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

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ACKNOWLEDGEMENT

- Di Wu, Department of Biostatistics, UNC Chapel Hill
- Kimon Divaris, Pediatric and Public Health, Adams School of Dentistry
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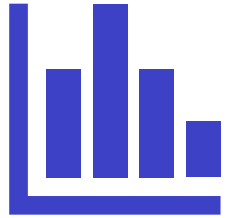
- Meredith Davis, Pediatric Dentistry, Adams School of Dentistry

OVERVIEW

Objective

Methods

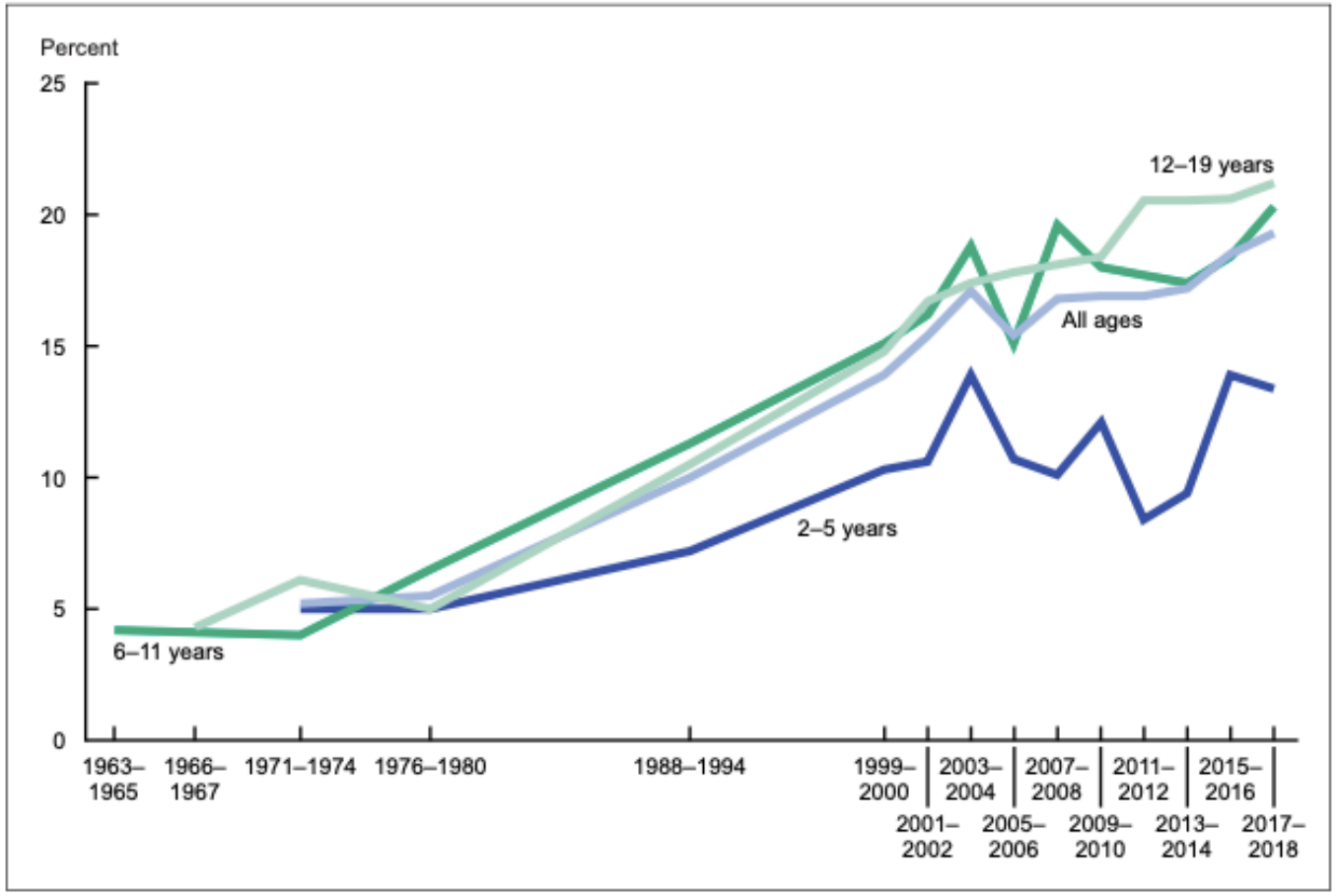


Results

CHILDHOOD OBESITY

- *Cause*: "imbalance between energy intake and expenditure"[1]
- *Measure*[2]: body mass index (BMI), [kg/m^2]
  - Obese = 95th percentile among same age and gender group
- *Risks*[2]
  - Higher risk of chronic diseases in children
  - Higher likelihood of adult obesity

