Role of Microbiome in Asthma

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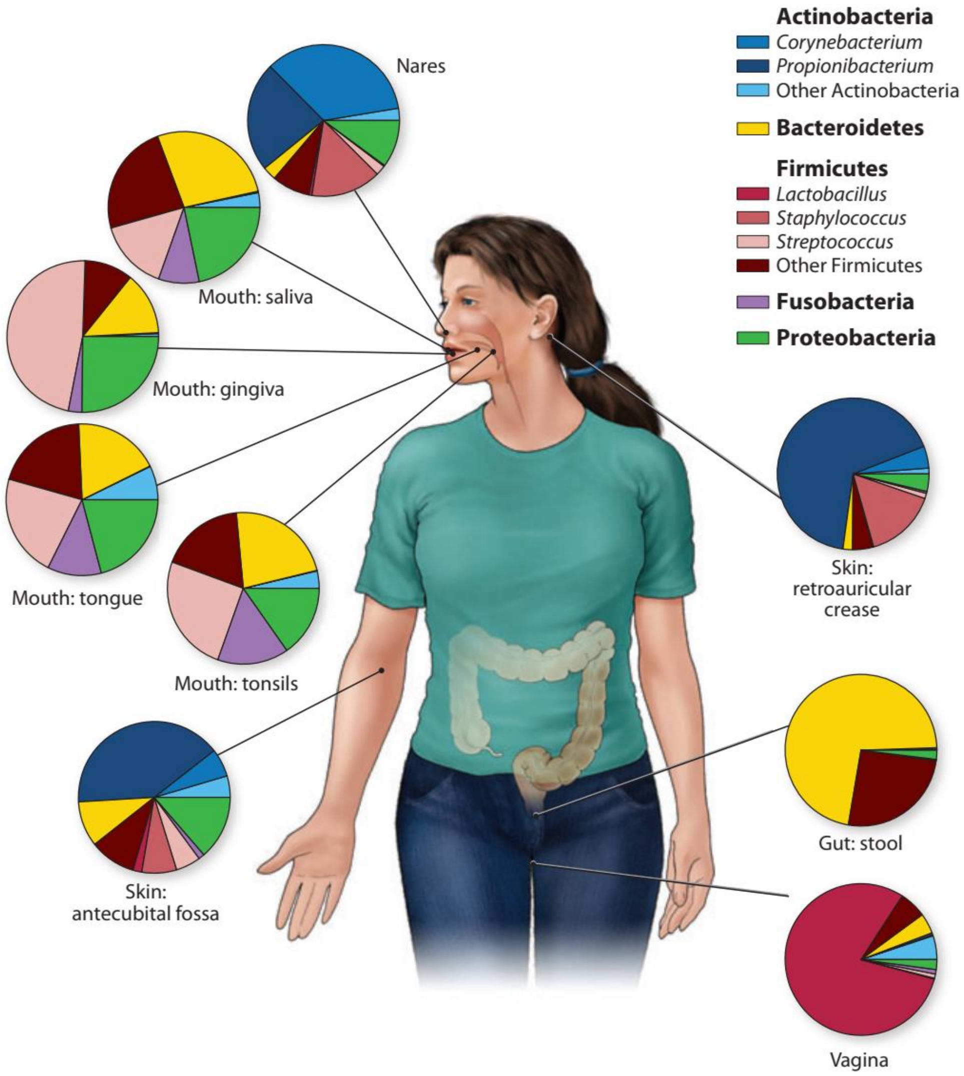
Human microbiome as the second genome



- Somatic cells of human body: 10^{13}
- Commensal microorganisms in human body: 10^{14}

- **Role of microbiome**
  - Source of genetic diversity
  - Important component of immune function
  - Functional unit to regulate the drug response and metabolism

