The Role of Taste & Oral Microbiome on Caries Risk in Young Children

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Healthy Smile Happy Child
The Manitoba Collaborative Project for the Prevention of Early Childhood Tooth Decay

the Children's Hospital Research Institute of Manitoba
a division of the Children's Hospital Foundation of Manitoba

Outline

- Risk factors for tooth decay
- Taste receptors
- Taste and tooth decay risk
- Bacterial and fungal species in children's mouths
- Sex- and gender-based differences in risk factors for tooth decay
- The association between taste, the oral microbiome and tooth decay risk

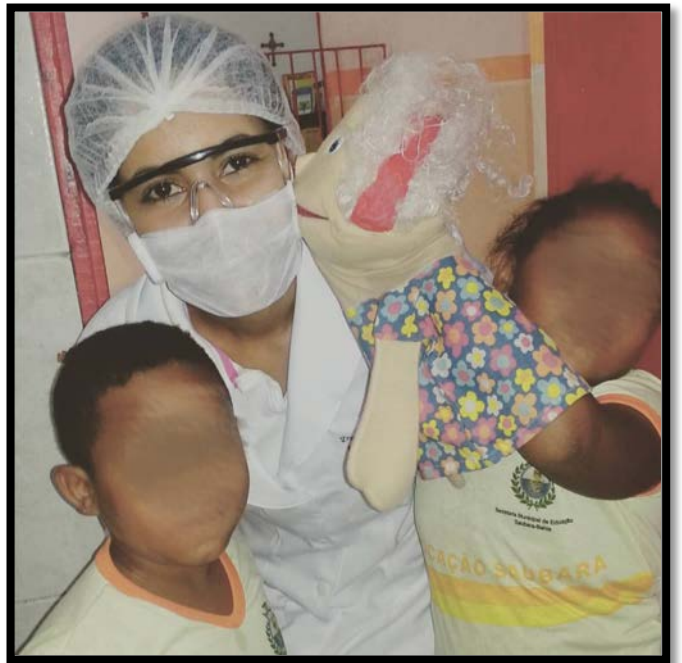
Conflict of Interest Statement:

- There are **no conflicts of interest** to be reported.

My Trajectory

- 2009-2015 Graduated as a Doctor of Dental Surgery
  - 2010 Started doing research at UFBA
  - 2013 Summer research at the U of M
- 2015-2016 Worked as a dentist in a small community
- 2017 Masters of Oral Biology program
- 2018 Transferred to the PhD program

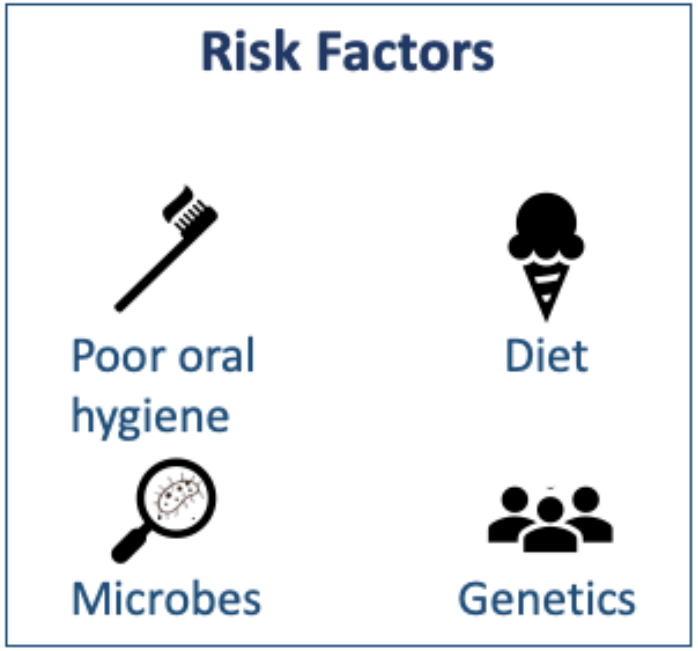
Oral Health Education at Brazilian Public Schools




ESCOVÓDROMO

Tooth Decay Dental Caries

- Demineralization of dental hard tissues
- WHO:
  - One of the most prevalent diseases (+ 530 million children)
  - Functional limitation, pain, and anxiety
  - Expensive treatment
- In Canada, it is common among:
  - Children from low-income households
  - Newcomer/refugee families
  - Rural and remote regions



(GBD. Lancet. 2017. 392:1789–1858; Schroth et al. Journal of Canadian Dental Association. 2016. 82:g20; Peres et al. J Dent Res. 2016. 95(4):388–394)

Severe Early Childhood Caries (S-ECC)

- **Early Childhood Caries** is defined as any caries experience in the primary dentition in those <72 months of age.

- **Severe Early Childhood Caries (S-ECC)** can lead to long-term complications and reduced quality of life.

Photos: Dr. Schroth

(Schroth et al. Journal of Canadian Dental Association. 2016. 82, g20)

Taste Receptors

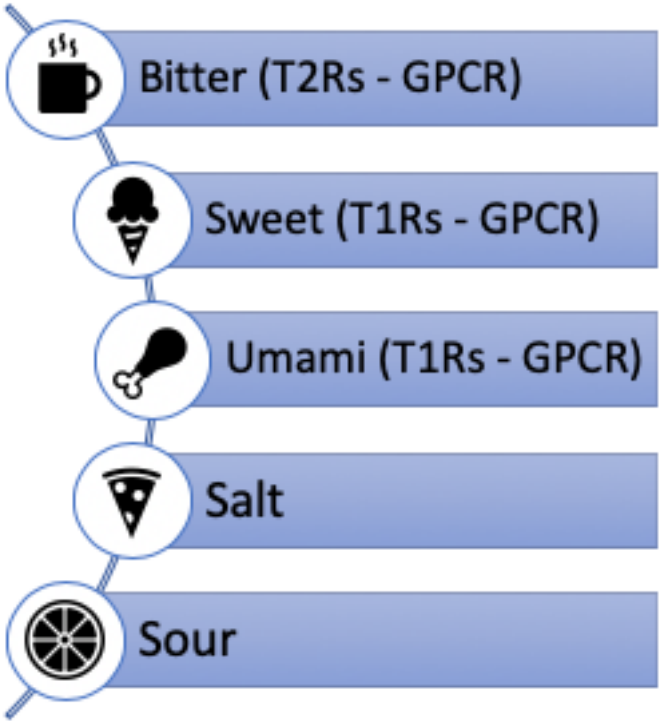
Sugar intake

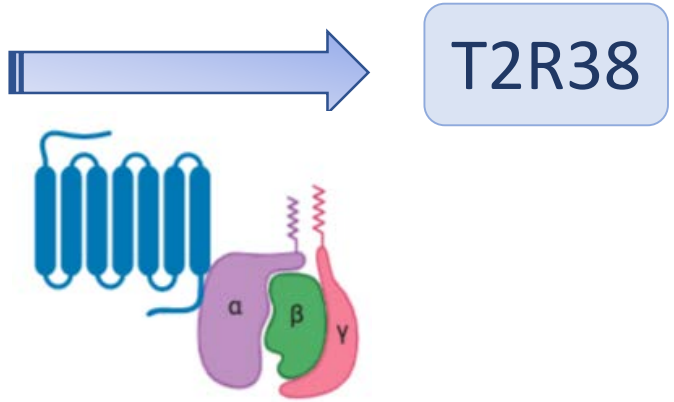
↓

⬆ Caries risk

- Genetic factors

Basic tastes:

