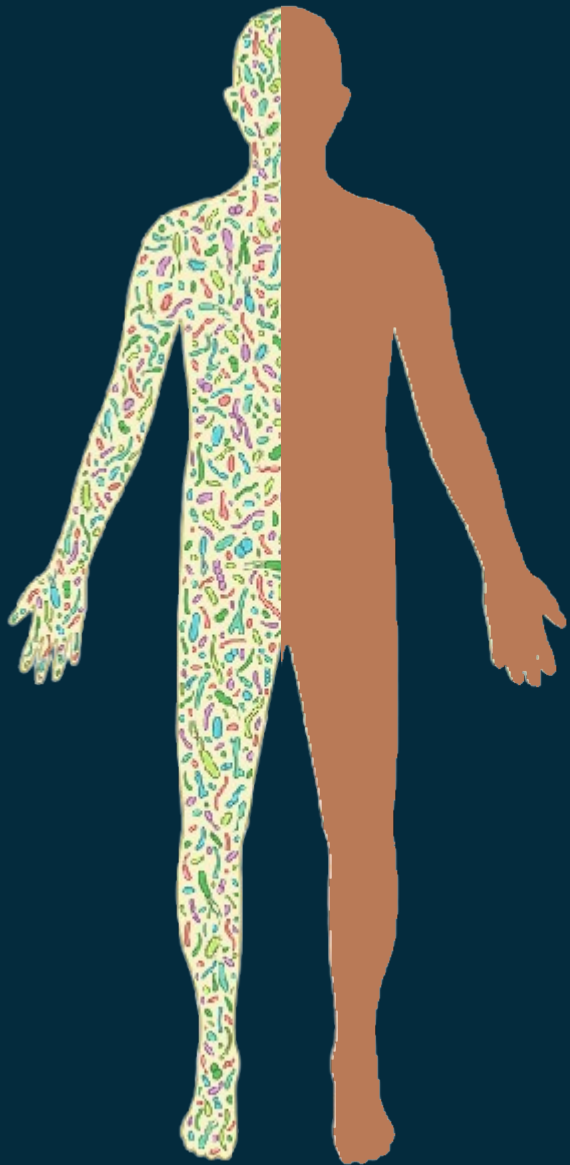


# The Salivary Microbiome and Cognitive Function

John Robert Warren, Stephen Johnson, Michael Culbertson, Bruce Paster, Eric Grodsky, Chandra Muller, Bharat Thyagarajan, Adam M. Brickman, Jennifer Manly, Ryan T. Demmer



In your body...