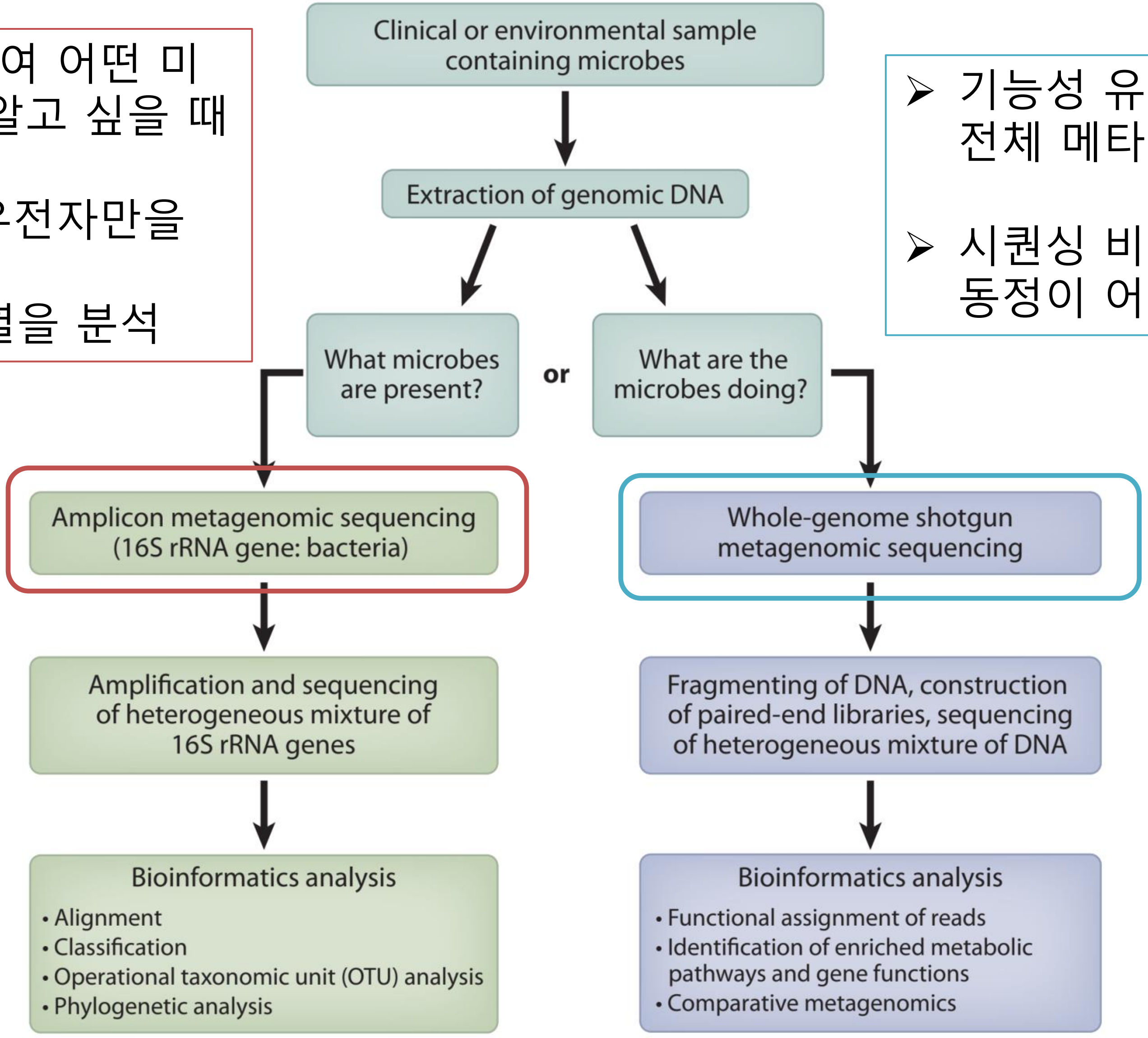
Grice EA, et al. Annu Rev Genomics Hum Gent 2012;13:151-170.

Definitions for common terms in microbiome investigation

Term	Definition
Microbiota	The assemblage of all microorganisms present in a defined site or niche. Current usage often implies only bacteria.
Microbiome	The entire habitat, including the microbiota, their genomes (genes), and the surrounding site-specific conditions Current usage often refers only to bacterial members of the "biome"
Mycobiota Mycobiome	Variation on the above definitions referring specifically to fungi present in a habitat
Metagenome Metagenomics	Collection of genomes and genes from the members of a microbiota, characterized by the process of 'metagenomics' (ie, whole-genome shotgun DNA sequencing, amplicon sequencing) to obtain information on potential functions of the microbiota
Dysbiosis	Descriptive for imbalance in a microbiome, such as lack of homeostasis in microbial composition or functions
Diversity	Multiple types of measures exist. A calculated index or measure reflecting the types, numbers, and distribution of microbiota present within a sample or site (α-diversity) or between different samples or sites (β-diversity)

Workflow for metagenomic sequencing and analysis

- 미생물 군집 분석을 하여 어떤 미생물이 존재하는지를 알고 싶을 때
- 종 동정을 위한 표지 유전자만을 선택적으로 증폭한 후
그 증폭산물의 염기서열을 분석



Clinical or environmental sample containing microbes

Extraction of genomic DNA

What microbes are present?

or

What are the microbes doing?

Amplicon metagenomic sequencing (16S rRNA gene: bacteria)

Amplification and sequencing of heterogeneous mixture of 16S rRNA genes

Bioinformatics analysis
- Alignment
- Classification
- Operational taxonomic unit (OTU) analysis
- Phylogenetic analysis