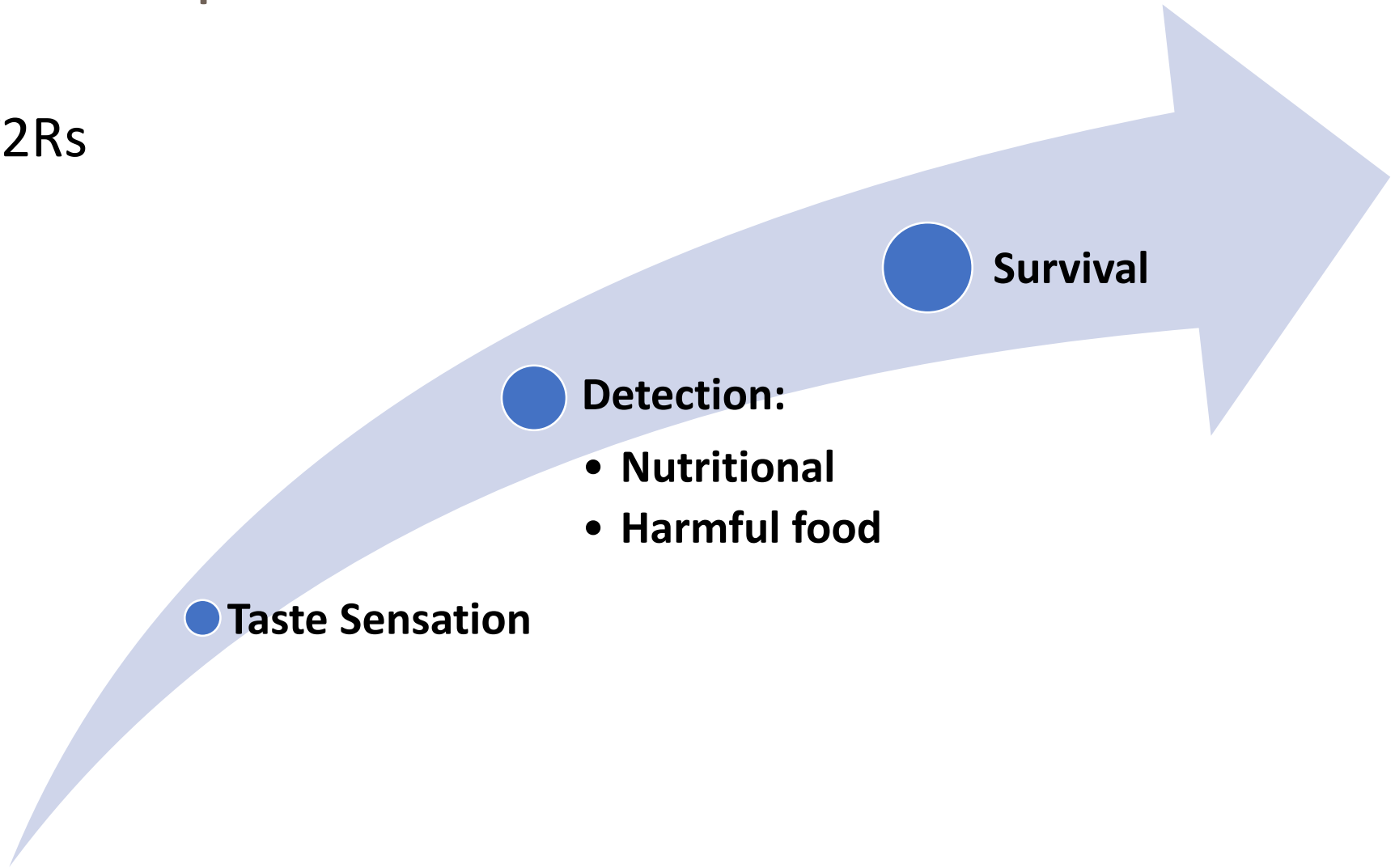




Bachmanov AA and Beauchamp GK. Annu. Rev. Nutr. 2007. 27:389–414

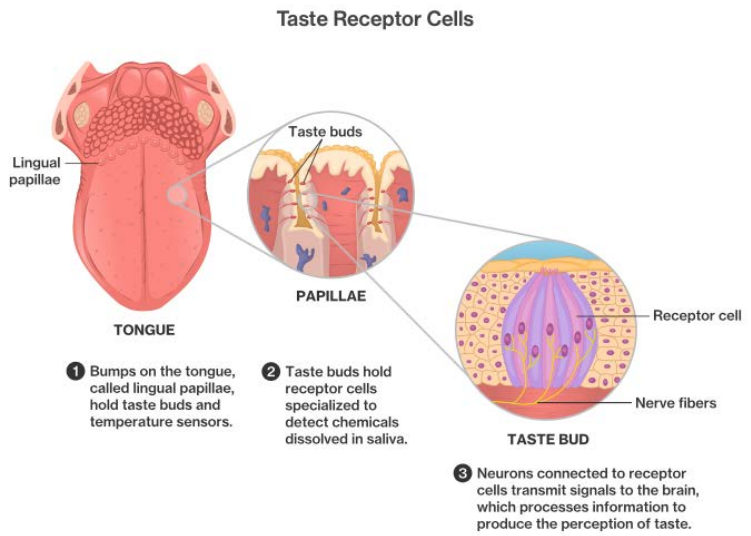
Bitter Taste Receptors – T2Rs

- 25 human T2Rs



T2Rs: Function

- Taste receptors function as chemoreceptors.
- They interact with molecules (ligands), to initiate an afferent signal transmitted to the brain, which results in taste sensation.

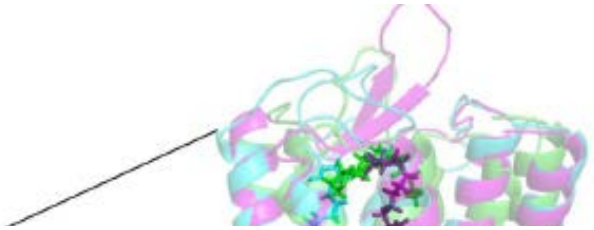


https://www.coursehero.com/sg/introduction-to-psychology/taste-and-smell/

ACS Infectious Diseases
Article
Cite This: ACS Infect. Dis. 2018, 4, 1146–1156
pubs.acs.org/journal/aidcbc

Characterization of the Binding Sites for Bacterial Acyl Homoserine Lactones (AHLs) on Human Bitter Taste Receptors (T2Rs)

Appalaraju Jaggupilli, Nisha Singh, Vivianne Cruz De Jesus, Kangmin Duan, and Prashen Chelikani*

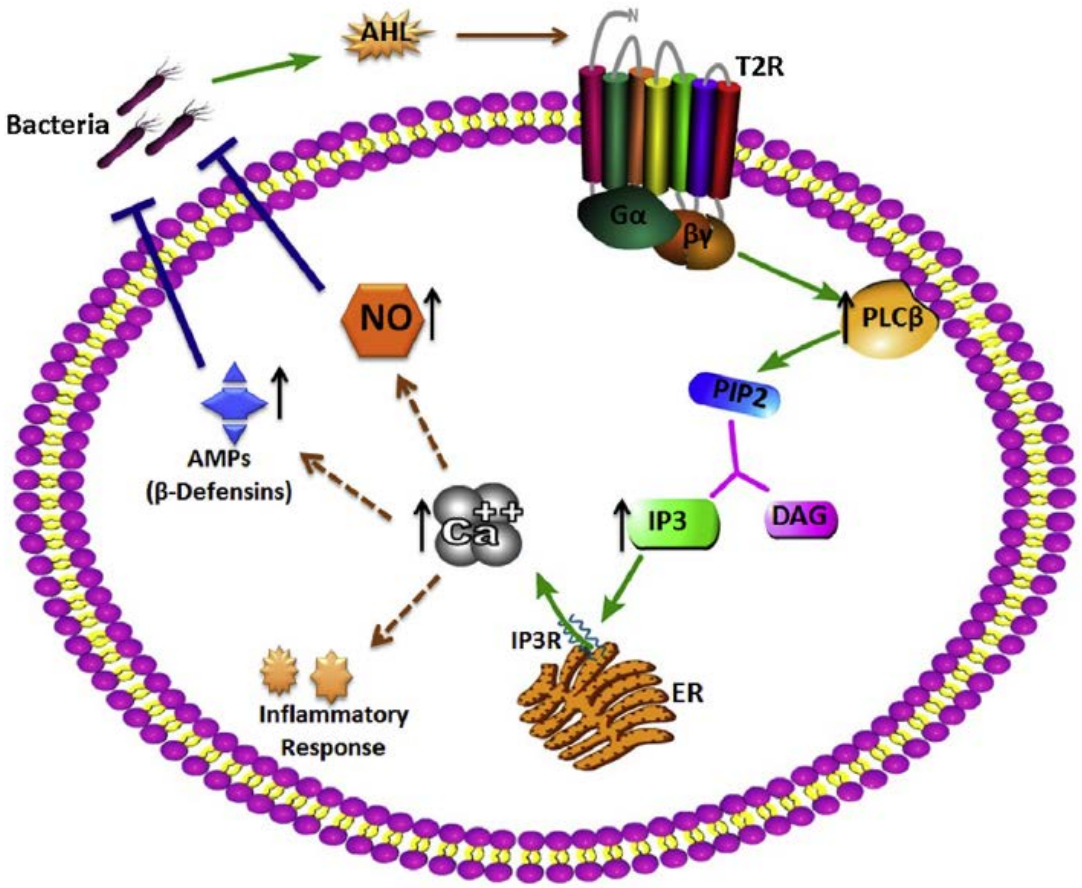


Chemosensory bitter taste receptors (T2Rs) are activated by multiple antibiotics

Appalaraju Jaggupilli, Nisha Singh, Vivianne Cruz De Jesus, Mohamed Soussi Gounni, Premnath Dhanaraj, and Prashen Chelikani[1]
Manitoba Chemosensory Biology Research Group and Department of Oral Biology, University of Manitoba, Children's Hospital Research Institute of Manitoba (CHRIM), Winnipeg, Manitoba, Canada

T2Rs: Function

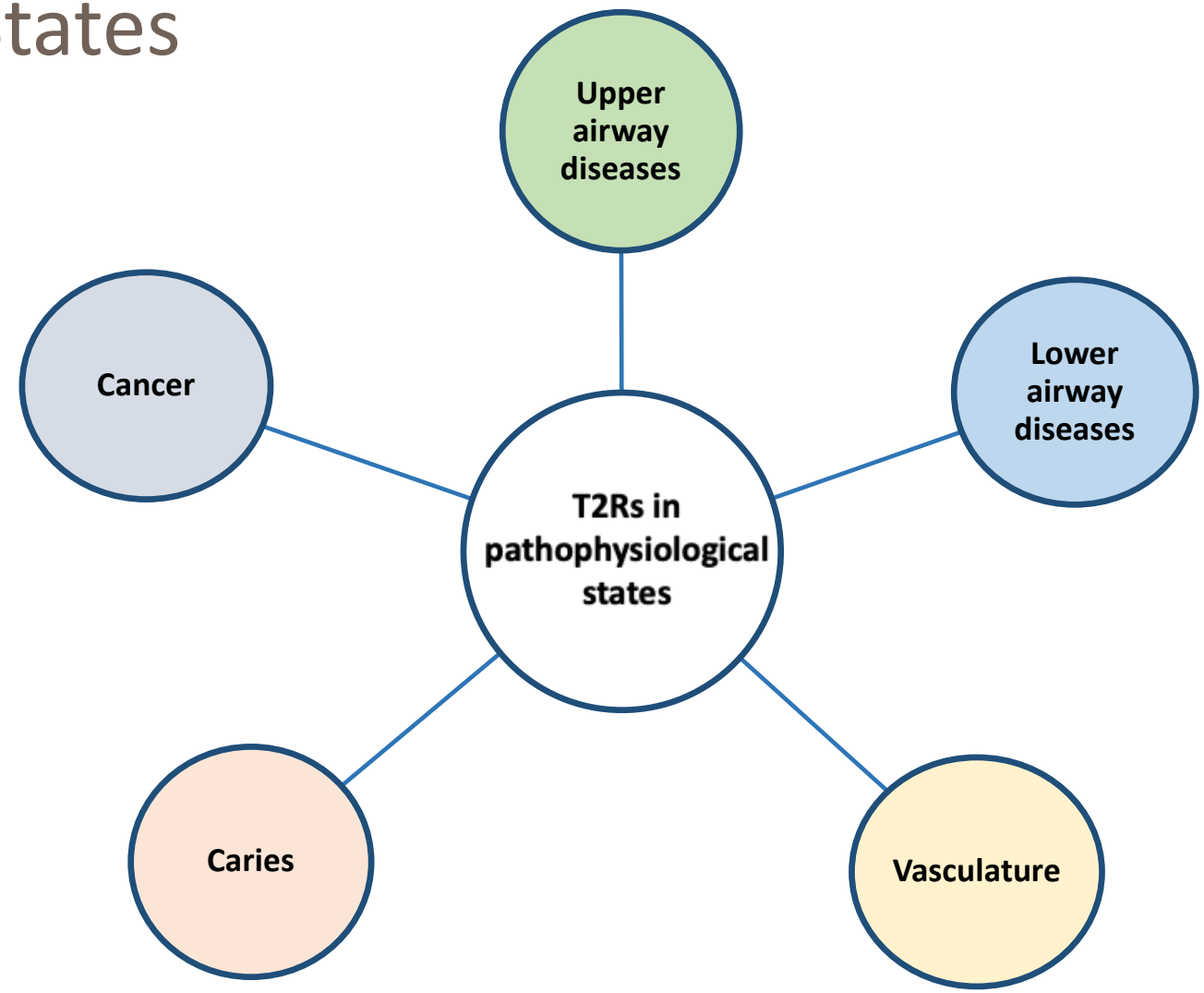
- The bacteria produce some molecules that can activate T2Rs, resulting in the increase of the production of NO and antimicrobial peptides (AMPs) that kill the bacteria.