*Cells*

*Genes*

**Human**

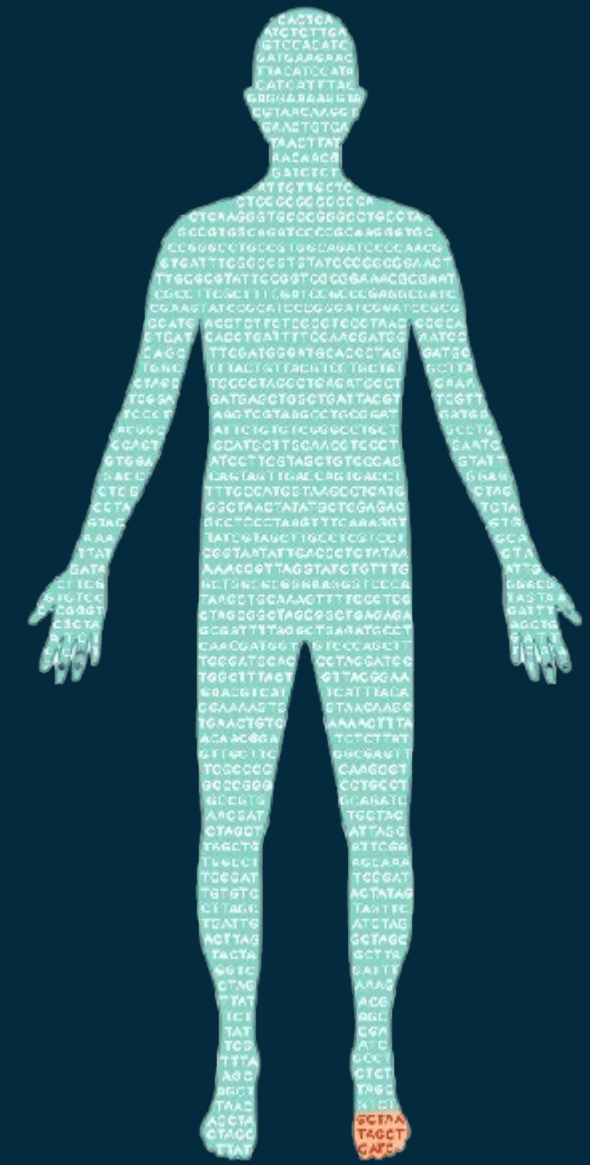
~30 trillion

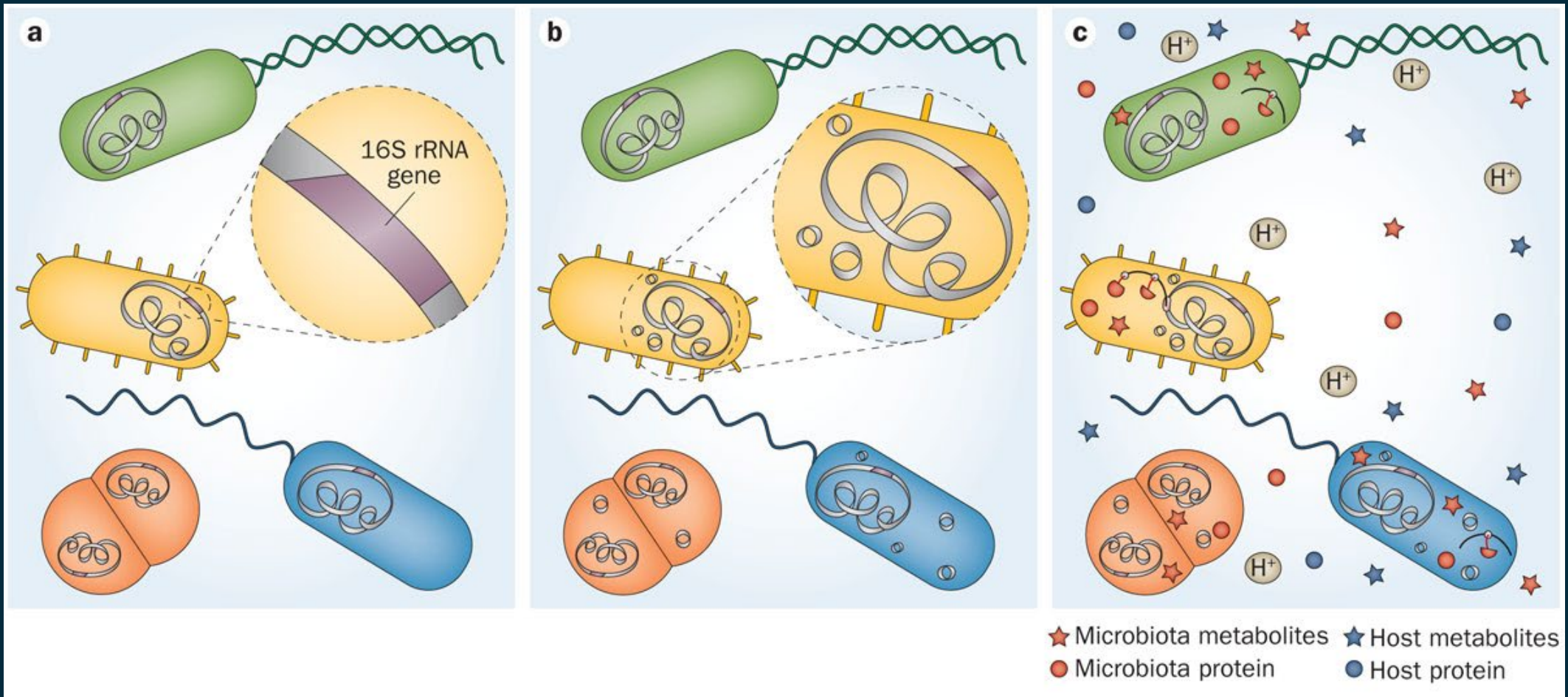
~20,000

**Microbes**

~30 trillion

~2 million





## Microbiota

*Who organisms are there?*

Taxonomic identification  
(e.g., 16S rRNA)

## Metagenome

*What are they doing?*

Genes and genomes of  
microbiota

## Microbiome

*The whole stew*  
Microbes, genomes,  
genes, proteins...

# Oral Dysbiosis

1. Microbial imbalance defined by comparing microbiota observed in diseased people to those among healthy controls
2. Focus on overabundance ("dominance") of select taxa



# Today's Research Questions

How is the composition of oral microbiota associated with...

1. Midlife cognitive functioning
2. ADRD pathophysiology
3. Early socioeconomic, educational, spatial, and demographic factors

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How is the composition of oral microbiota associated with...

1. Midlife cognitive functioning

~~2. ADRD pathophysiology~~

~~3. Early socioeconomic, educational, spatial, and demographic factors~~

# 1. Oral Microbiota and Cognition

# Oral Microbiota potentially...

- ... increase systemic and neuroinflammation (perhaps via mechanisms like bacterial lipopolysaccharides )
- ... influence cardiometabolic diseases (via nitrogen metabolism and other pathways)
- ... directly infect the brain (e.g., via the cranial trigeminal nerves, via) **with neurotoxic consequences**

# Prior Research

- Oral microbiota associated with systemic inflammation, diabetes, cardiovascular disease, stroke