Whole-genome shotgun metagenomic sequencing

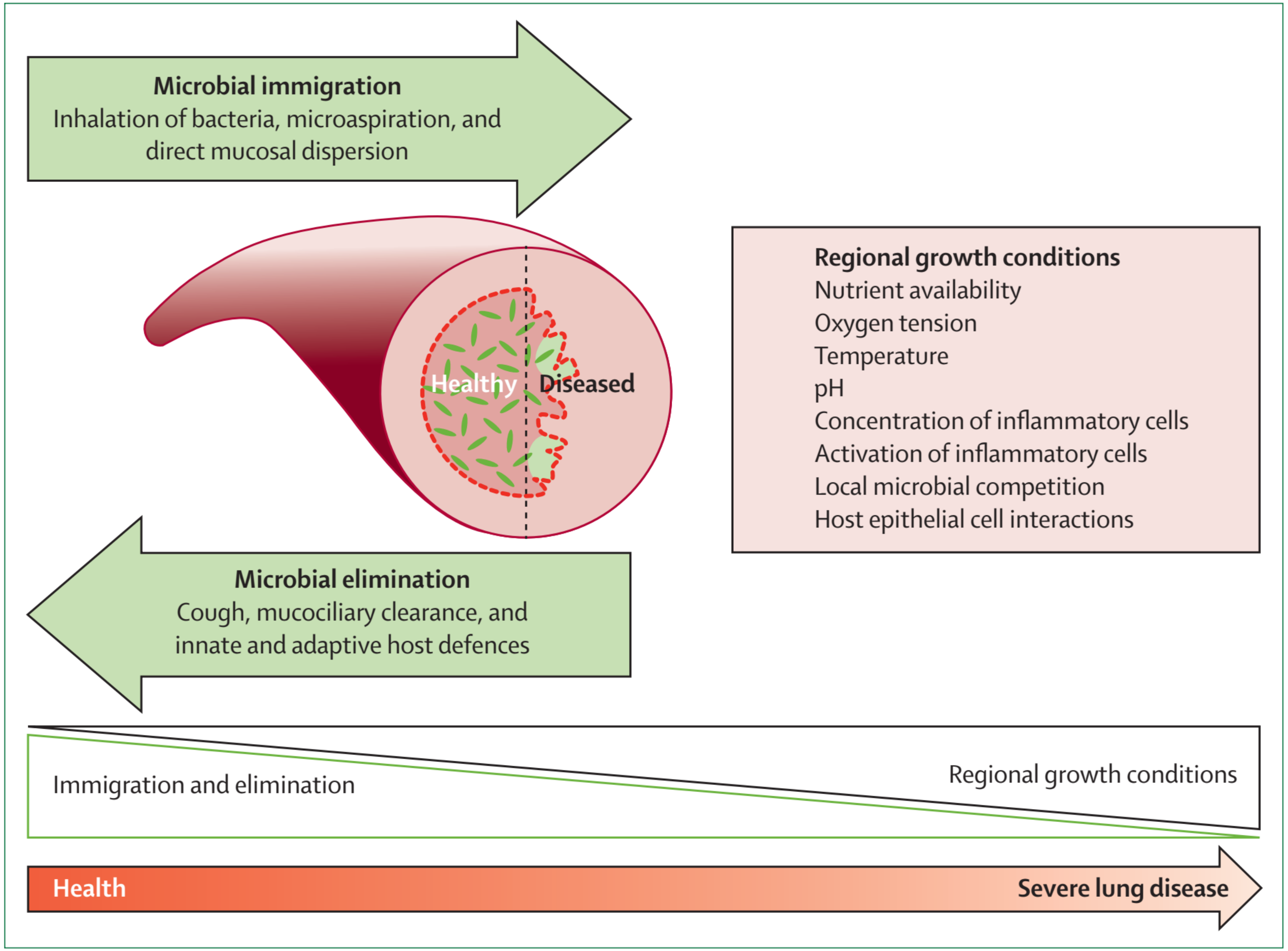
Fragmenting of DNA, construction of paired-end libraries, sequencing of heterogeneous mixture of DNA

Bioinformatics analysis
- Functional assignment of reads
- Identification of enriched metabolic pathways and gene functions
- Comparative metagenomics

- 기능성 유전자들의 구성을 포함하여 전체 메타게놈 그대로를 볼 수 있음
- 시퀀싱 비용이 많이 들고 시퀀스의 동정이 어려움

Grice EA, et al. Annu Rev Genomics Hum Gent 2012;13:151-170.

Determinants of the respiratory microbiome



Microbial immigration
Inhalation of bacteria, microaspiration, and direct mucosal dispersion

Regional growth conditions
Nutrient availability
Oxygen tension
Temperature
pH
Concentration of inflammatory cells
Activation of inflammatory cells
Local microbial competition
Host epithelial cell interactions

Microbial elimination
Cough, mucociliary clearance, and innate and adaptive host defences

