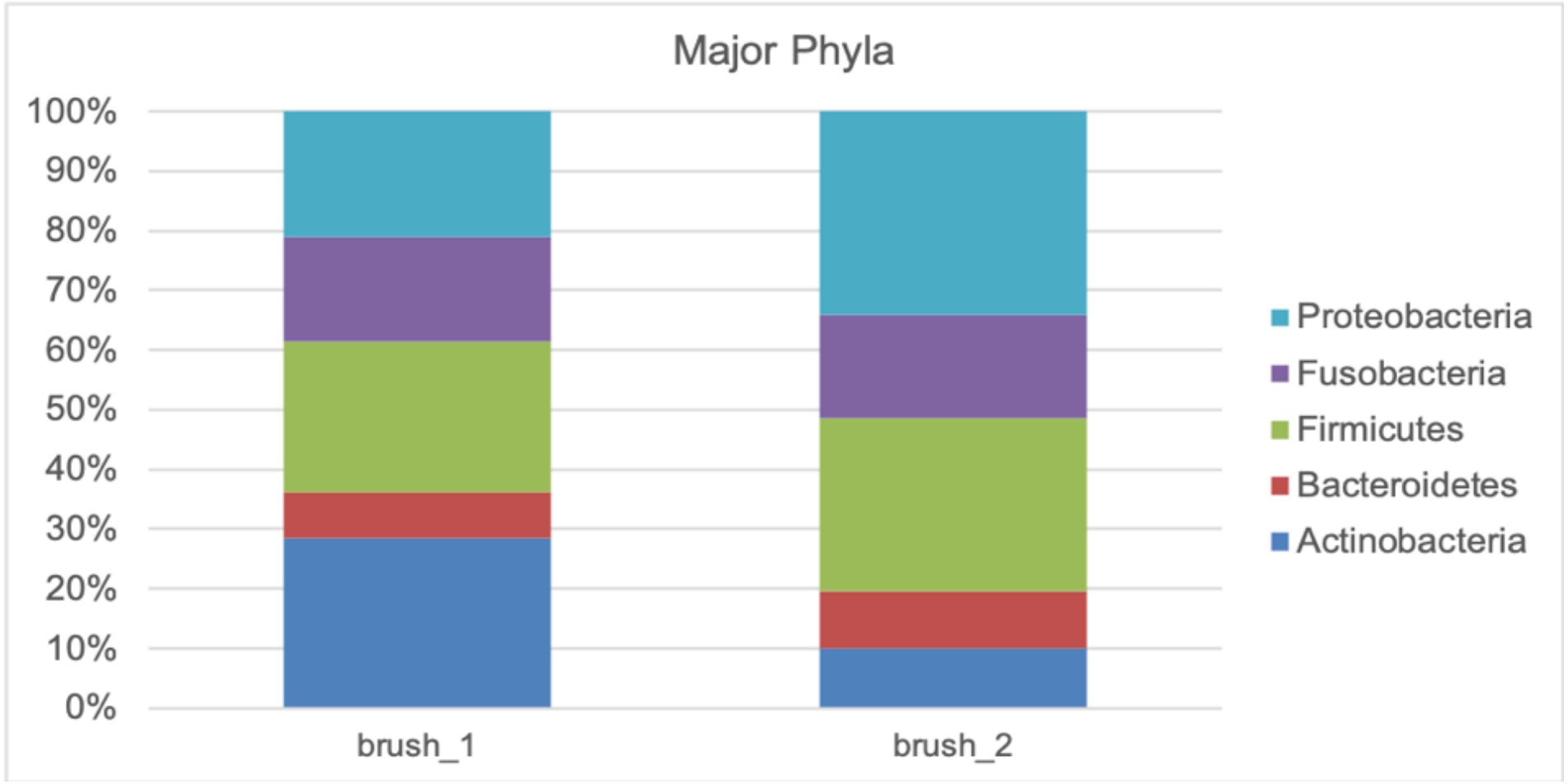
# Oral Health Behaviors N= 16

| Oral Health Behaviors | Frequency | Percentage |
|---|---|---|
| **Brushing** | Every day | 43.8% |
| | Twice daily | 43.8% |
| | More than twice | 6.3% |
| | Others | 6.3% |