Figure. Trends in obesity among children and adolescents aged 2–19 years, by age: United States, 1963–1965 through 2017–2018



Adopted from: Fryar CD, Carroll MD, Afful J. Prevalence of Overweight, Obesity, and Severe Obesity Among Children and Adolescents Aged 2-19 Years: United States, 1963-1965 Through 2017-2018. *NCHS Health E-Stats*. 2020.

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 acid[4]
- *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

ZOE 2.0[8]

- 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

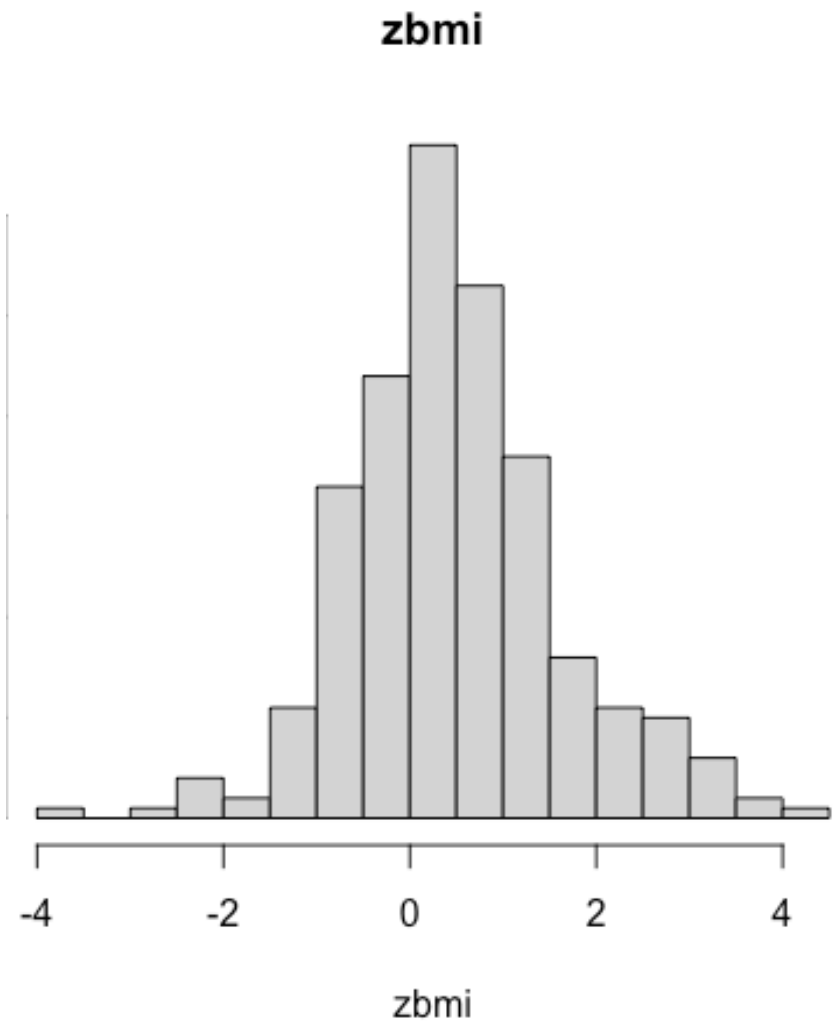
zbmi ~ species + age + gender + batch (t6 = 1)

Model 4

t6 ~ species + age + gender + batch

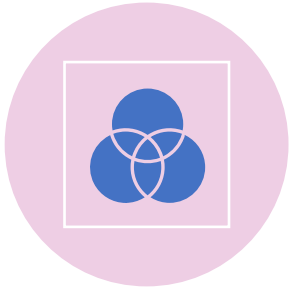
VARIABLES & DATA DISTRIBUTION

Variable	Type	Description
zbmi	Continuous	Z-score transformed age- and sex- adjusted body mass index (BMI)
species	Continuous	DNA/RNA sequence reads, normalized in transcripts per million (TPM), for the individual bacterial species
age	Continuous	Age in months at the time of exam
gender	Binary	Genetically verified gender (boy/girl)
batch	Categorical	Identification number for each sequencing batch
t6	Binary	Presence of established/severe dental caries, based on international standards (ICDAS ≥ 3)