Immigration and elimination

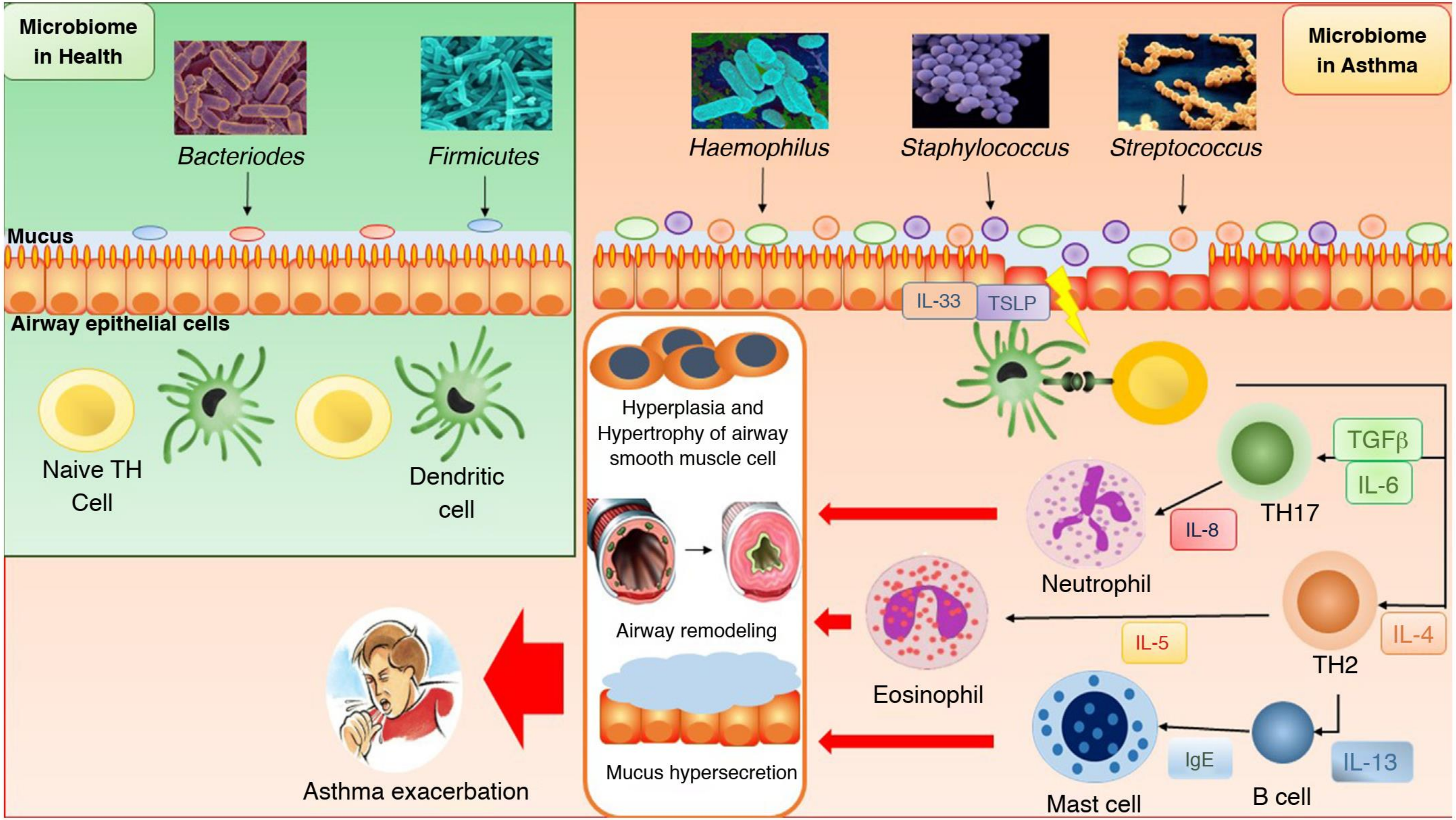
Regional growth conditions

Health

Severe lung disease

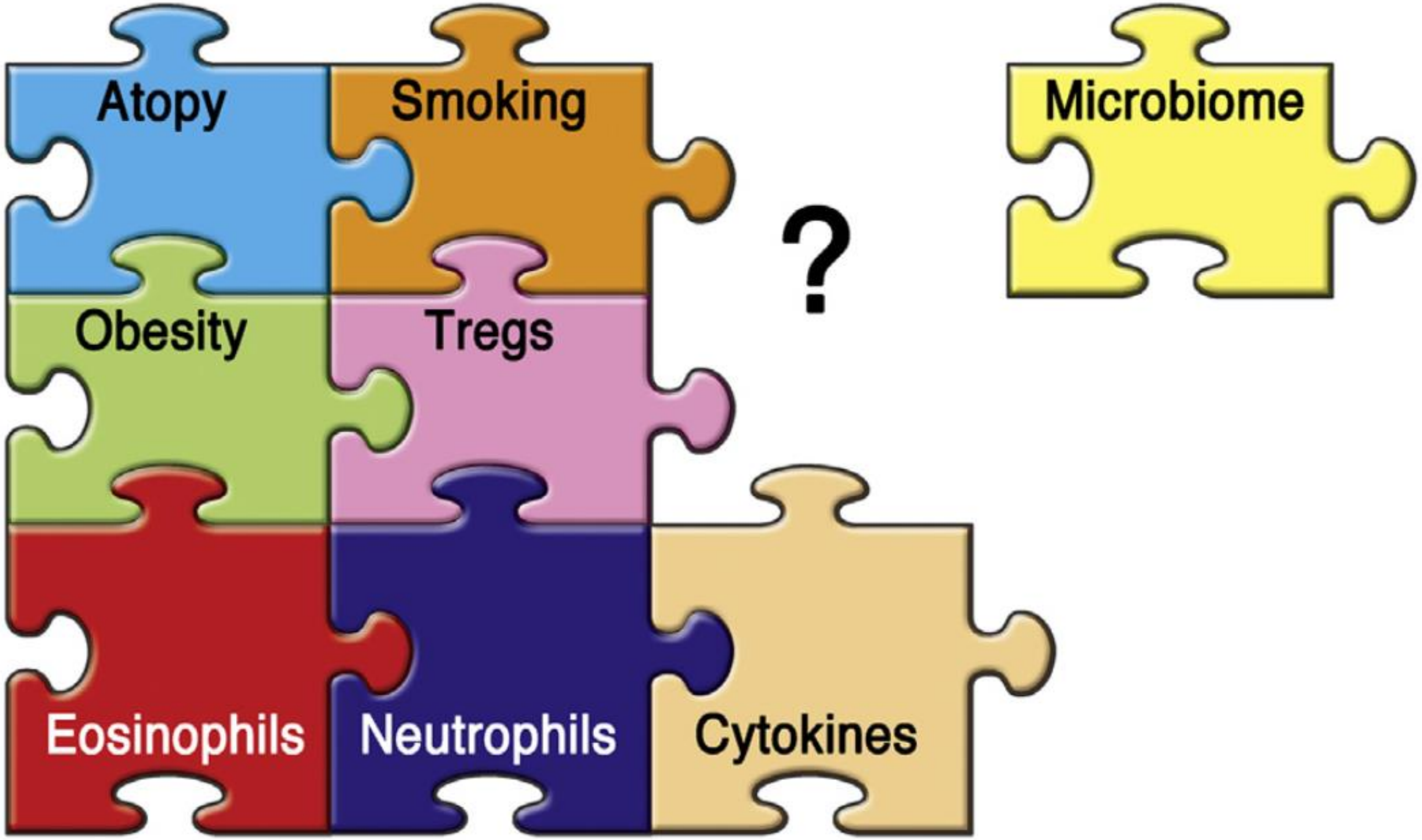
Dickson RP, et al. Lancet 2014;384(9944):691-702.