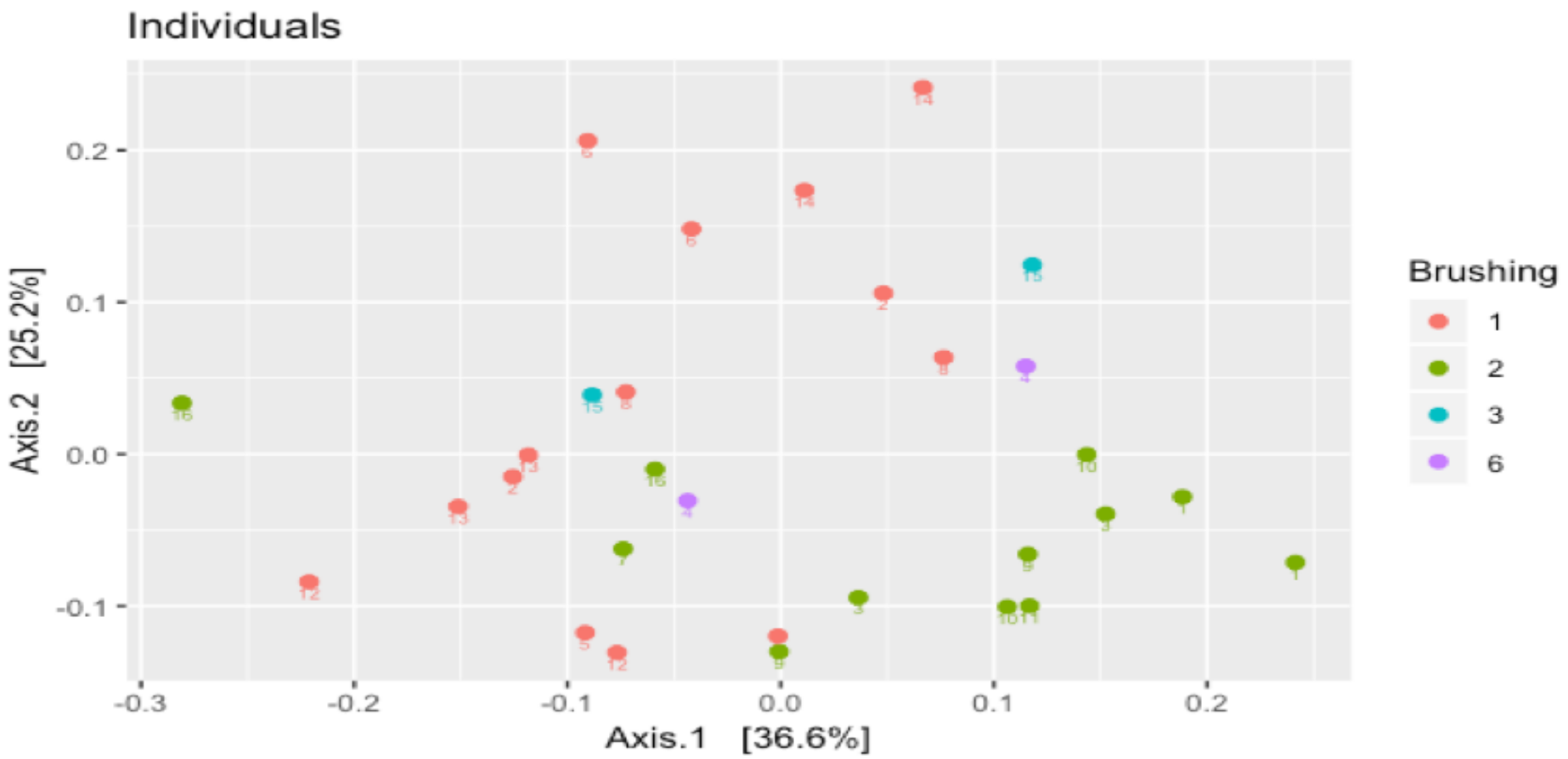
# Microbiome Results

- Tooth brushing frequency among individuals in the sample was once a day (brush1; 43.8%), twice a day (brush2; 43.8%), and more than twice a day (12.6%).
- 16S rRNA sequencing of microbiomes collected from teeth (upper & lower) revealed that the major phyla in the subjects were: *Proteobacteria*, *Fusobacteria*, *Firmicutes, Bacteroidetes*, and *Actinobacteria*.
- The prevalence of *Actinobacteria* significantly decreases from brush1 to brush2 ($p = 0.001045$). The prevalence of *Proteobacteria* significantly increases from brush1 to brush2 ($p = 0.02571$).
- The effect of tooth brushing frequency on microbiome beta diversity (i.e. community composition) is also highly significant ($p = 0.015$ by PERMANOVA and $p = 0.001$ by ANOSIM).

# Identified Phyla



# Beta Diversity Ordination



# Limitations

- Cross sectional study in a small sample size
- Low budget constraints to run the study
- Sample from local site representing a section of the population

# Conclusion

- The findings from this study demonstrate that tooth brushing frequency can affect the proportional composition of the plaque microflora.
- The implication of these changes and caries risk will require additional research.
- Developing tooth brushing interventions aimed at optimizing the oral microbiota may decrease the risk for both oral and systematic diseases.

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# Thank you!