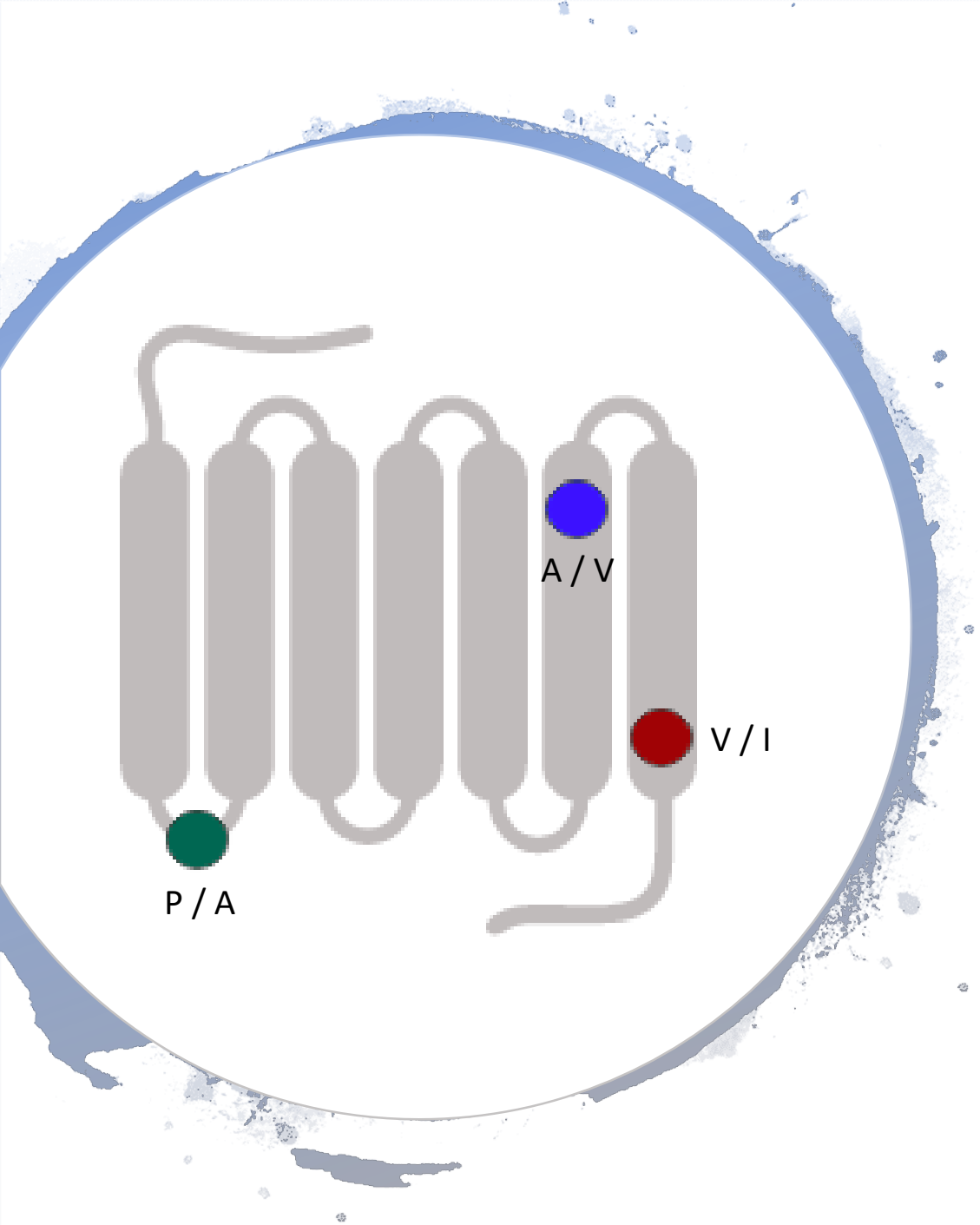
F.A. Shaik et al. / The International Journal of Biochemistry & Cell Biology 77 (2016) 197–204

T2Rs in Pathophysiological States

- Wendell et al. (2010): TAS1R2 and TAS2R38 → associated with caries protection and risk
- Kulkarni et al. (2013): TAS1R2 → high caries risk
- Haznedaroğlu et al. (2015): TAS1R3 and TAS1R2 → high caries risk



Haznedaroglu E et al. Caries Res. 2015. 49:275–281; Kulkarni GV et al. Caries Res. 2013. 47:219–225; Wendell S et al. J Dent Res. 2010. 89:1198–1202; Shaik FA et al. Int J Biochem Cell Bio. 2016. 77, 197-204

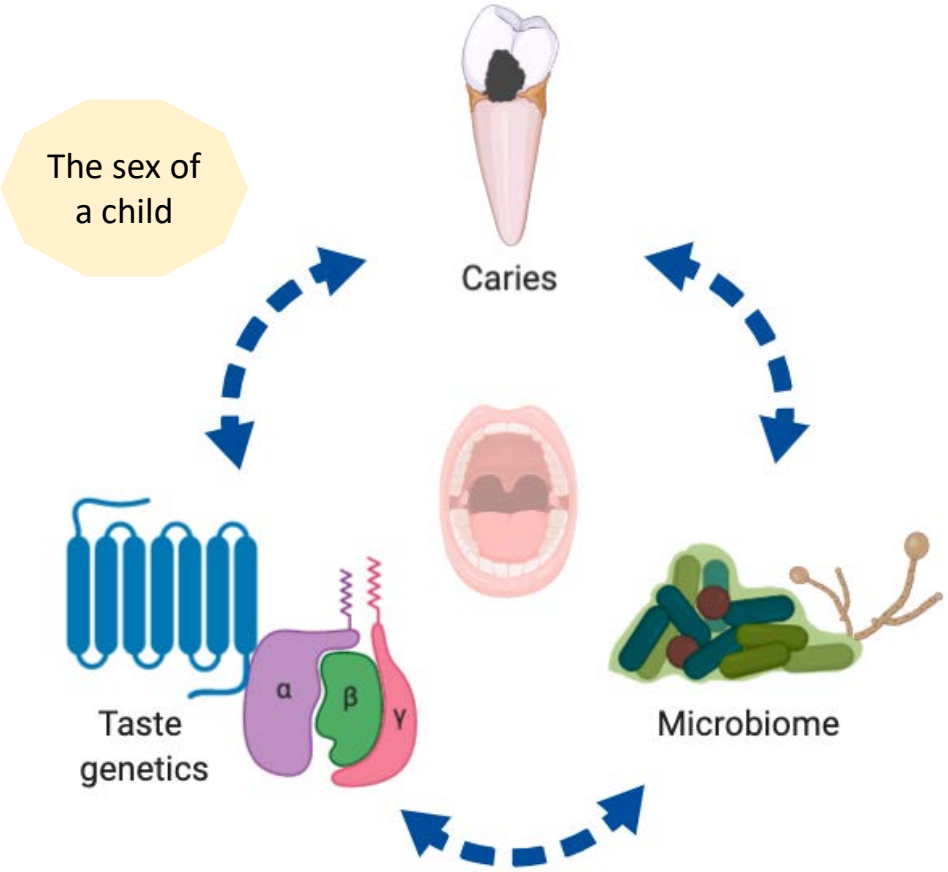


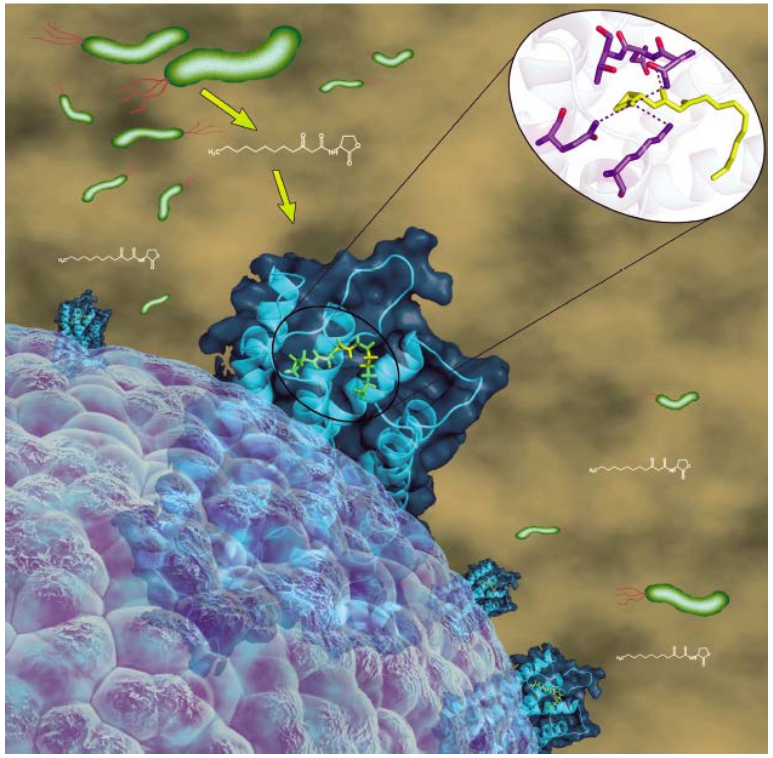
T2R38 genotypes & taster status

AVI/AVI	Non-taster
PAV/AVI	Taster
PAV/PAV	Super taster

SNPs = single nucleotide polymorphisms

Taste, Oral Microbiome & Tooth Decay





Aim: ***Determine the association between taste genetics and plaque microbiome and their influence on caries risk in young children.***

Taste, Oral Microbiome & Tooth Decay

Objective 1: Identify which species of bacterium and fungus are in the children`s mouth.

Does the sex of a child play a role in the composition of the oral microbiome?

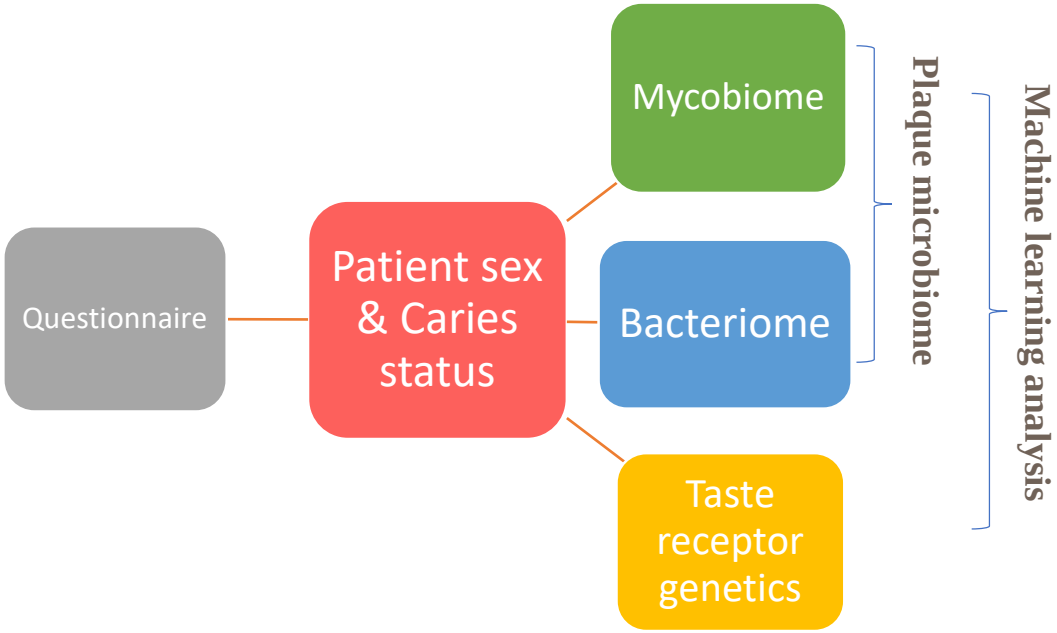
Objective 2: Identify the children's T2R38 taster status and to investigate the presence of SNPs in other receptors.