Role of lung microbiome in health and asthma



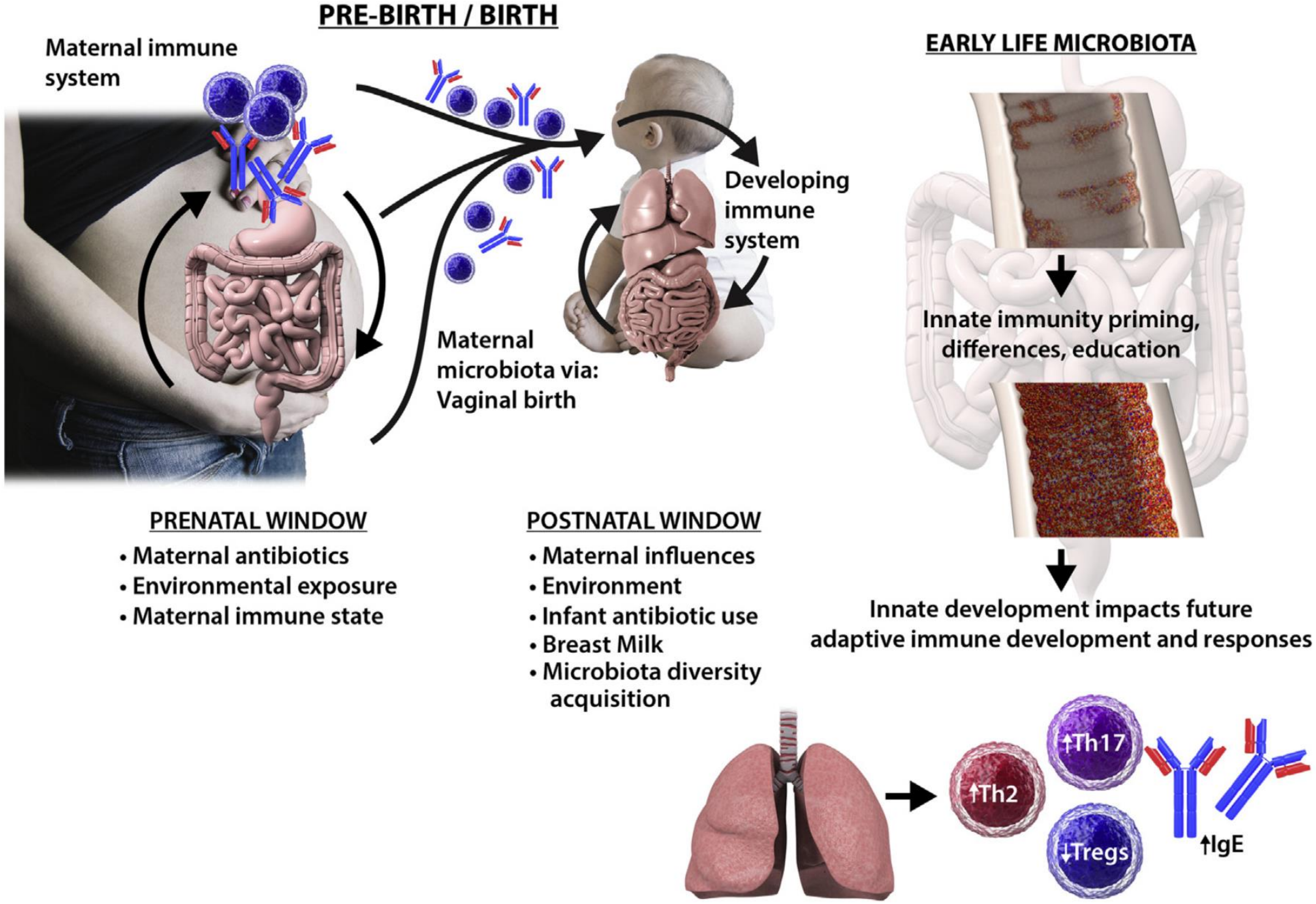
Shukla SD, et al. Curr Opin Pharmacol 2019;46:143-149.

Microbiome in asthma endotyping



Trujillo J, et al. J Allergy Clin Immunol 2022;149(2):533-534.