STATISTICAL ANALYSIS

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

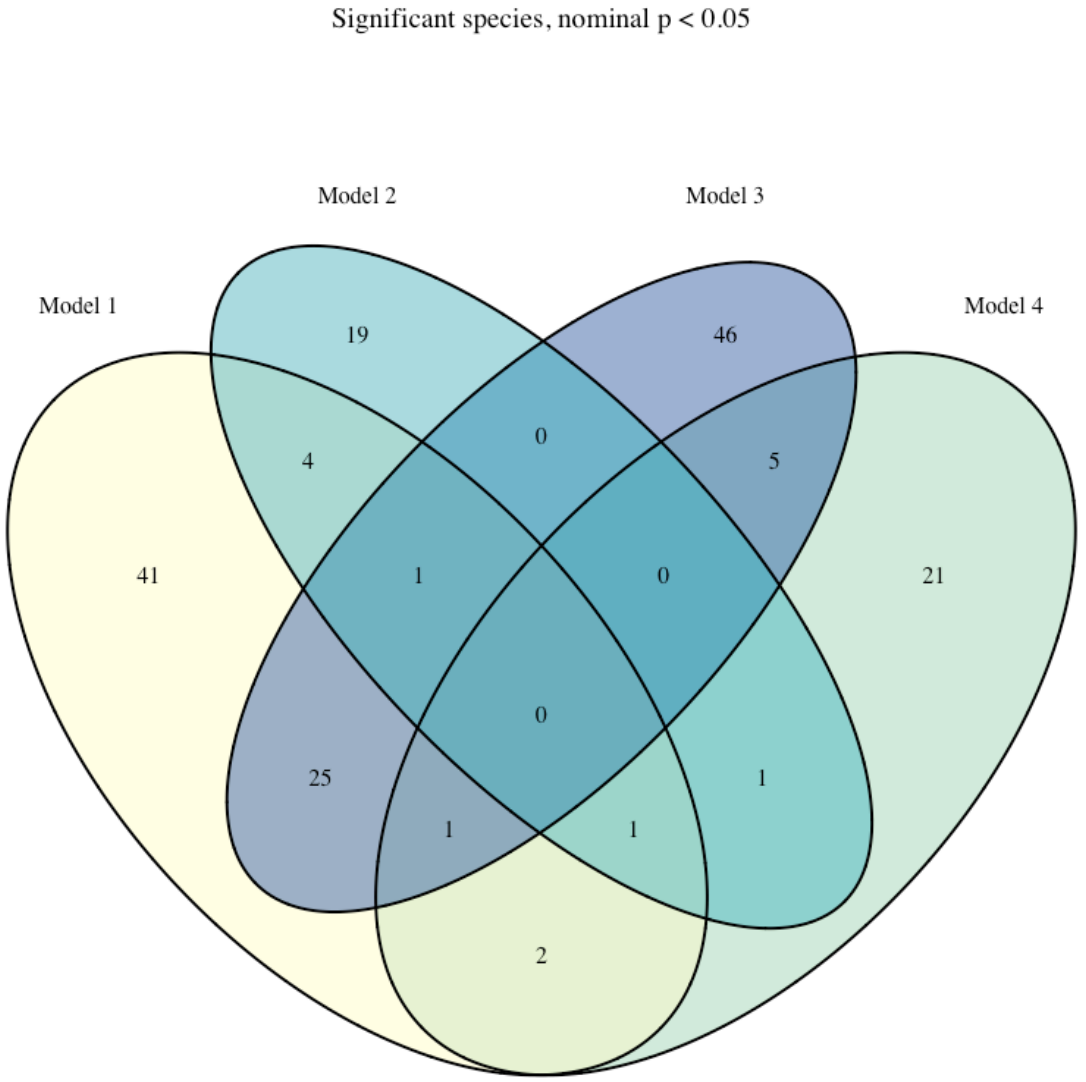
Model	p < 0.05	FDR adjusted p < 0.2
1	75	5
2	26	0
3	78	29
4	31	11