Objective 3: Study the association between taste genetics, the oral microbiome and tooth decay risk.

Approach

Pilot study:

- 80 children, <72 months of age
  - 40 caries-free and 40 with severe tooth decay (S-ECC)
  - Saliva and dental plaque samples



Characteristics of the participants

By caries-status

Variable	Caries Status: Caries-Free (*n* = 40)	Caries Status: S-ECC (*n* = 40)	*p*-value
Age (months)[a]	46.2 ± 14.2	45.6 ± 11.4	0.84
Ever breast-fed[b,c]			
Yes	33 (82.5)	21 (52.5)	**0.004**
No	7 (82.5)	19 (47.5)	
Ever bottle-fed[b]			
Yes	30 (75.0)	40 (100.0)	**0.001**
No	10 (25.0)	0 (0.0)	
Put to bed with bottle[b,c]			
Yes	7 (17.5)	25 (62.5)	**<0.0001**
No	33 (82.5)	15 (37.5)	
Snacks before bedtime[b,c]			
Yes	16 (40.0)	30 (75.0)	**0.001**
No	24 (60.0)	10 (10.0)	
Sweet preference[c]			
Do not prefer	12 (30.0)	3 (7.5)	**0.03**
Prefers occasionally[1]	24 (60.0)	28 (70.0)	
Prefers frequently[2]	4 (10.0)	9 (22.5)	
Oral health[b]			
Very good/Good	39 (97.5)	10 (25.0)	**<0.0001**
Fair/Poor/Very poor	1 (2.5)	30 (75.0)	
Uses toothpaste[b]			
Yes	39 (97.5)	32 (80.0)	**0.03**
No	1 (2.5)	8 (20.0)	
Tooth brushing frequency[b,c]			
≥ twice/day	27 (67.5)	11 (27.5)	**0.0003**
< twice/day	13 (32.5)	29 (72.5)	
Age at the first dental visit (months)[a]	20.5 ± 10.4	21.4 ± 13.2	0.98
Age when mouth started to be cleaned (months)[a]	10.7 ± 9.9	13.3 ± 7.2	0.19

Characteristics of the participants

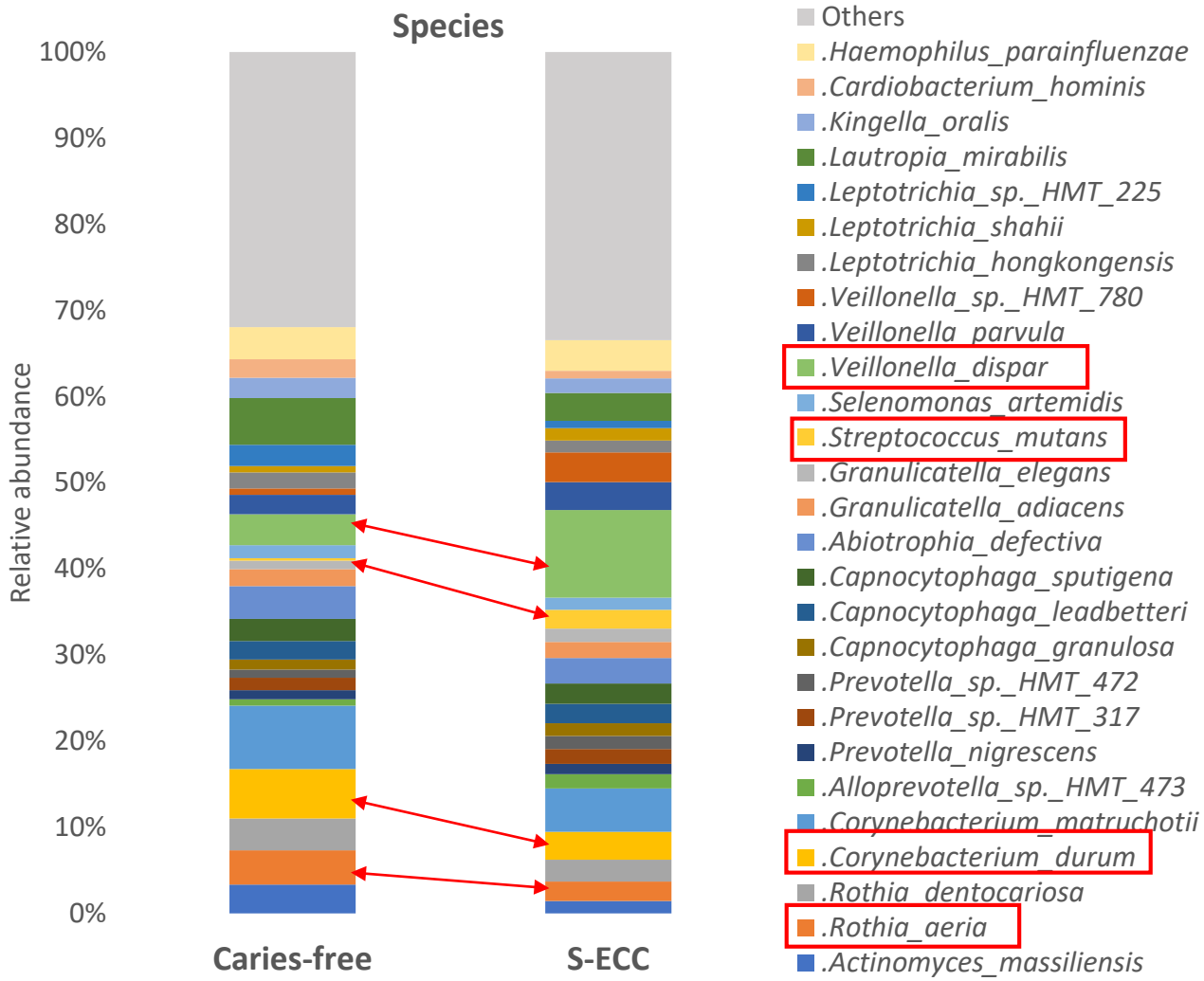
By caries-status & sex

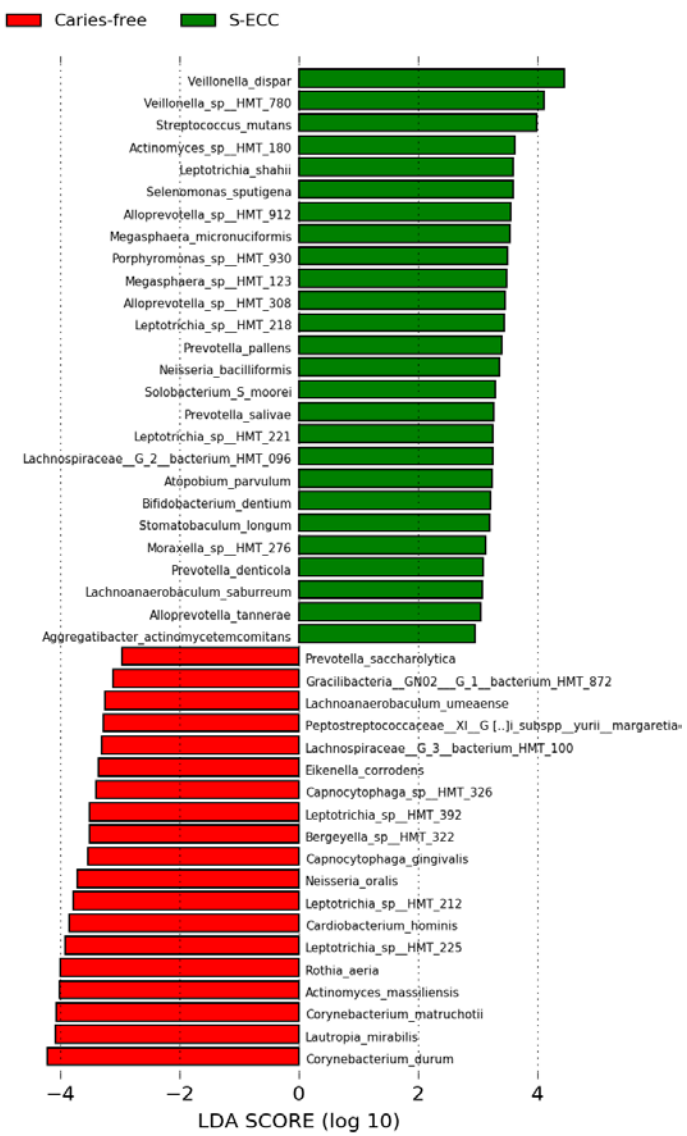
Variable	Caries Status and Sex					
	Caries-Free		*p*-value	S-ECC		*p*- value
	Girls (*n* = 21)	Boys (*n* = 19)		Girls (*n* = 25)	Boys (*n* = 15)	
Age (months)[a]	47.9 ± 15.1	44.3 ± 13.2	0.42	45.7 ± 11.5	45.4 ±11.6	0.93
Ever breast-fed[b,c]						
Yes	18 (85.7)	15 (79.0)	0.69	14 (56.0)	7 (46.7)	0.57
No	3 (14.3)	4 (21.0)		11 (44.0)	8 (53.3)	
Ever bottle-fed[b]						
Yes	14 (66.7)	16 (84.2)	0.28	25 (100.0)	15 (100)	1.0
No	7 (33.3)	3 (15.8)		0 (0.0)	0 (0.0)	
Put to bed with bottle[b,c]						
Yes	2 (9.5)	5 (26.3)	0.23	17 (68.0)	8 (53.3)	0.35
No	19 (90.5)	14 (73.7)		8 (32.0)	7 (46.7)	
Snacks before bedtime[b,c]						
Yes	8 (38.1)	8 (42.1)	0.8	17 (68.0)	13 (86.7)	0.27
No	13 (61.9)	11 (57.9)		8 (32.0)	2 (13.3)	
Sweet preference[c]						
Do not prefer	5 (23.8)	7 (36.8)	0.64	2 (8.0)	1 (6.7)	0.89
Prefers occasionally[1]	14 (41.7)	10 (52.6)		18 (72.0)	10 (66.7)	
Prefers frequently[2]	2 (9.52)	2 (10.5)		5 (20.0)	4 (26.7)	
Uses toothpaste[b]						
Yes	21 (100.0)	18 (94.7)	0.47	21 (84.0)	11 (73.3)	0.44
No	0 (0.0)	1 (5.3)		4 (16.0)	4 (26.7)	
Oral health[b]						
Very good/Good	20 (95.2)	19 (100)	1.0	6 (24.0)	4 (26.7)	1.0
Fair/Poor/Very poor	1 (4.8)	0 (0.0)		19 (76.0)	11 (73.3)	
Tooth brushing frequency[b,c]						
≥ twice/day	14 (66.7)	11 (57.9)	0.57	7 (28.0)	4 (26.7)	1.0
< twice/day	7 (33.3)	8 (42.1)		18 (72.0)	11 (73.3)	
Age at the first dental visit (months)[a]	21.8 ± 11.7	19 ± 8.8	0.41	26.3 ± 14.4	13.5 ± 4.9	**0.0004**
Age when mouth started to be cleaned (months)[a]	12.7 ± 8.17	10.7 ± 5.4	0.38	13.26 ± 7.3	6.86 ± 3.7	**0.0007**

Objective 1: Identify which species of bacterium and fungus are in the children`s mouth.