- Evidence is from small-scale clinical samples; not population representative, little ability to adjust for confounders
- Evidence about cognition or ADRD risk universally uses periodontal disease as a proxy
- Oral microbiota and AD neuropathology?
- *Beneficial* oral microbiota?

# Innovations

1. First direct assessments of the oral microbiota in a large, population representative cohort study
2. Assessment of a range of beneficial *and* detrimental influences of the oral microbiota
3. First analyses of the early life factors that shape the oral microbiota using data from a large, population representative sample
4. Among the first analyses of the association between oral microbiota *itself* and (a) cognitive function and (b) AD neuropathology

# Innovations

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2. Allows for assessment of a range of beneficial *and* detrimental influences of the oral microbiota
- ~~3. First analyses of the early life factors that shape the oral microbiota using data from a large, population representative sample~~
4. Among the first analyses of the association between oral microbiota *itself* and (a) cognitive function ~~and (b) AD neuropathology~~

## 2. Introduction to High School & Beyond: 1980



## Sophomores (n=30,030)

Year 1980

Age 16

## Seniors (n=28,240)

Year 1980

Age 18

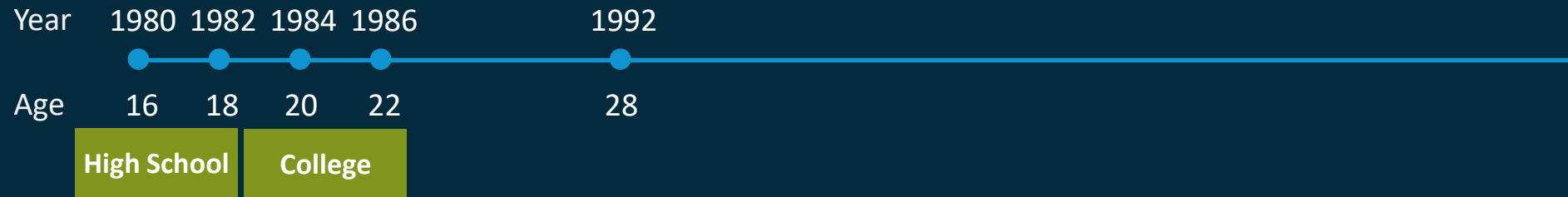
## Sophomores (n=14,830)



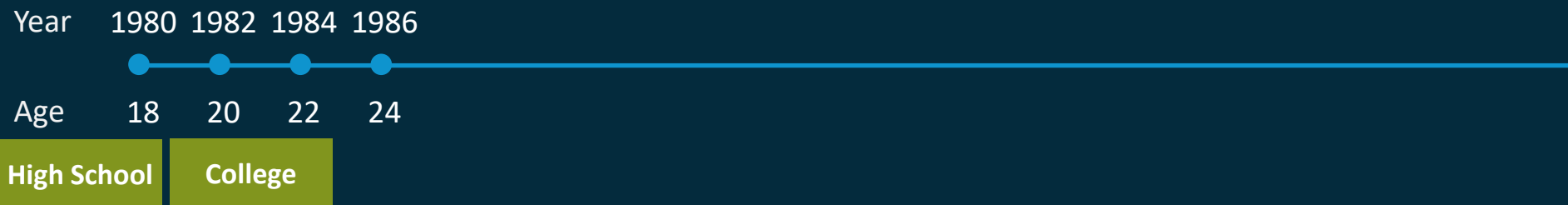
## Seniors (n=12,000)