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



RESULTS

Total number of significant species across the 4 models: 167

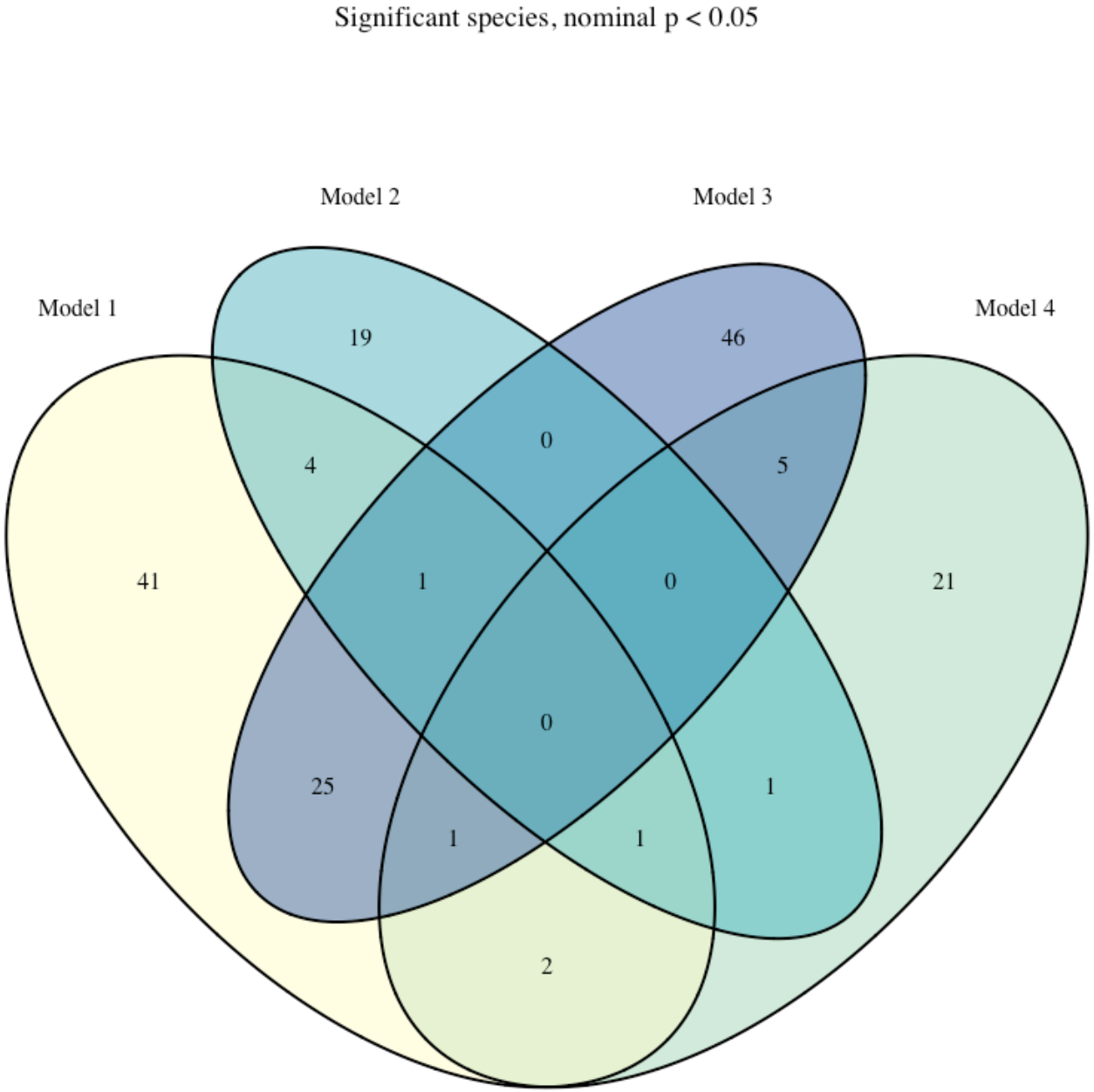
- Obesity associated: 146
- Obesity + ECC associated: 104

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)