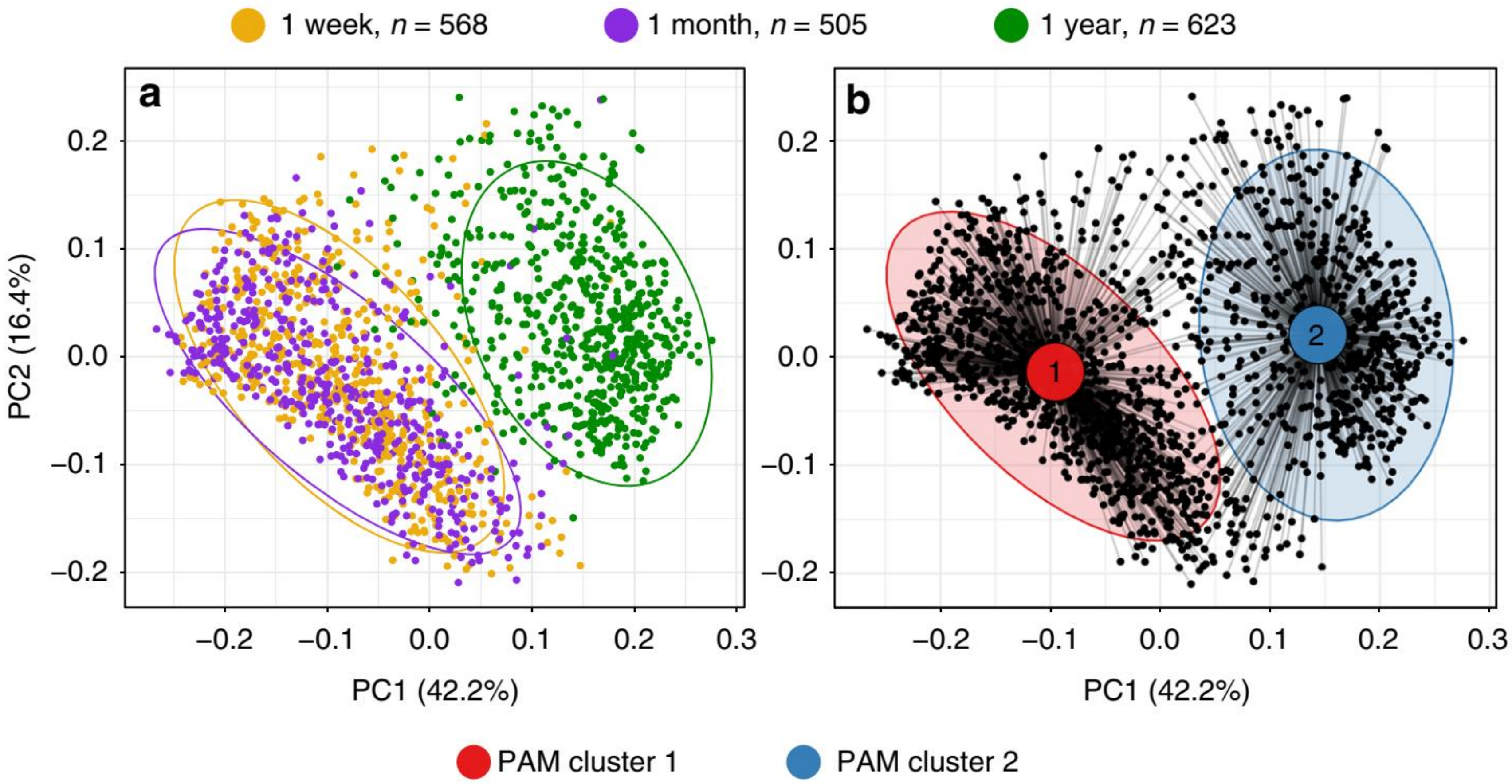
Early-life influences on the developing microbiome



Borbet TC, et al. J Allergy Clin Immunol 2019;144(6):1457-1466.