## Sophomores (n=14,830)



## Seniors (n=12,000)



# Surveys + Administrative Records

## Sophomores (n=14,830)



## Seniors (n=12,000)



Surveys +  
Health Visits +  
Blood & Saliva +  
Administrative Records

### Sophomores (n=14,830)



### Seniors (n=12,000)



Cognition, Education, Work,  
Income, Health, COVID,  
Demographics, More

Surveys +  
Health Visits +  
Blood & Saliva +  
Administrative Records

### Sophomores (n=14,830)



### Seniors (n=12,000)



Height, Weight, Hip and  
Waist Circumference, Blood  
Pressure, Pulse

Surveys +  
Health Visits +  
Blood & Saliva +  
Administrative Records

### Sophomores (n=14,830)



### Seniors (n=12,000)



Mortality, Prescriptions,  
Consumer Credit, More

Surveys +  
Health Visits +  
Blood & Saliva +  
Administrative Records

### Sophomores (n=14,830)



### Seniors (n=12,000)

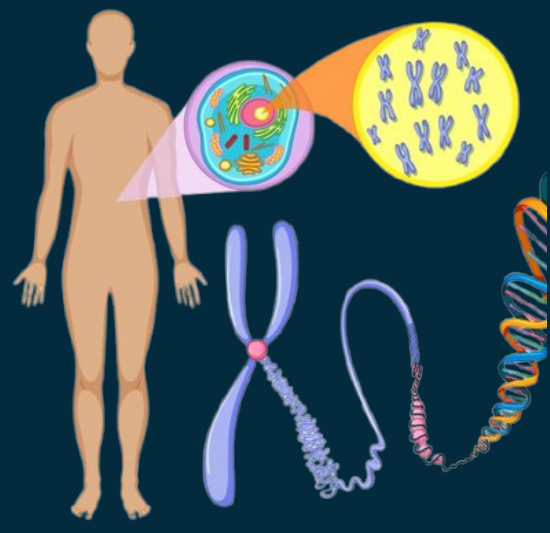


Markers of Neuropathology,  
Inflammation, Immunology,  
Systemic Aging, Metals, More

Surveys +  
Health Visits +  
Blood & Saliva +  
Administrative Records



Surveys +  
Health Visits +  
Blood & **Saliva** +  
Administrative Records



Surveys +  
Health Visits +  
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# 3. Research Design

# HS&B:80 DATA

- $n=5,880$  who responded in 2021 and provided a saliva sample
- Sampling weights
- Clustering

# COGNITIVE FUNCTIONING MEASURE

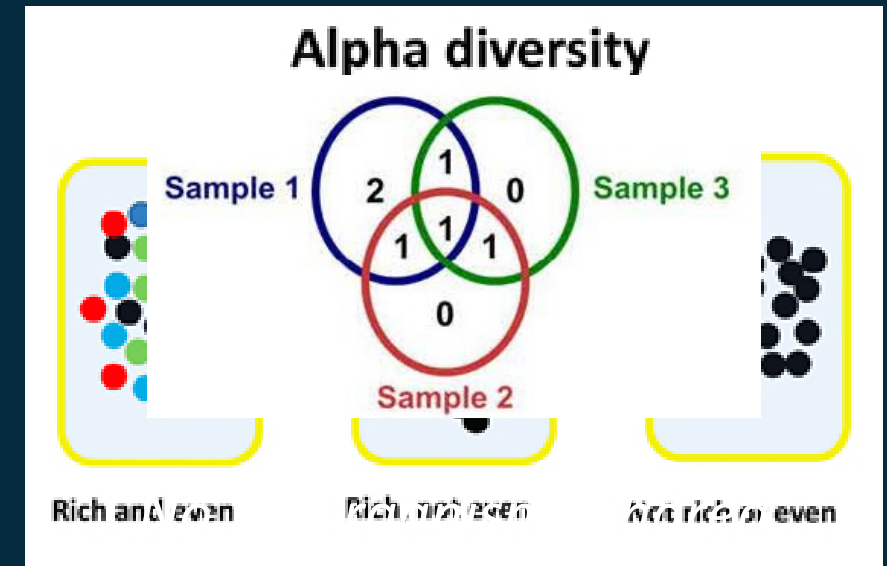
- Four cognitive tasks on telephone survey:
  - (1) Immediate and delayed word recall
  - (2) Forward and backward digit span
  - (3) Phonemic and (4) Semantic fluency
- Two cognitive tasks on the web survey:
  - (5) visual and (6) verbal paired associates
- Composite measure of cognitive functioning based on all six tasks using a hierarchical explanatory IRT model

# MICROBIOTA MEASURES

- Omnigene kit; unstimulated drooling
- DNA extracted and sequenced at ARDL/UMGC
- Samples paired-end sequenced at a read length of 300 nucleotides (nt) on the Illumina NextSeq
  - Preprocessing median (range): 71,800 (191 – 206,177)
  - Post-filtering median (range): 71,710 (191 – 206,121)
  - Anybody sample w/ <20,000 sequence reads removed

# MICROBIOTA MEASURES

- Alpha Diversity (Shannon Index)
- Beta Diversity (Bray-Curtis)
- Treponema : Corynebacterium Ratio



between people; people  
with similar (richness) have  
balanced (evenness) of  
organisms within a sample

# 4. Results

# Reality Check 1

	<b>Coefficient</b>	<b>95% CI</b>	<b>p</b>
Age	-0.03	-0.05, -0.01	0.001
Male	-0.37	-0.42, -0.33	<0.0001
Education (vs. HS or less)			
Some college	0.29	0.22, 0.35	<0.001
Two-year Degree	0.40	0.28, 0.52	<0.001
Four-year Degree	0.50	0.43, 0.58	<0.001
Graduate Degree	0.65	0.57, 0.74	<0.001
Grade 12 Achievement	0.55	0.52, 0.58	<0.001
Diabetes	-0.19	-0.11, -0.27	<0.001
Hypertension	-0.10	-0.04, -0.16	<0.001