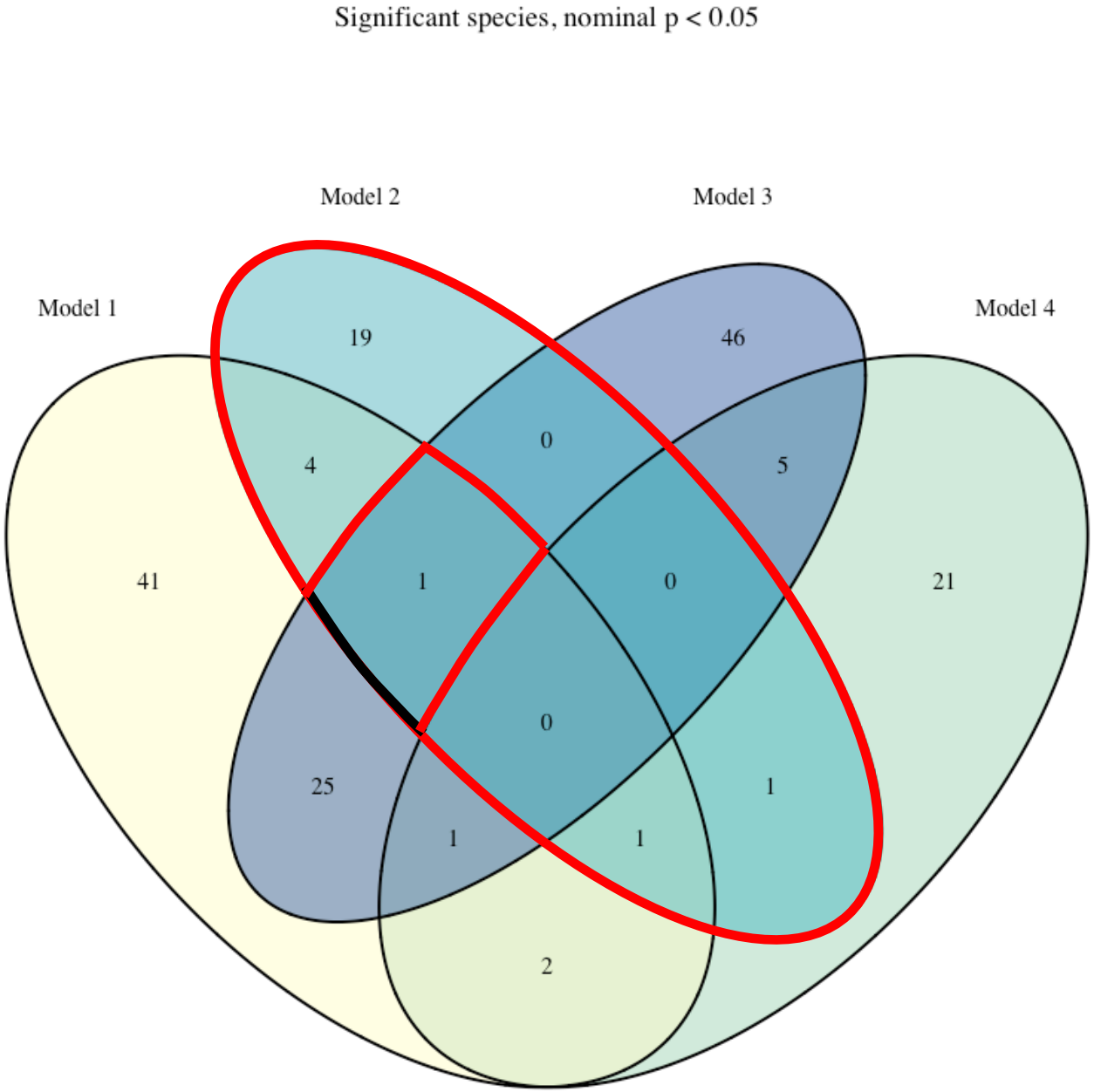
Model 4: t6 ~ species + age + gender + batch



