CHILDHOOD OBESITY, EARLY CHILDHOOD CARIES, AND THE ORAL MICROBIOME: A SECONDARY BIOSTATISTICAL ANALYSIS OF ZOE 2.0

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- Meredith Davis, Pediatric Dentistry, Adams School of Dentistry
OVERVIEW

Objective

Methods

Results
CHILDHOOD OBESITY

• **Cause:** “imbalance between energy intake and expenditure”\(^1\)

• **Measure\(^2\):** body mass index (BMI), \([\text{kg/m}^2]\)
  - Obese = 95\(^{th}\) percentile among same age and gender group

• **Risks\(^2\)**
  - Higher risk of chronic diseases in children
  - Higher likelihood of adult obesity

EARLY CHILDHOOD CARIES (ECC)

- **Definition:** “the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of 6”³

- **Cause:** disrupted balance of demineralization and remineralization at the tooth-biofilm interface, where the microbiome ferments carbohydrates and produces acid⁴

- **Measure:** International Caries Detection and Assessment System (ICDAS)⁵
  - Sensitive: ICDAS ≥ 1
  - Established/severe: ICDAS ≥ 3
# THE ROLE OF ORAL MICROBIOME

## ECC
- Gives rise to dental caries via its metabolic activity\(^4\)

## Childhood obesity
- Benahmed et al. (2020)\(^6\) – modulating effects on the gut microbiome, which plays an important role in the development of obesity
- Craig et al. (2018)\(^7\) – stronger indicator of infant weight gain than the gut microbiome
OBJECTIVE

i. Explore the potential of oral microbiome as an indicator of childhood obesity

ii. Explore the potential of oral microbiome as a common biomarker for childhood obesity and early childhood caries
• Genetic epidemiological study of oral health in preschool-age (3-5 years) children enrolled in Head Start centers in North Carolina

• Data includes:
  • Family history and demographics
  • Oral health status (dental caries)
  • Environmental factors
  • Some, oral microbiome

• 8,059 enrolled, 6,404 with dental clinical data
STUDY SAMPLE

302 subjects
- 4 subjects excluded from the DNA analysis
- 7 subjects excluded from the RNA analysis

666 core species
- 418 DNA core species
- 632 RNA core species
- 384 overlap
MULTIPLE LINEAR REGRESSION MODELS

Model 1
zbmi ~ species + age + gender + batch

Model 2
zbmi ~ species + age + gender + batch (t6 = 0)

Model 3
zbmi ~ species + age + gender + batch (t6 = 1)

Model 4
t6 ~ species + age + gender + batch
# VARIABLES & DATA DISTRIBUTION

<table>
<thead>
<tr>
<th>Variable</th>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>zbmi</td>
<td>Continuous</td>
<td>Z-score transformed age- and sex- adjusted body mass index (BMI)</td>
</tr>
<tr>
<td>species</td>
<td>Continuous</td>
<td>DNA/RNA sequence reads, normalized in transcripts per million (TPM), for the individual bacterial species</td>
</tr>
<tr>
<td>age</td>
<td>Continuous</td>
<td>Age in months at the time of exam</td>
</tr>
<tr>
<td>gender</td>
<td>Binary</td>
<td>Genetically verified gender (boy/girl)</td>
</tr>
<tr>
<td>batch</td>
<td>Categorical</td>
<td>Identification number for each sequencing batch</td>
</tr>
<tr>
<td>t6</td>
<td>Binary</td>
<td>Presence of established/severe dental caries, based on international standards (ICDAS ≥ 3)</td>
</tr>
</tbody>
</table>
Each linear regression model run with DNA sequence data and RNA sequence data. P-values and coefficients extracted. P-values adjusted for multiple regression using the FDR method. List of significant species from each model compared.
RESULTS

Total number of significant species across the 4 models: 167

- Obesity associated: 146
- Obesity + ECC associated: 104

Model 1: zbmi ~ species + age + gender + batch
Model 2: zbmi ~ species + age + gender + batch (t6 = 0)
Model 3: zbmi ~ species + age + gender + batch (t6 = 1)
Model 4: t6 ~ species + age + gender + batch

<table>
<thead>
<tr>
<th>Model</th>
<th>p &lt; 0.05</th>
<th>FDR adjusted p &lt; 0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>11</td>
</tr>
</tbody>
</table>

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Model 4: t6 ~ species + age + gender + batch
RESULTS

Associated with obesity only in the absence of dental caries

- 25 species

Model 1: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch}$
Model 2: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch} \ (t6 = 0)$
Model 3: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch} \ (t6 = 1)$
Model 4: $t6 \sim \text{species} + \text{age} + \text{gender} + \text{batch}$
RESULTS

Associated with obesity only in the presence of caries

- 77 species
- 24 species with FDR adjusted p < 0.2

Model 1: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch}$
Model 2: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch} (t6 = 0)$
Model 3: $zbmi \sim \text{species} + \text{age} + \text{gender} + \text{batch} (t6 = 1)$
Model 4: $t6 \sim \text{species} + \text{age} + \text{gender} + \text{batch}$
RESULTS

Only associated with obesity, no ECC association

• 42 species
• 2 species with FDR adjusted \( p < 0.2 \)
  • *Glaesserella sp. 15-814* (-)
  • *Actinobacillus pleuropneumoniae* (+)

Model 1: \( \text{zbmi} \sim \text{species} + \text{age} + \text{gender} + \text{batch} \)
Model 2: \( \text{zbmi} \sim \text{species} + \text{age} + \text{gender} + \text{batch} (t6 = 0) \)
Model 3: \( \text{zbmi} \sim \text{species} + \text{age} + \text{gender} + \text{batch} (t6 = 1) \)
Model 4: \( t6 \sim \text{species} + \text{age} + \text{gender} + \text{batch} \)
RESULTS

Associated with obesity and ECC on separate terms

• 2 species

• 1 species with FDR adjusted p < 0.2
  • *Veillonella dispar* (-/-)

Model 1: zbmi ~ species + age + gender + batch
Model 2: zbmi ~ species + age + gender + batch (t6 = 0)
Model 3: zbmi ~ species + age + gender + batch (t6 = 1)
Model 4: t6 ~ species + age + gender + batch

**Significant species, nominal p < 0.05**
CONCLUSION

I. *Glaesserella sp. 15-184* and *Actinobacillus pleuropneumoniae* may potentially serve as an indicator of childhood obesity.

II. *Veillonella dispar* may potentially serve as a common biomarker for childhood obesity and early childhood caries.

III. Other species need further investigation for conclusive results.
LIMITATIONS

+ FUTURE DIRECTIONS

- Limitation: limited sample size, resulting in limited testing power

- Future directions
  1) Contradicting directionality of correlation from stratified sampling
  2) Biological mechanisms of identified species in their roles in childhood obesity and ECC
  3) Metabolomics of the oral biofilm with regards to the two conditions
REFERENCES


THANK YOU!