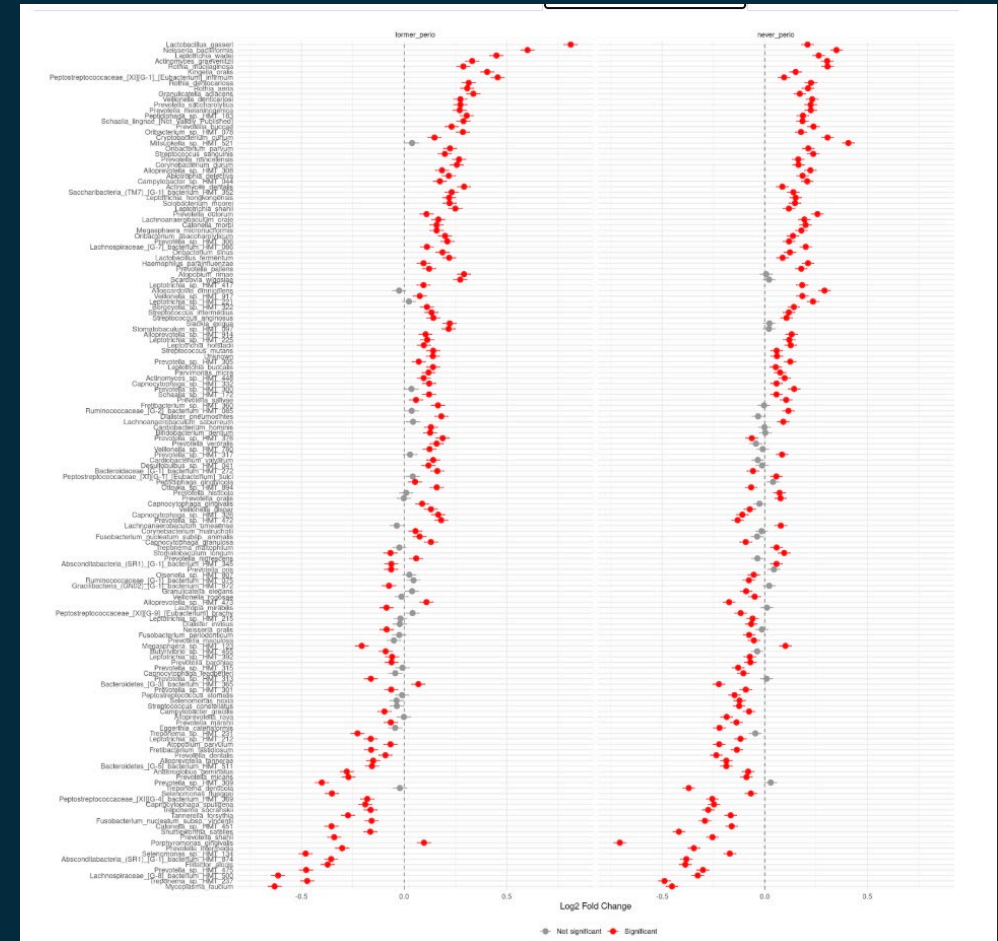
# Reality Check 2

Key species enriched (*more abundant*) among people without periodontal disease

- *R. mucilaginosa*
- *C. durum*

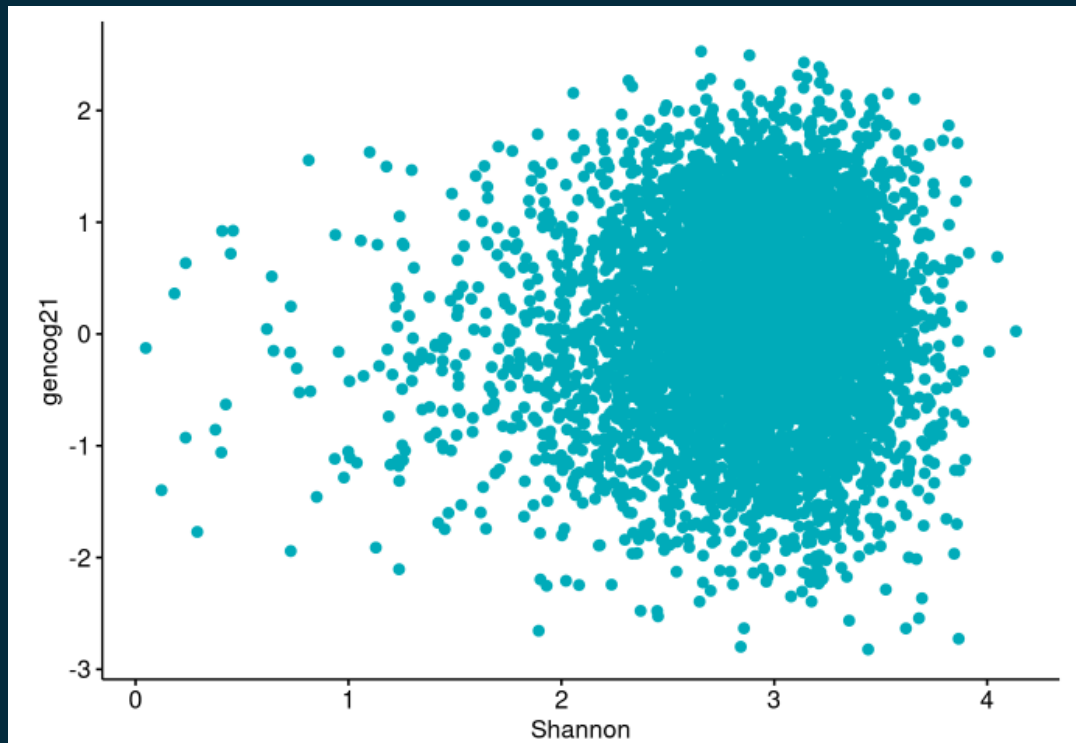
Key species enriched (*more abundant*) among people with periodontal disease

- *P. gingivalis*
- *F. nucleatum*
- *T. forsythia*
- *F. alocis*
- *Treponema spp.*