RESULTS

Associated with obesity only in the absence of dental caries

- 25 species



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

RESULTS

Associated with obesity only in the presence of caries

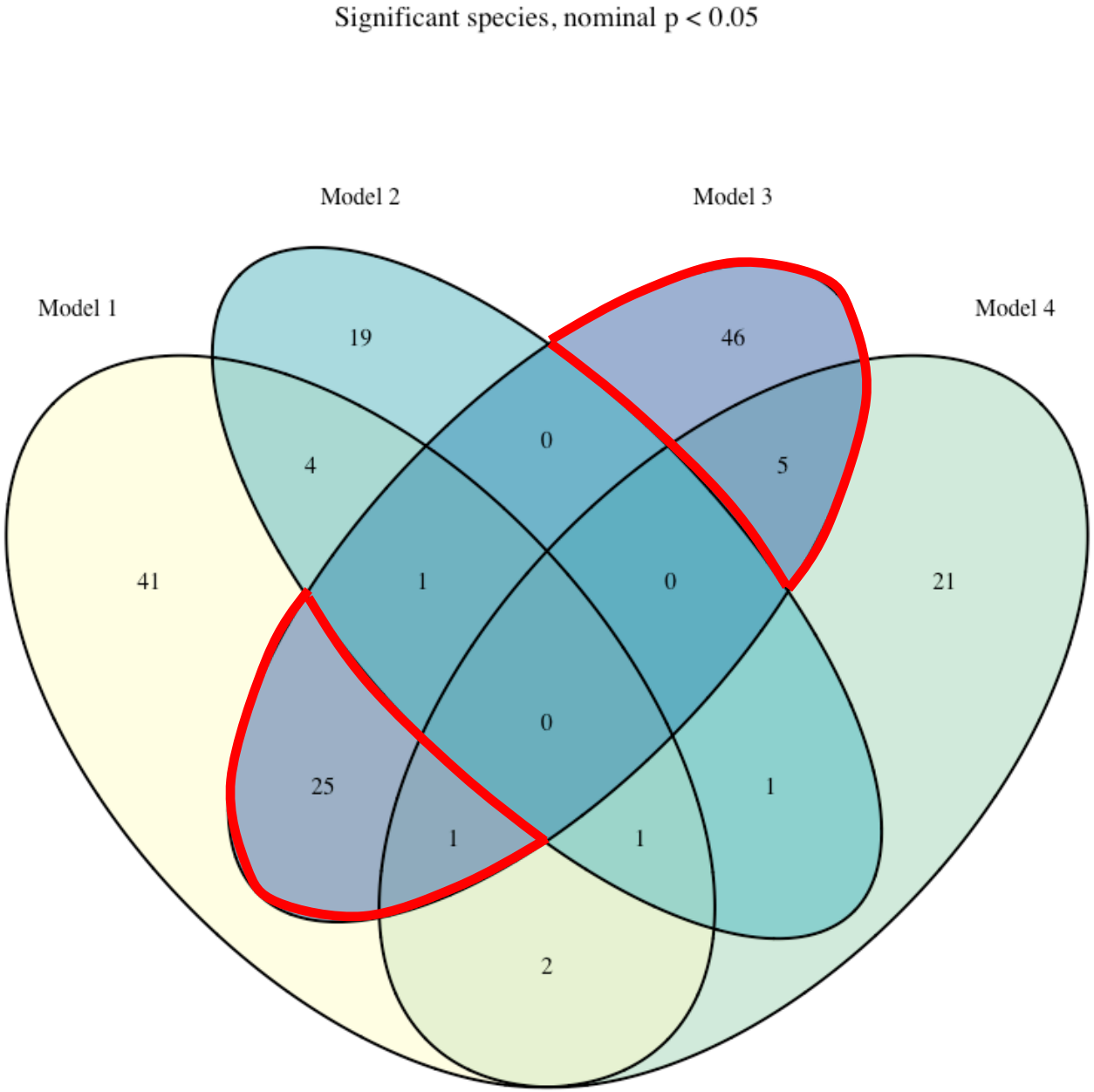
- 77 species
- 24 species with FDR adjusted p < 0.2