Does the sex of a child play a role in determining what kind of bacteria is in their mouth?

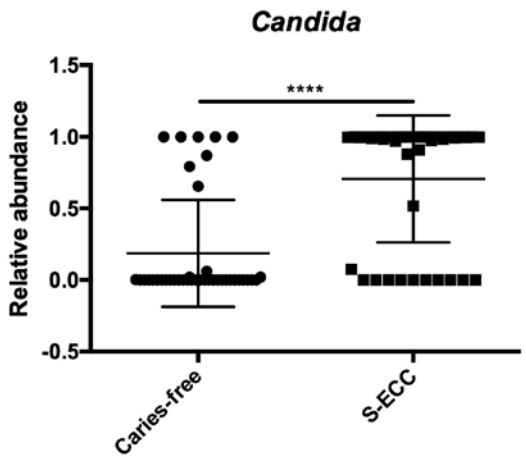
Bacteria in Children`s Mouths

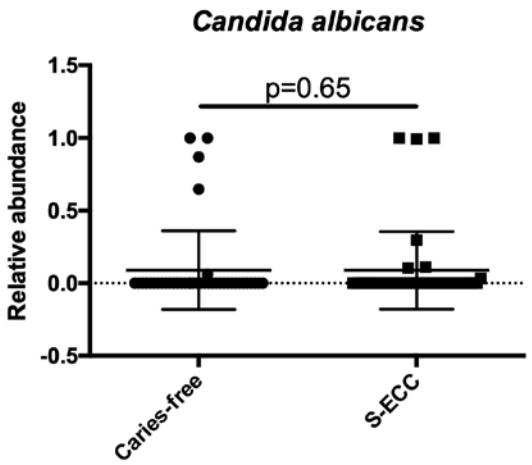


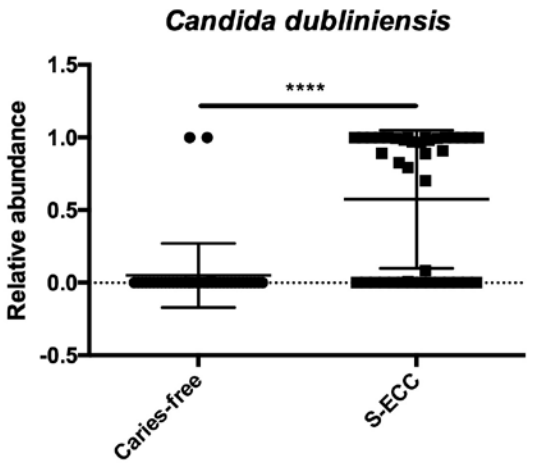


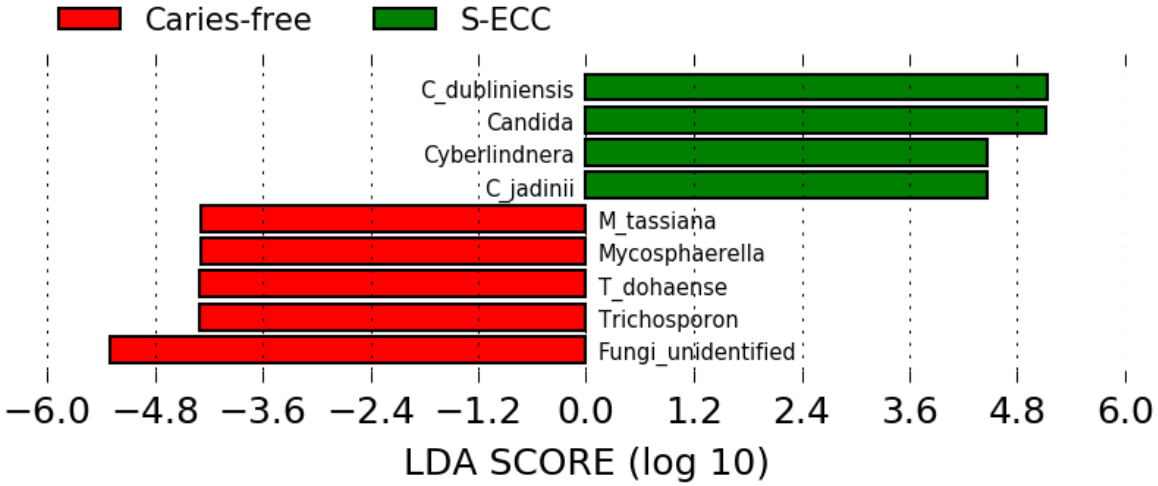
Fungus in Children`s Mouths

Questions?









- ✓ *Candida dubliniensis* was the most abundant species found in the caries-active group.
- ✓ No differences were seen in the abundance of *C. albicans*.

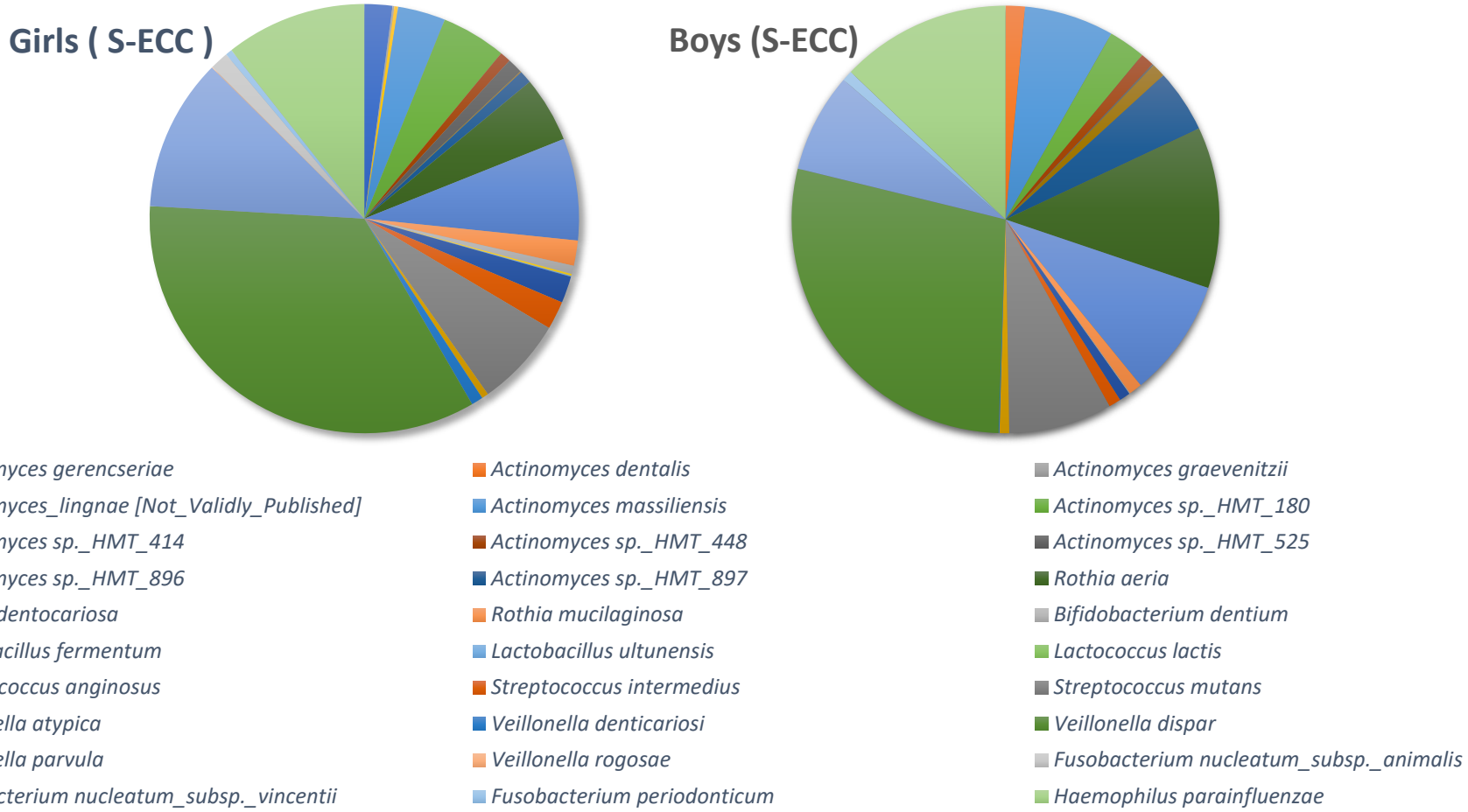
Who is at Greater Risk for Tooth Decay?