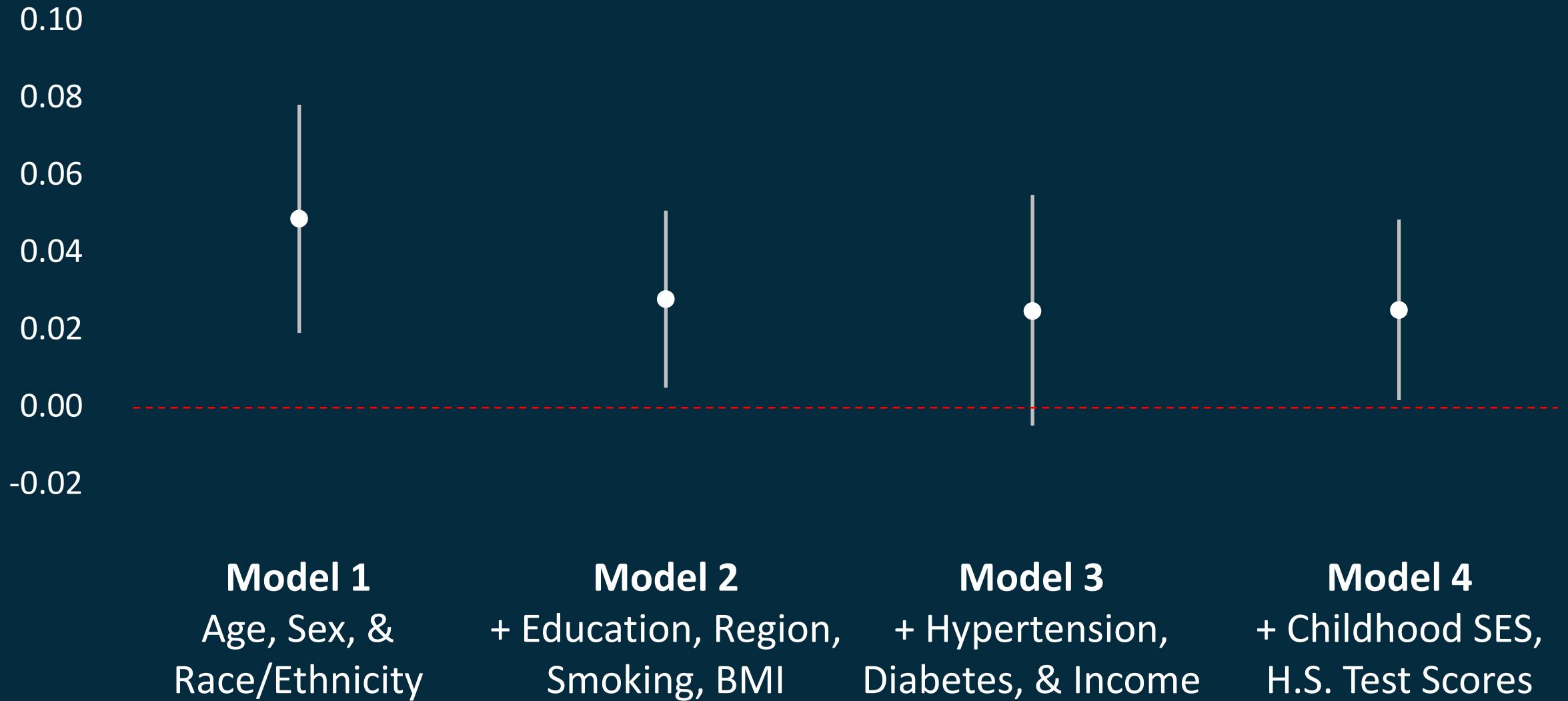
# Alpha Diversity & Cognitive Function

Modest positive association



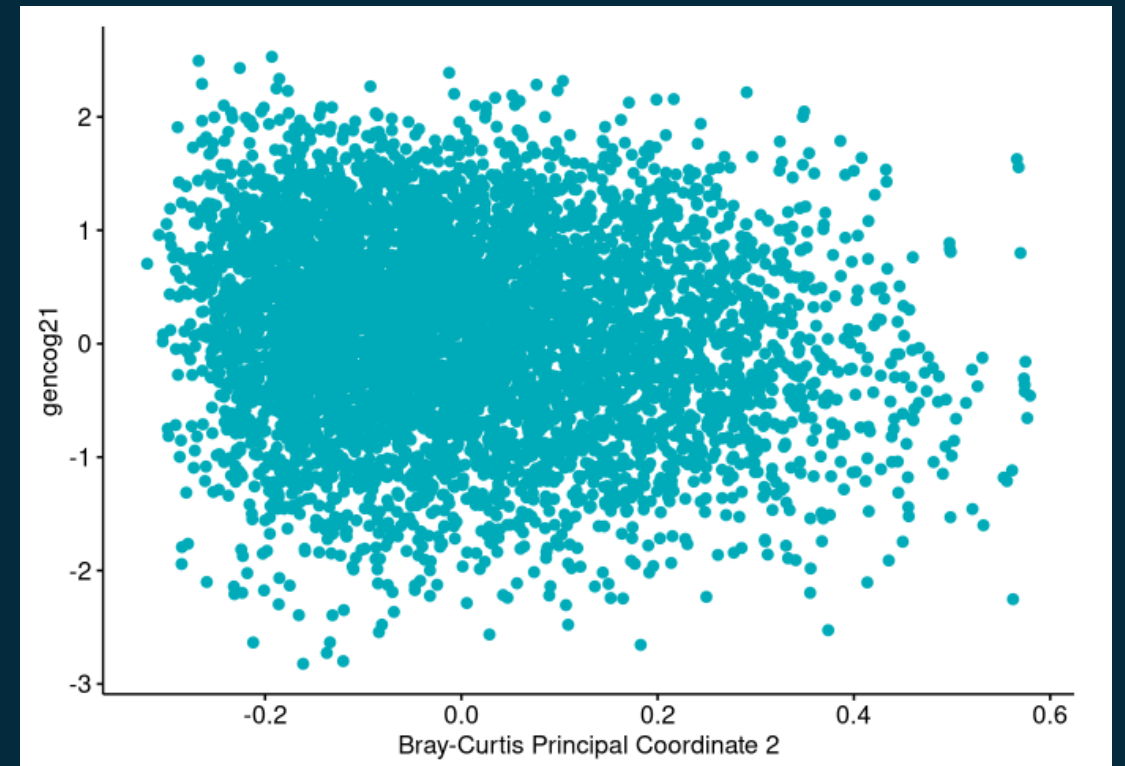
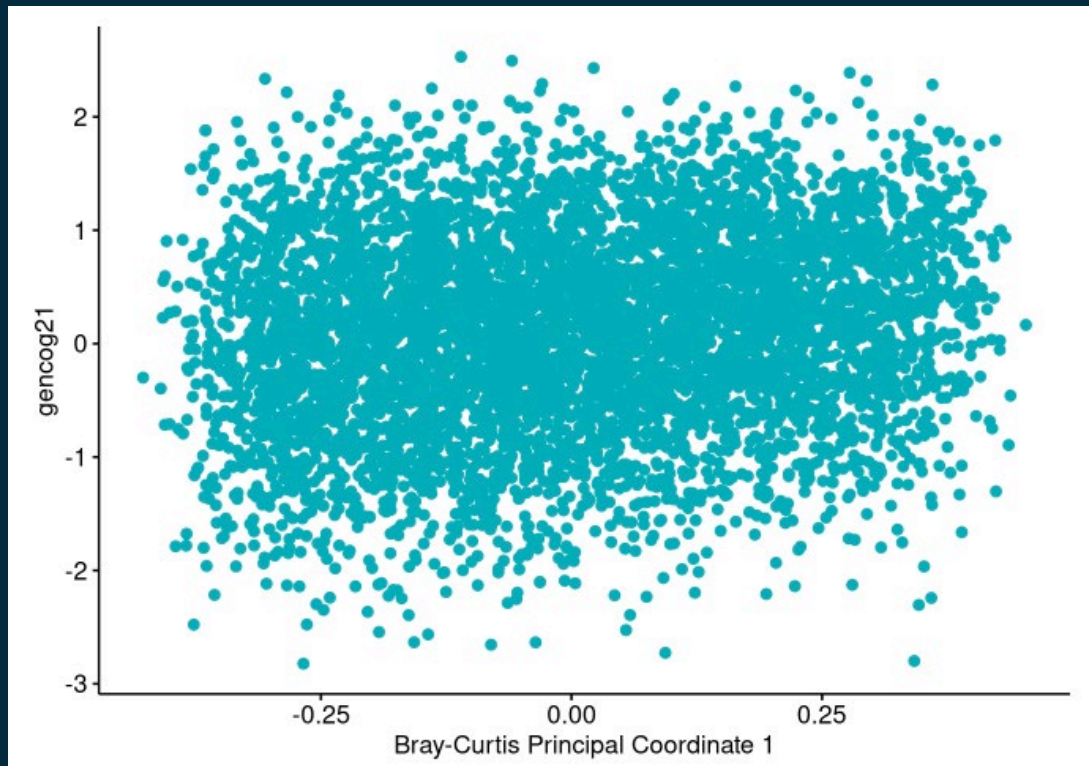
# Alpha Diversity