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



RESULTS

Only associated with obesity, no ECC association

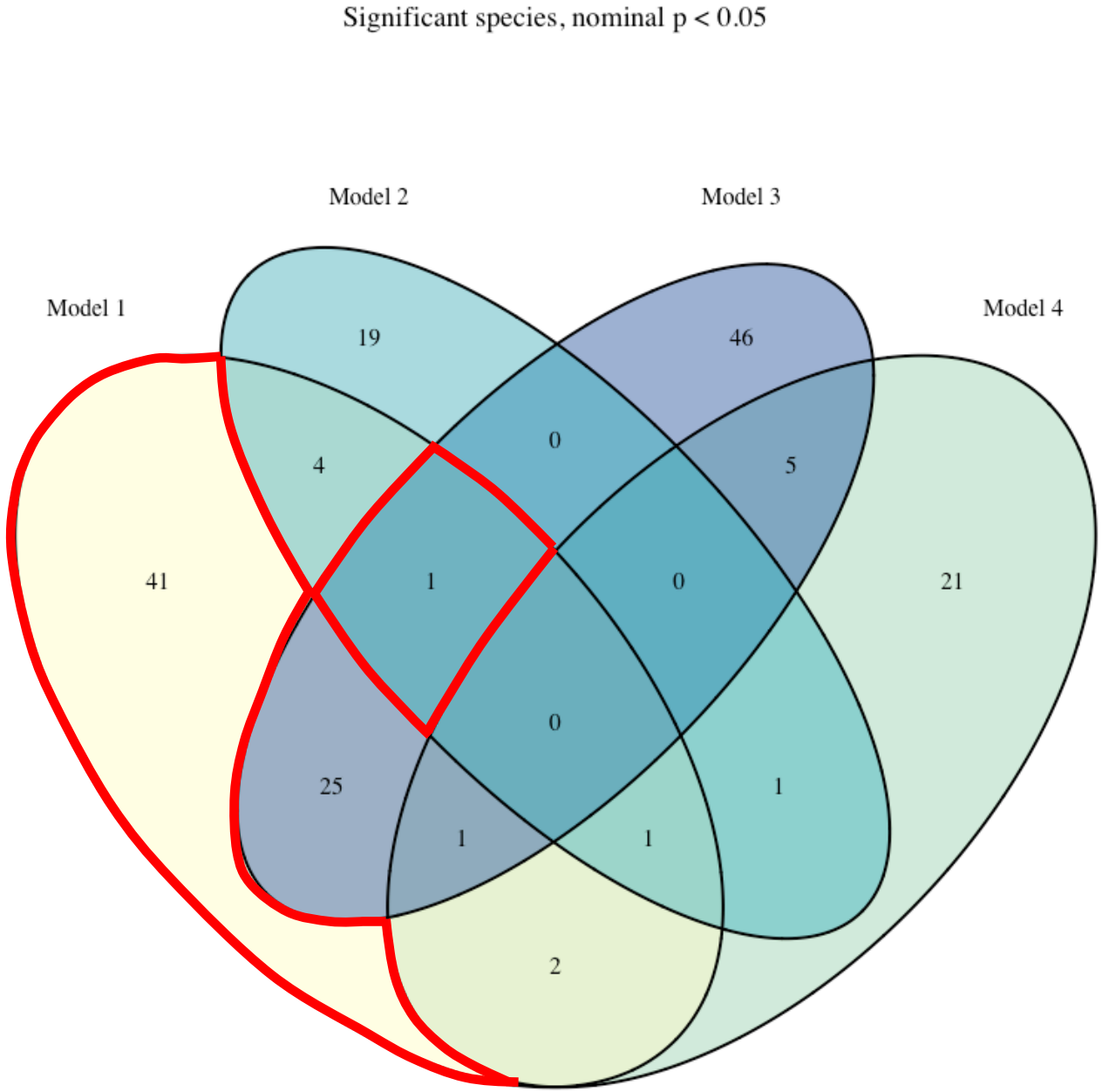
- 42 species
- 2 species with FDR adjusted p < 0.2
  - *Glaesserella sp. 15-814* (-)
  - *Actinobacillus pleuropneumoniae* (+)