Maturation of gut microbiome and childhood asthma

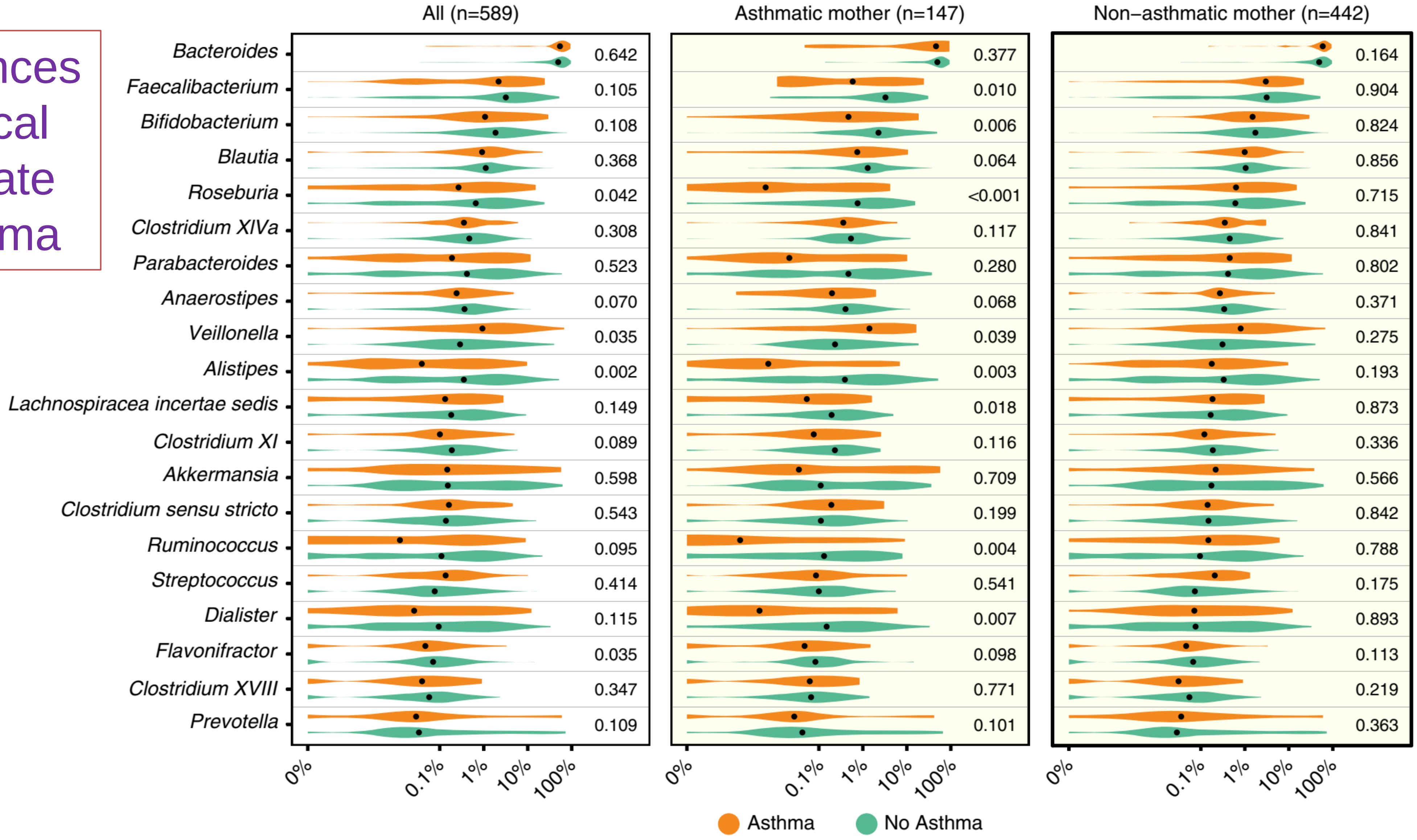
- ➢ 690 Children from Copenhagen prospective birth cohort (COPSAC$_{2010}$)
- ➢ Fecal samples, 16S rRNA gene amplicon sequencing



Stokholm J, et al. Nat Commun 2018;9(1):141.

Early-life gut microbiome and later asthma

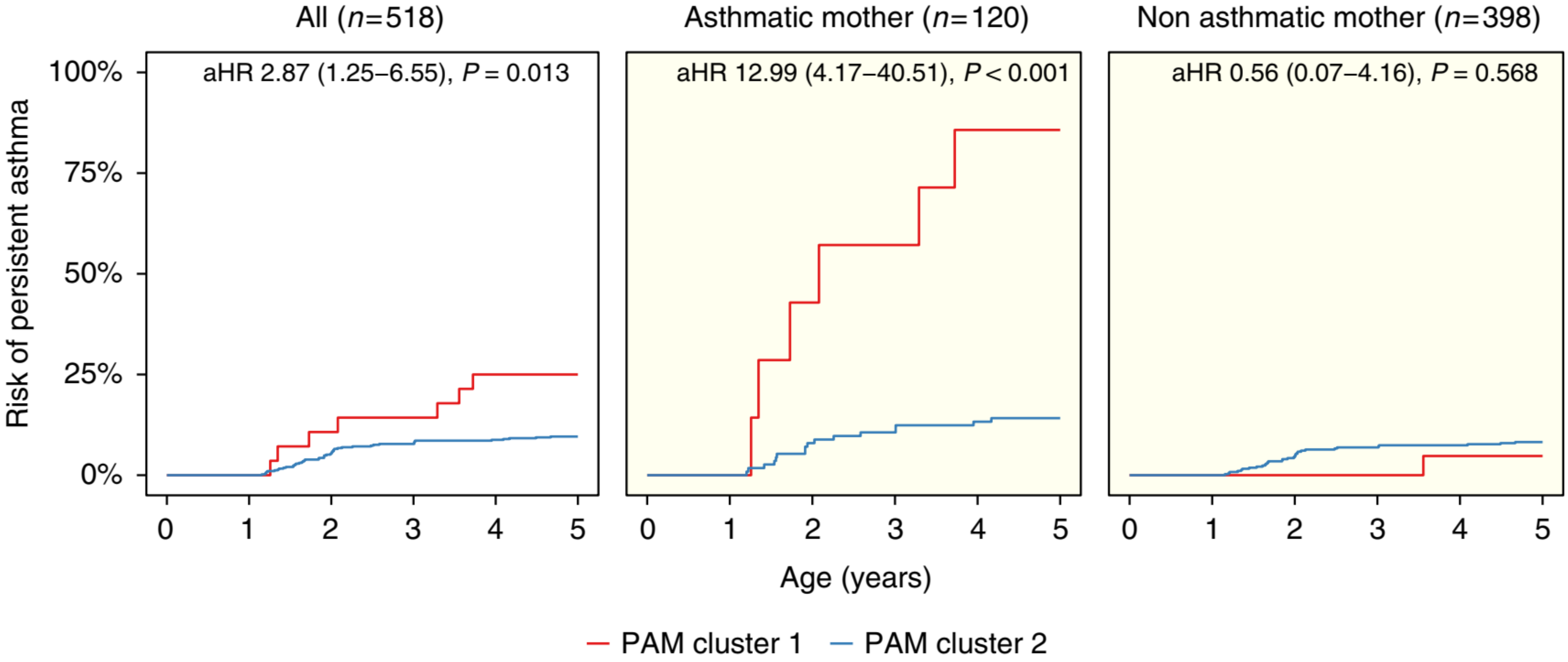
Relative abundances in the 1-year fecal samples associate with 5-year asthma