(Shannon Index)



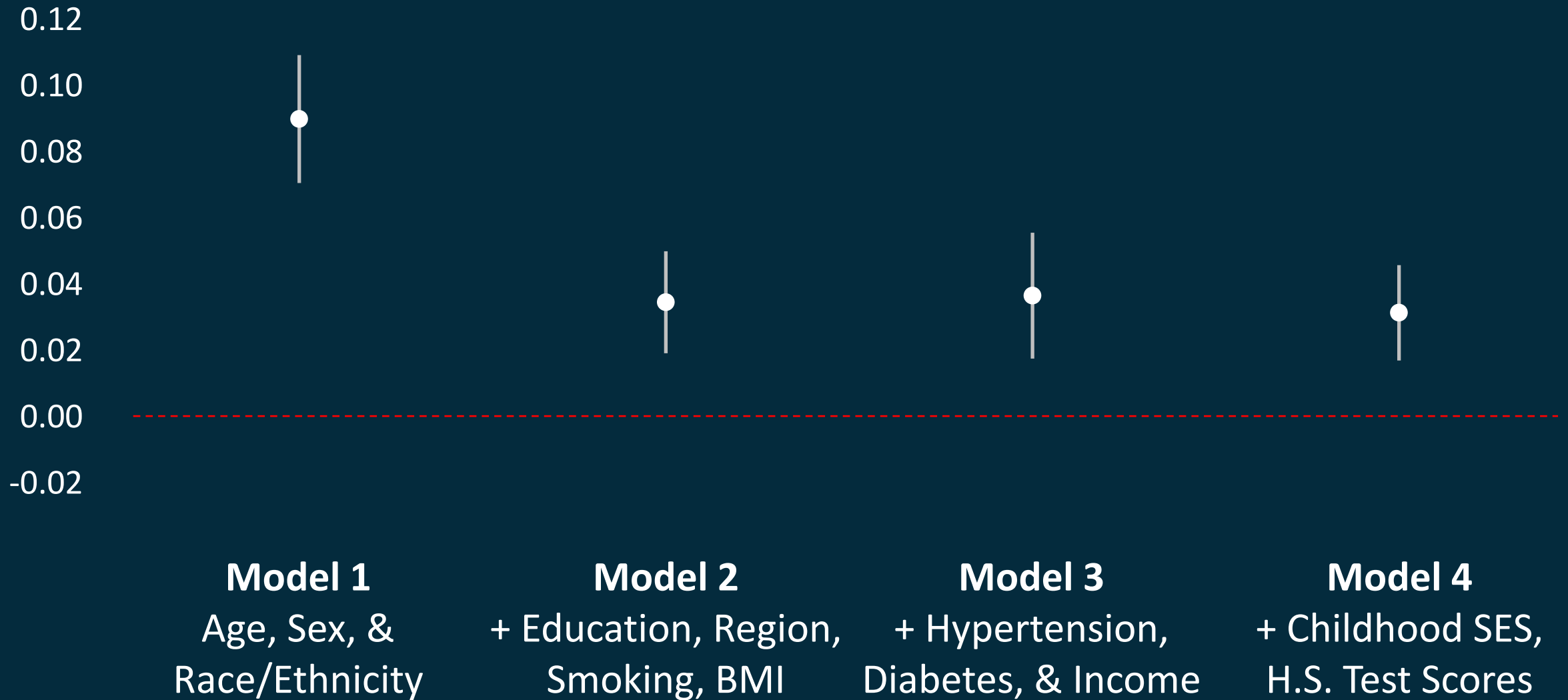
# Beta Diversity & Cognitive Function

Modest positive association with PCo1, negative association with PCo2



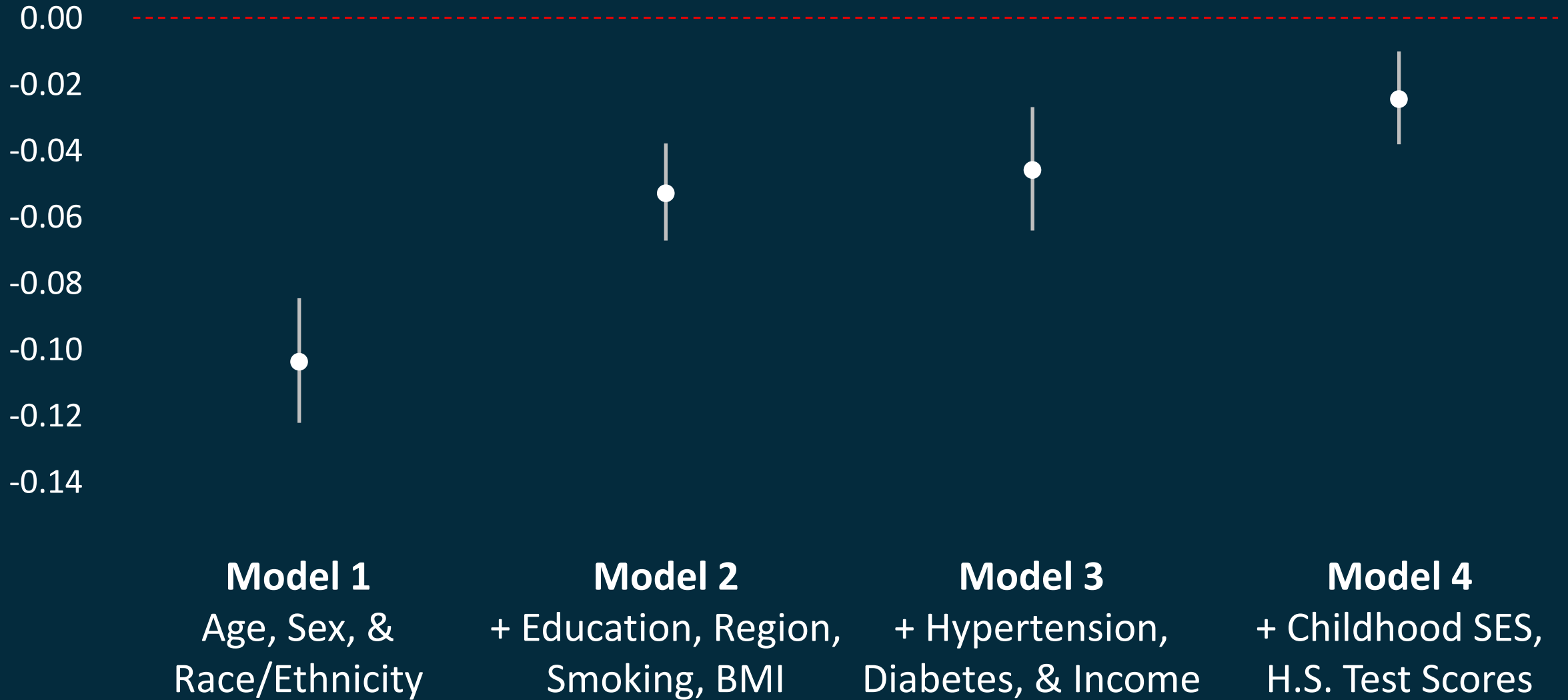
# Beta Diversity

(Bray-Curtis PCo1)



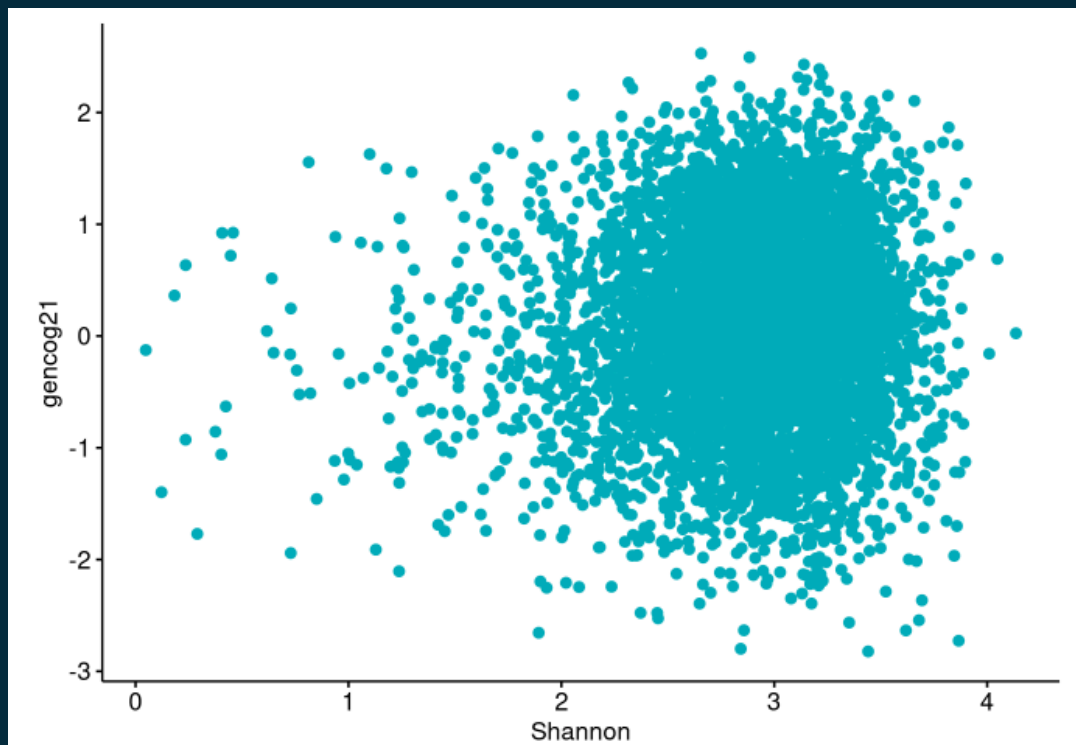
# Beta Diversity

(Bray-Curtis PCo2)