Model 1: zbmi ~ species + age + gender + batch

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

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

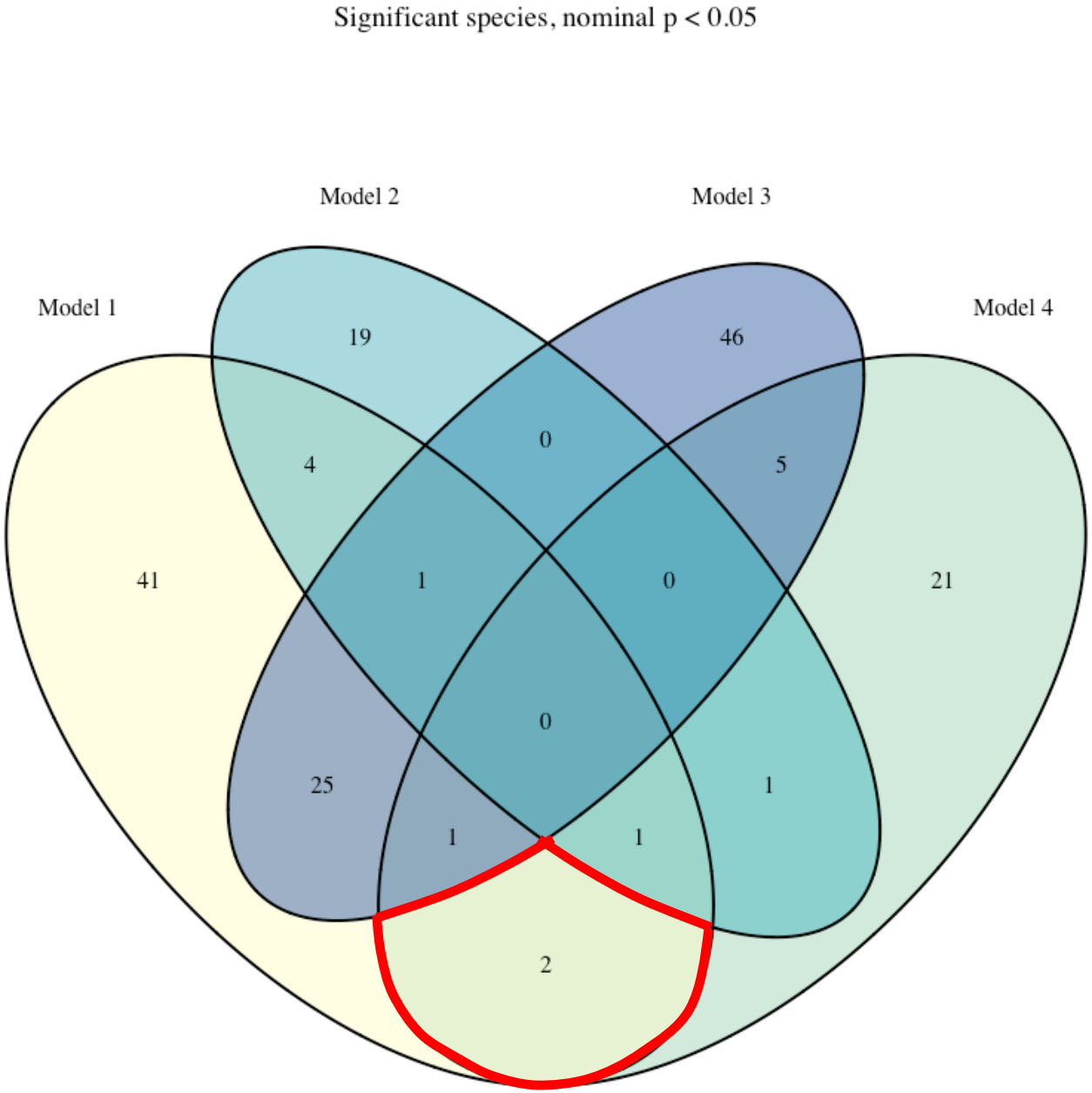
Model 4: t6 ~ species + age + gender + batch



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

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

1. Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, Bhadoria AS. Childhood obesity: causes and consequences. *J Family Med Prim Care*. 2015;4(2):187-192. doi:10.4103/2249-4863.154628

2. Overweight & Obesity. cdc.gov. https://www.cdc.gov/obesity. Updated March 23, 2021. Accessed July 1, 2021.

3. Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and Reporting Early Childhood Caries for Research Purposes. *J Public Health Dent*. 1999; 59(3):192-197.

4. Divaris K. Predicting Dental Caries Outcomes in Children: A "Risky" Concept. *J Dent Res.* 2016;95(3):249-254. doi:10.1177/0022034515620779.

5. Pitts NB, Ekstrand KR; ICDAS Foundation. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) – methods for staging of the caries process and enabling dentists to manage caries. *Community Dent Oral Epidemiol*. 2013;41(1):41-52. doi:10.1111/cdoe.12025.

6. Benahmed AG, Gasmi A, Doşa A, Chirumbolo S, Mujawdiya PK, Aaseth J, Dadar M, Bjørklund G. Association between the gut and oral microbiome with obesity. *Anaerobe*. 2020;70:102248. doi:10.1016/j.anaerobe.2020.102248.

7. Craig SJ, Blankenberg D, Parodi AC, Paul IM, Brich LL, Savage JS, Marini ME, Stokes JL, Nekrutenko A, Reimherr M, Chiaromonte F, Makova KD. Child Weight Gain Trajectories Linked To Oral Microbiota Composition. *Sci Rep*. 2018;8:14030. doi: 10.1038/s41598-018-31866-9.

8. Divaris K, Slade GD, Ferreira Zandona AG, Preisser JS, Ginnis J, Simancas-Pallares MA, Agler CS, Shrestha P, Karhade DS, Ribeiro AdA, Cho H, Gu Y, Meyer BD, Joshi AR, Azcarate-Peril MA, Basta PV, Wu D, North KE. Cohort Profile: ZOE 2.0—A Community-Based Genetic Epidemiologic Study of Early Childhood Oral Health. *Int J Environ Res Public Health*. 2020;17(21):8056. doi:10.3390/ijerph17218056.

THANK YOU!