- There is not enough evidence to say that boys are at greater risk for tooth decay than girls
- Male vs female differences associated with caries risk:
  - Reproductive hormone (fluctuating hormone levels)
    - Salivary flow and composition
    - Food cravings and aversions
  - Cultural and social differences
    - Domestic role in food preparation (frequent snacking)
    - Son preference
    - Earlier eruption of teeth
  - Genetic factors
    - Genome-wide association studies have found X-linked caries susceptible and caries protective genes

(Lukacs JR. Clin Oral Invest. 2011;15:649–656; Zeng Z. J Dent Res. 2013;92(5):432–437)

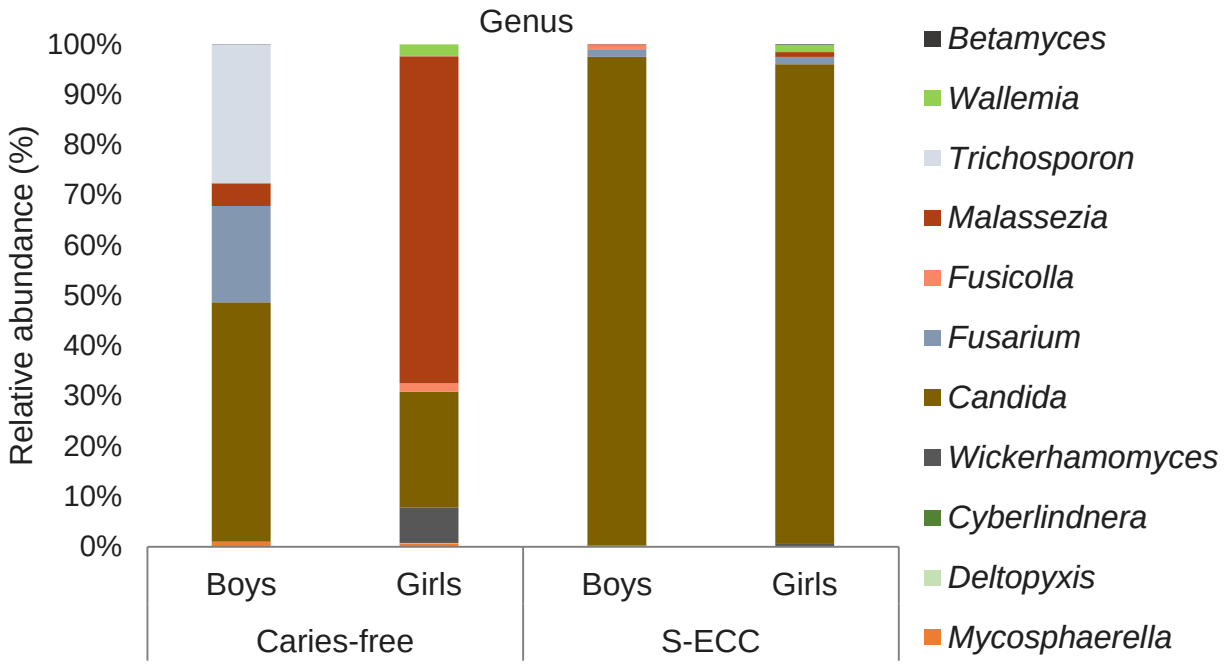
Differences Between Boys and Girls: Abundance of Bacterial Species

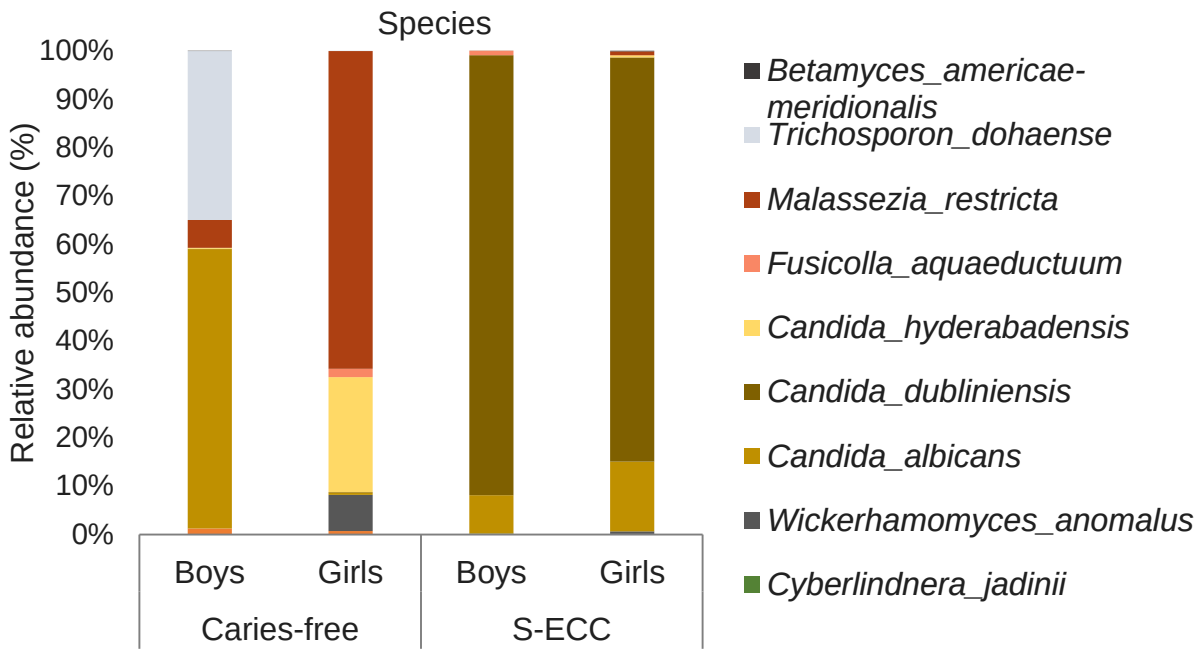
Taxonomic profiles of children according to their sex at species level



Differences Between Boys and Girls: Abundance of Fungal Species

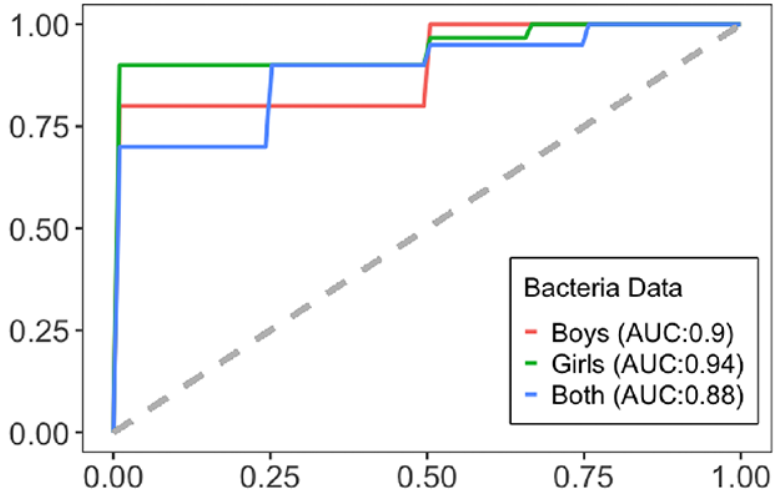
Taxonomic profiles of children according to caries status and sex at genus and species level

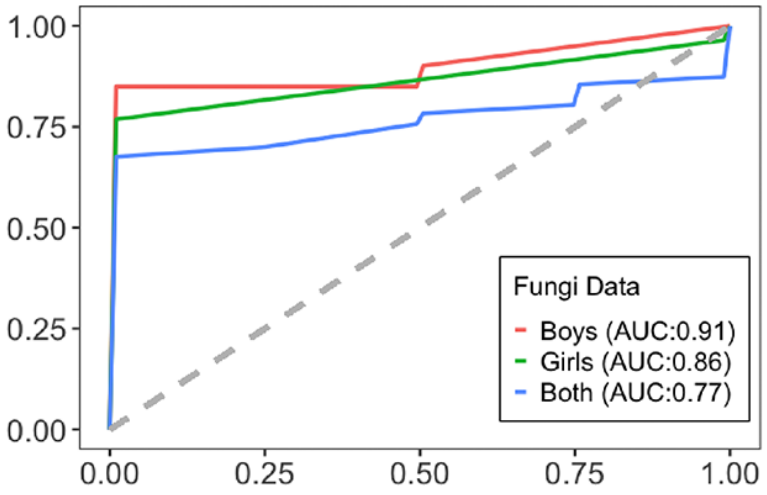




- *Malassezia* was the most abundant genus in caries-free girls, while *Candida* was the most abundant genus in caries-free boys.