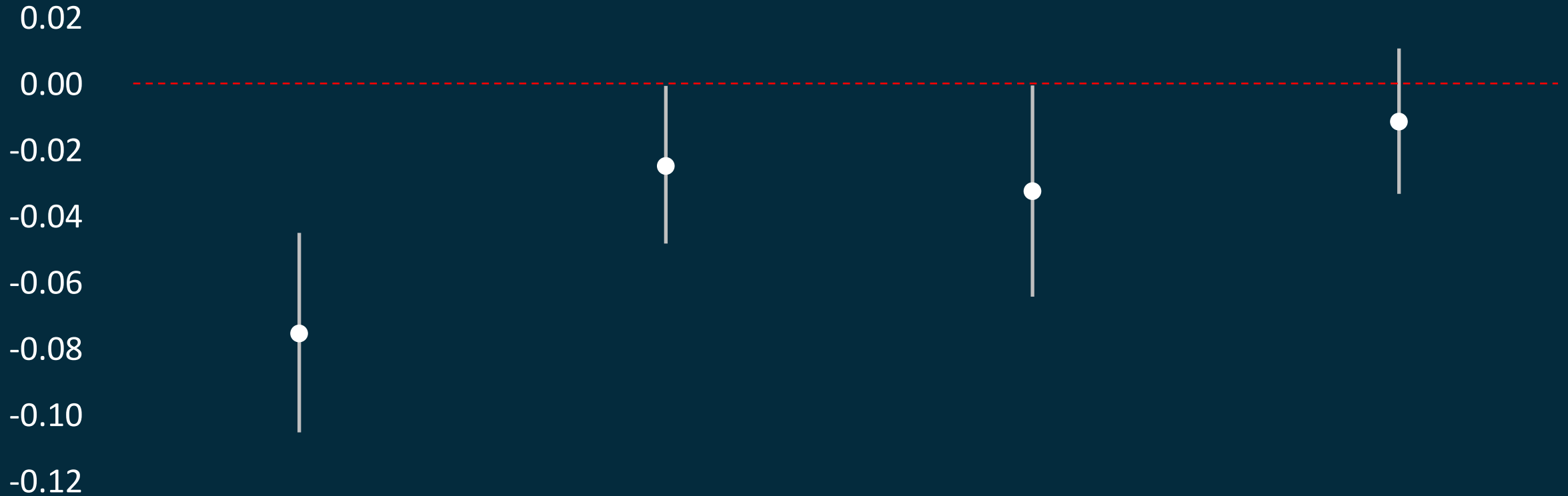


# T:C Ratio & Cognitive Function

Modest negative association



# T:C Ratio



## Model 1

Age, Sex, &  
Race/Ethnicity

## Model 2

+ Education, Region,  
Smoking, BMI

## Model 3

+ Hypertension,  
Diabetes, & Income

## Model 4

+ Childhood SES,  
H.S. Test Scores



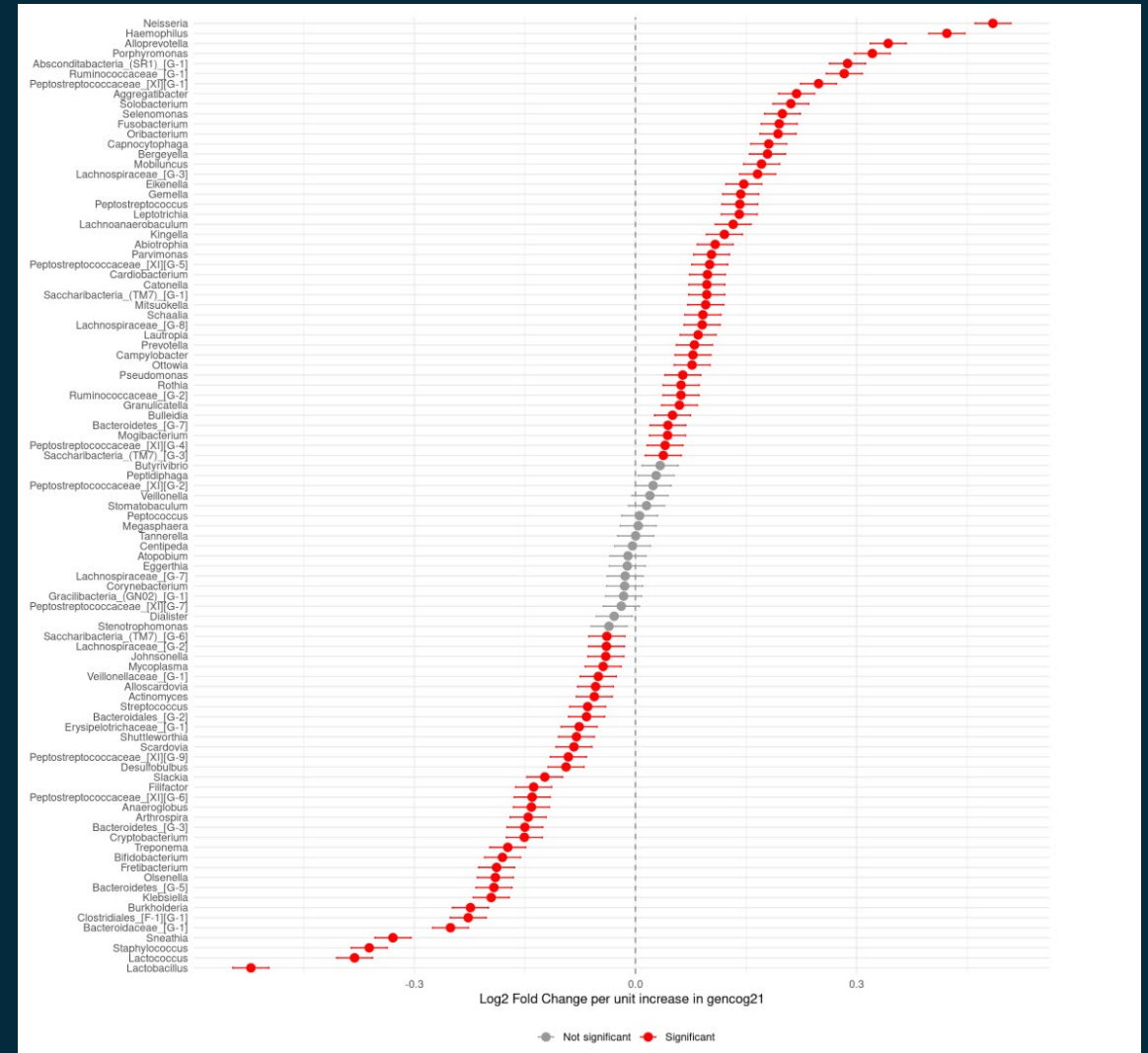
# Reality Check 3

Key taxa associated with better cognitive functioning

- *R. mucilaginosa*
- *V. parvula*

Key taxa associated with worse cognitive functioning

- *T. Forsythia*
- *T. denticola*
- *F. alocis*
- *P. gingivalis*



# 5. Conclusions

# Conclusions

1. Community-based, population representative samples can get oral microbiome samples along with saliva collected to get human DNA
2. High quality 16S data generated via a remote protocol
3. Multiple diversity metrics associated with cognitive function
4. Multiple expected periodontal pathogens and health-relevant organisms associated with cognitive function in expected directions
5. Some evidence of confounding by family SES, education
6. Stay tuned for NLS:72, HS&B:2029

# THANK YOU

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[edshareproject.org](https://edshareproject.org)



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