Classification of S-ECC using oral microbiome data

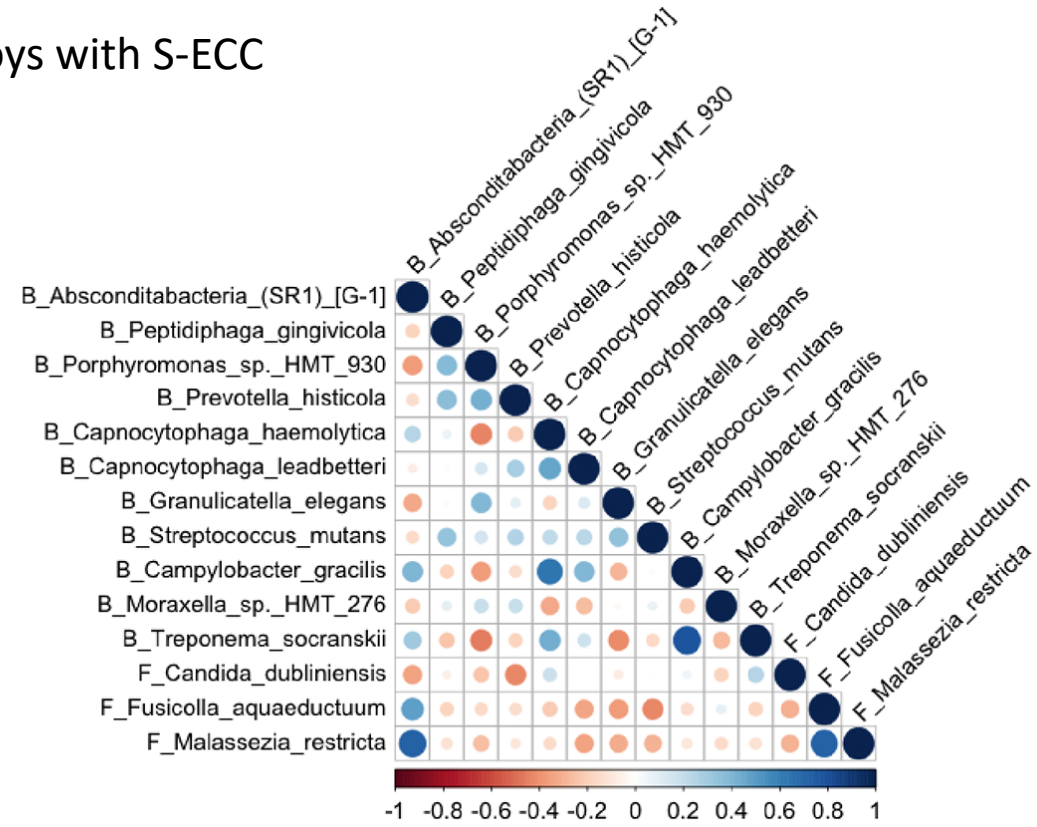




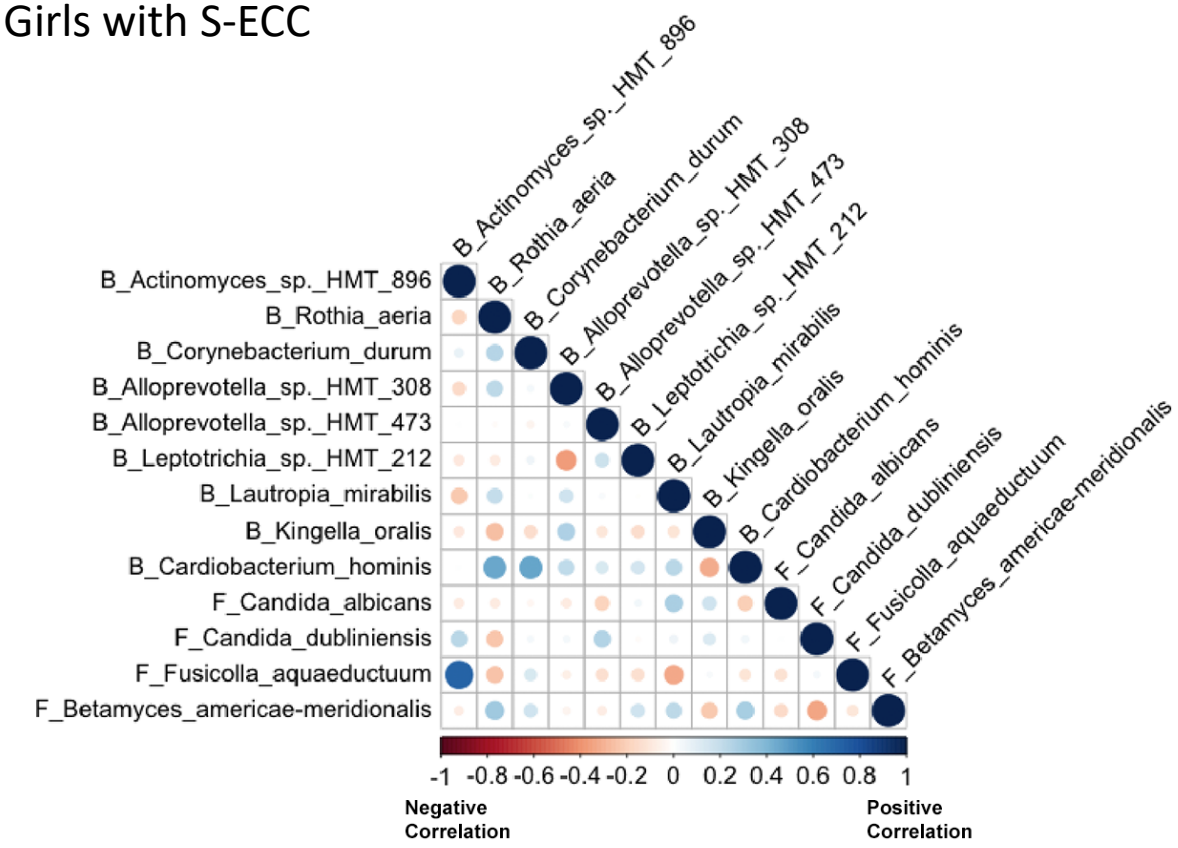
- ✓ When we do not stratify the data by sex the classification models are not as good as when the data is stratified by sex
- ✓ For girls, using bacterial counts results in a better classification model
- ✓ For boys, using fungal counts results in a better classification model

Correlation Between Fungi and Bacteria

Boys with S-ECC



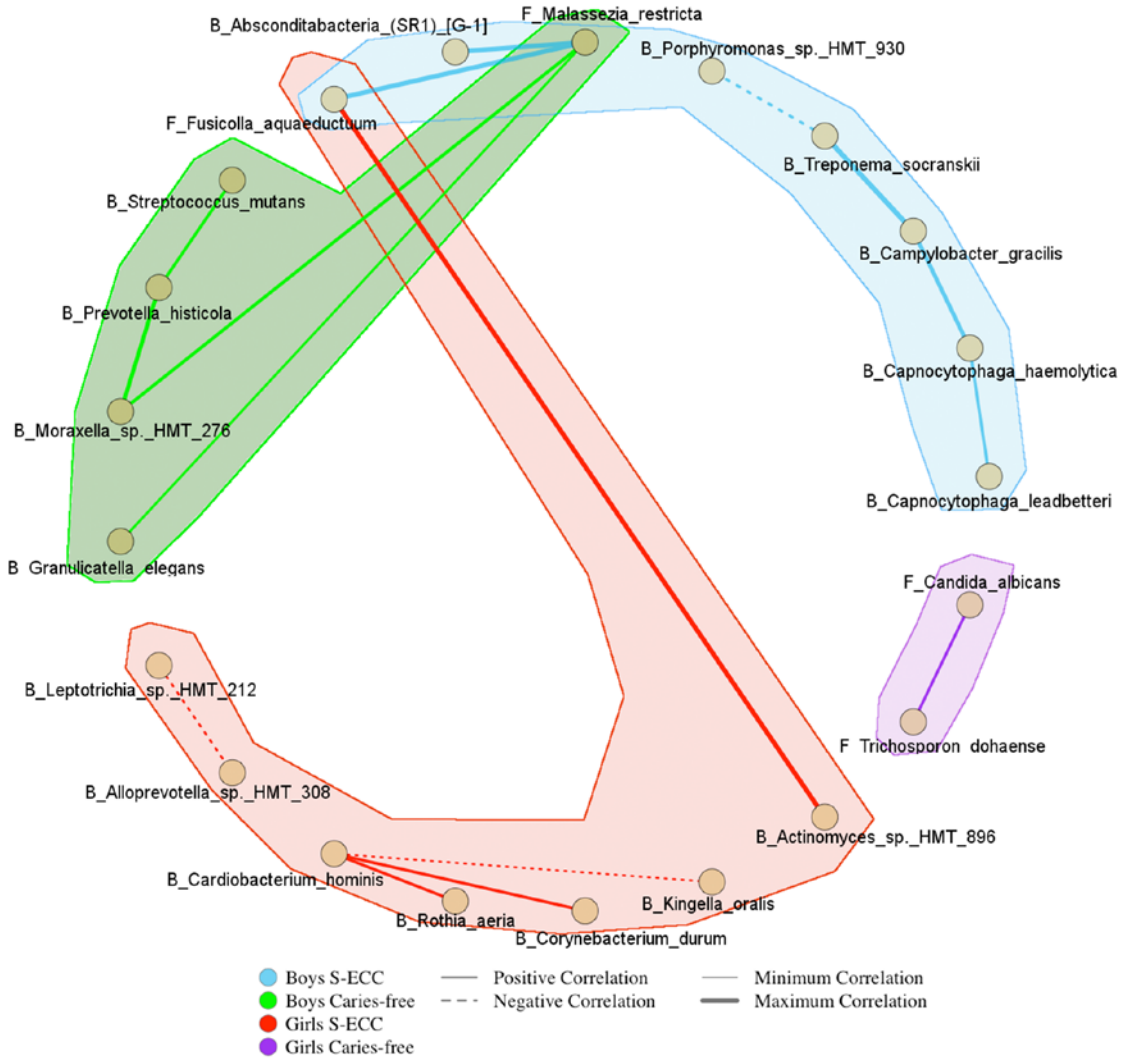
Girls with S-ECC



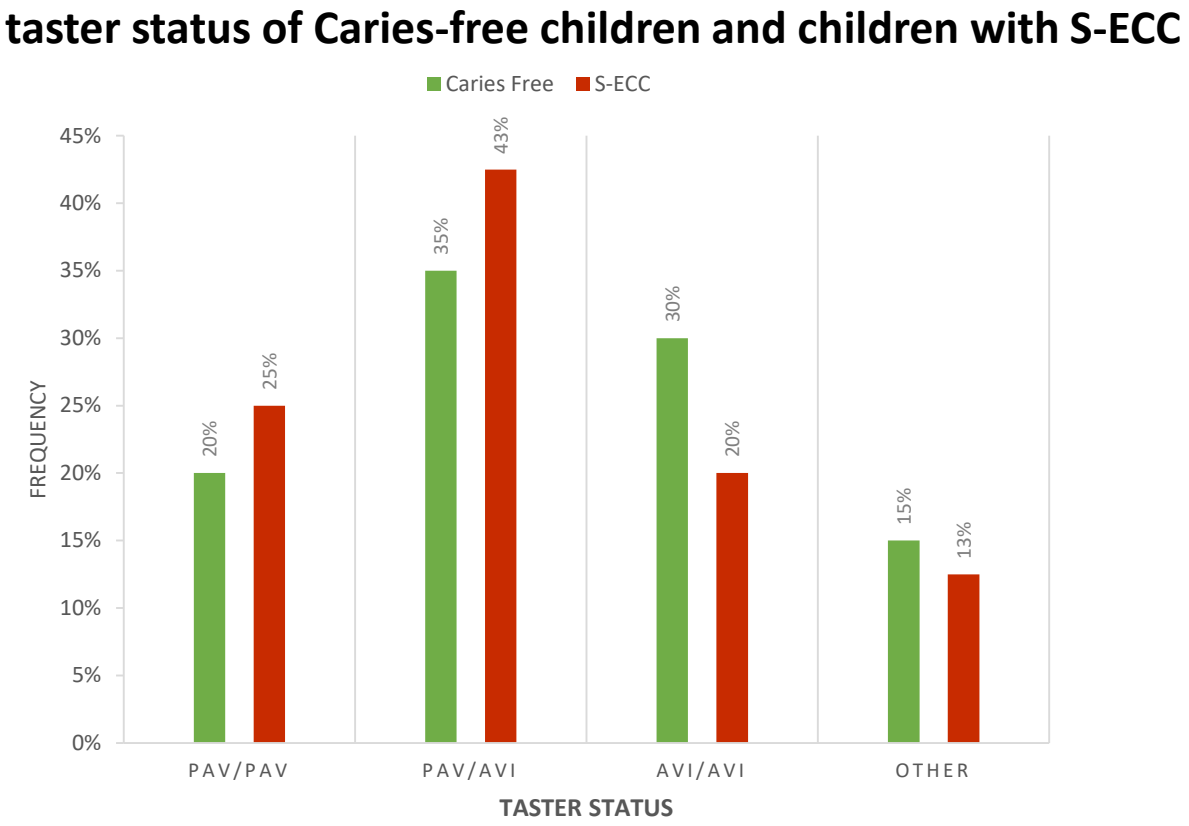
- Unique correlation plots were observed for boys and girls

Correlation Network of Fungal and Bacterial Species

- Positive and negative correlations were observed between bacterial and fungal species in different groups.

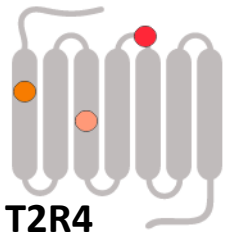
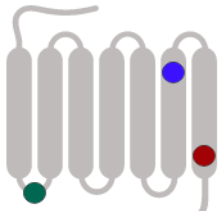


Objective 2: Identify the children's T2R38 taster status and to investigate the presence of SNPs in other receptors.



No significant differences were found when comparing caries status by TAS2R38 genotype.

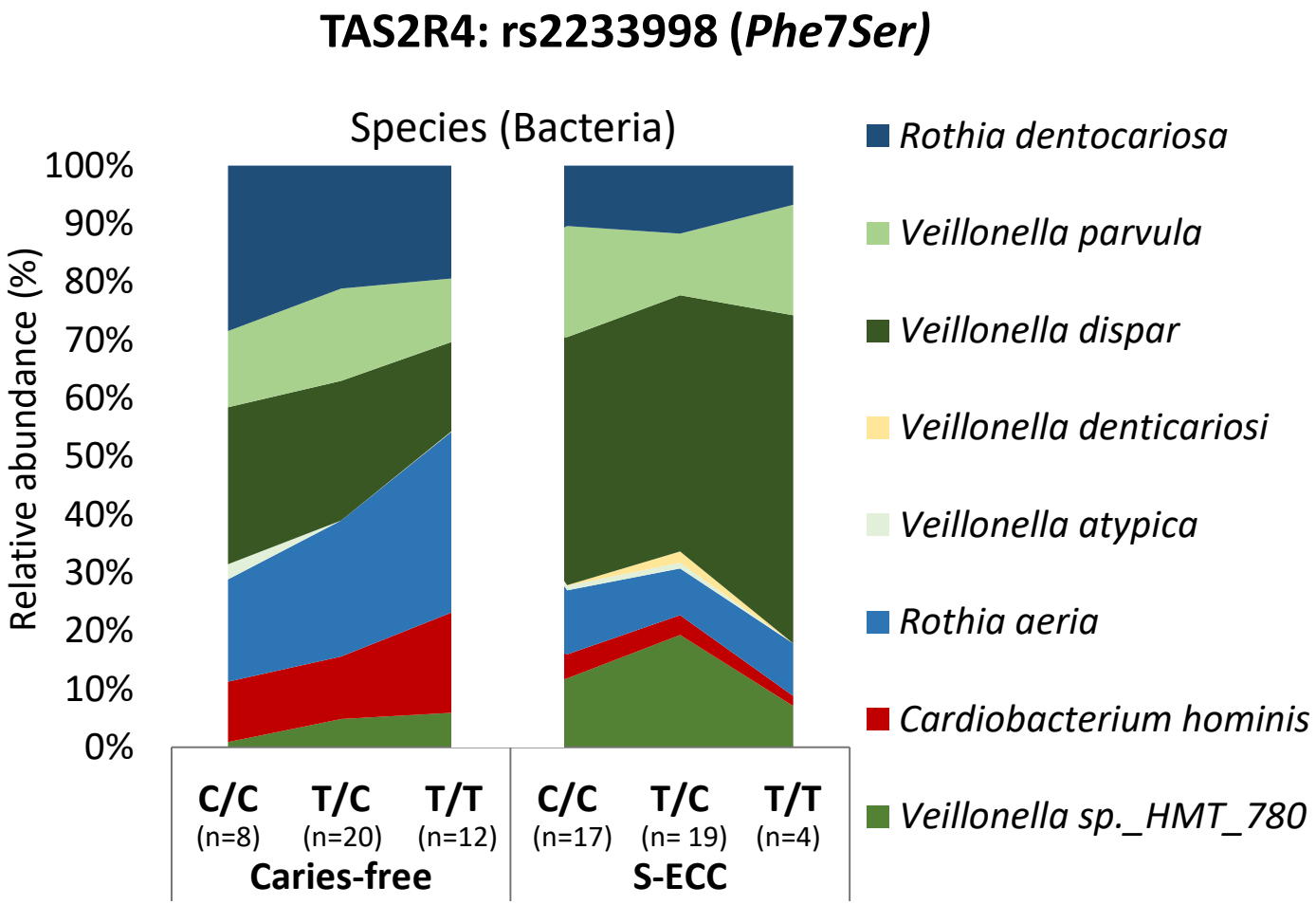
nsSNP analysis of bitter and sweet taste receptor genes

	Receptor	Gene	SNP			OR	P-value
Bitter	 T2R4	TAS2R4	rs2233998	*Phe7Ser*	20T→C	0.32	**<0.05**
			rs2234001	*Val96Leu*	286G→C	0.182	**<0.05**
			rs2234002	*Ser171Asn*	512G→A	0.19	**<0.05**
	 T2R38	TAS2R38	rs713598	*Ala49Pro*	*145C→G*	1.76	0.24
			rs1726866	*Val262Ala*	785A→G	1.13	0.81
			rs10246939	*Ile296Val*	886T→C	2.29	0.09
Sweet	 T1R2 T1R3	TAS1R2	rs9701796	*Ser9Cys*	26C→G	2.49	**0.05**
			rs35874116	*Ile191Val*	571A→ G	0.62	0.31
		TAS1R3	rs307377	*Arg757Cys*	2269C→T	0.38	0.19

✓ Among the analyzed nsSNPs, 4 are associated with S-ECC.

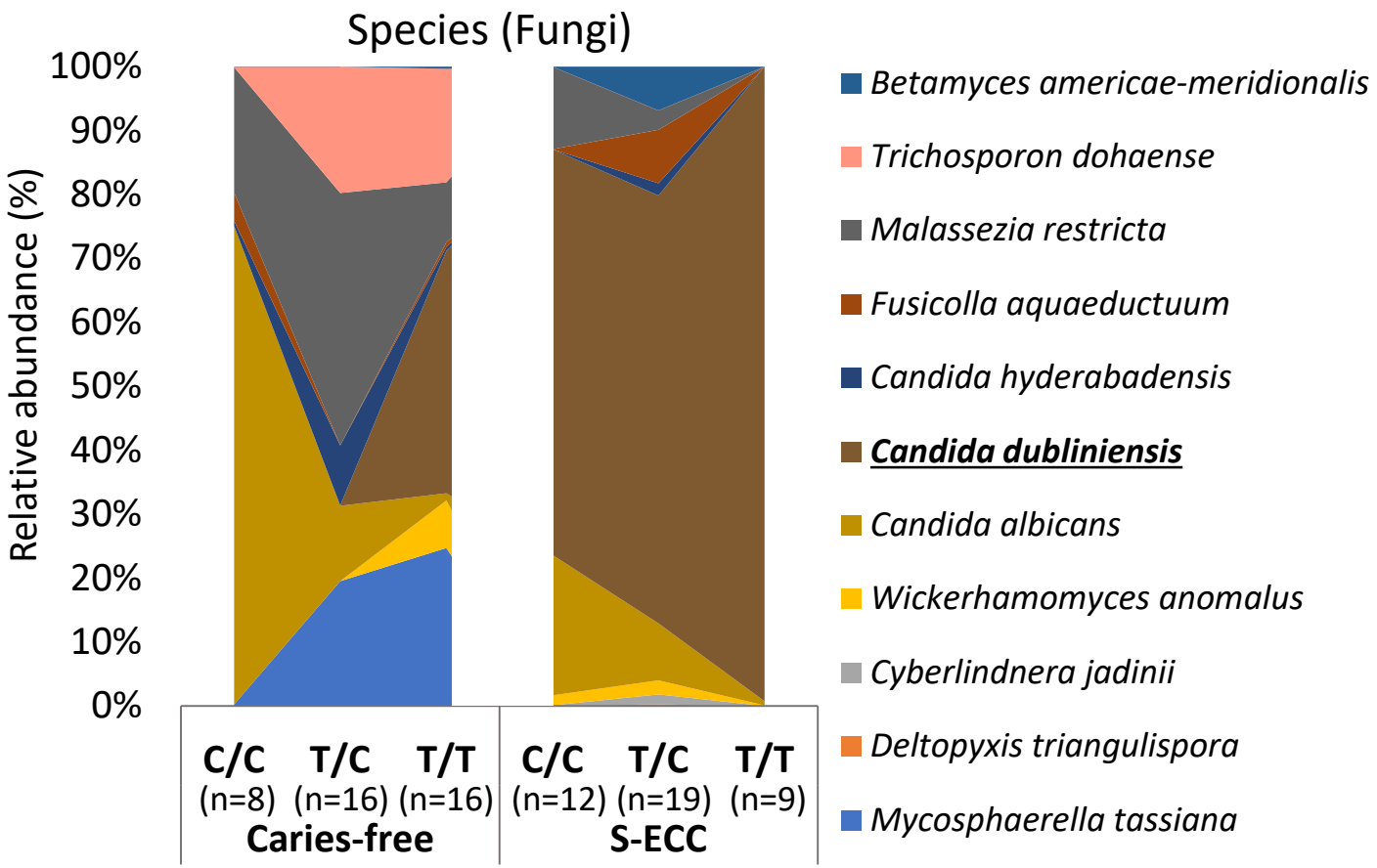
Objective 3: Study the association between taste genetics, the oral microbiome and tooth decay

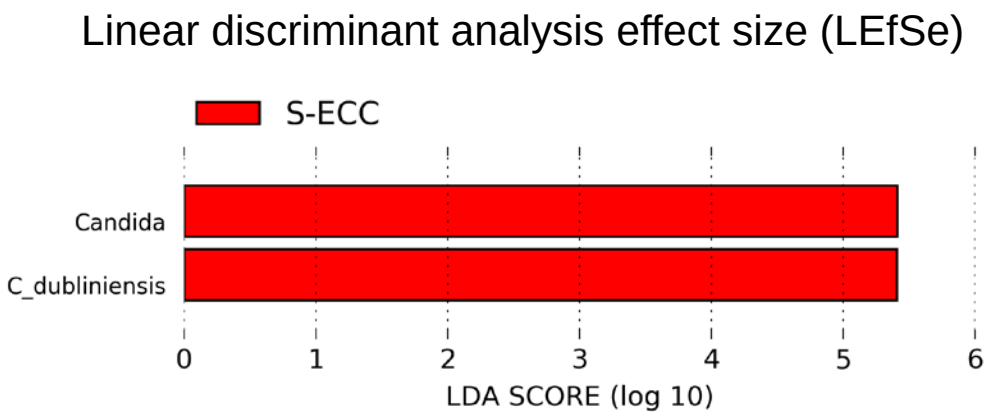
Plaque 16S rRNA: According to S-ECC status and T2R genotypes



Plaque ITS1 rRNA: According to S-ECC status and T2R genotypes

TAS2R38 rs10246939, Ile296Val





- ✓ The subgroup plaque mycobiome analysis revealed that the overabundant *C. dubliniensis is associated with S-ECC risk.*

Conclusion

Overall, these results show important evidence on the differences between the plaque microbiome of children with and without S-ECC and demonstrate that **sex and taste genetics may play a role on the composition of the plaque biofilm**.

Future goals

Use these results to contribute to greater screening of susceptible individuals and to develop targeted intervention strategies for dental caries in young children based on specific environmental modifications such as taste preferences.

Future goals

- We are recruiting children <72 months of age to participate in our new project.
- Target: recruiting 800 children.

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Thank you!