Stokholm J, et al. Nat Commun 2018;9(1):141.

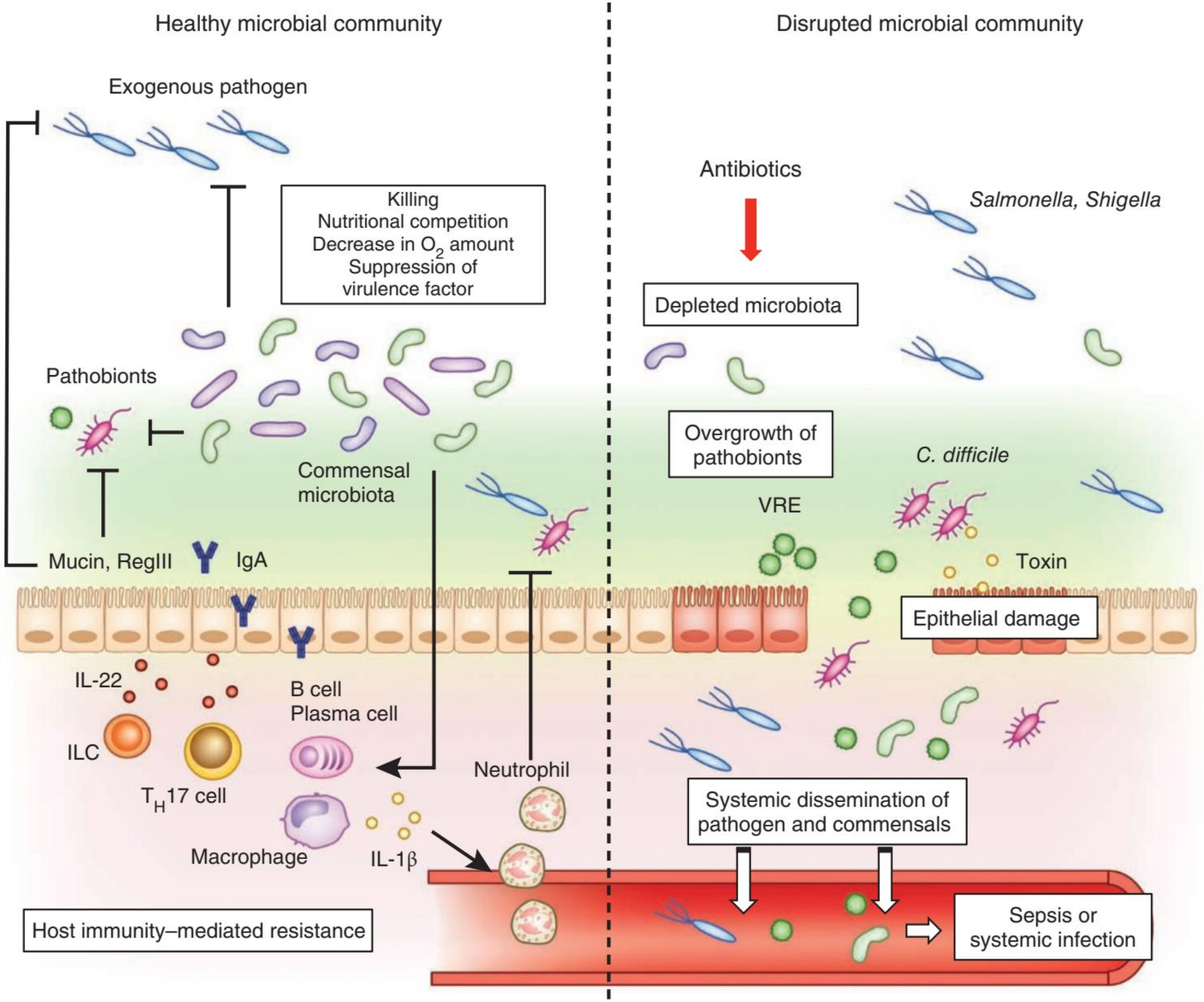
Maturation of gut microbiome and childhood asthma risk

- Interaction between maturation of gut microbiome and maternal asthma



Stokholm J, et al. Nat Commun 2018;9(1):141.

Gut microbiota and dysbiosis

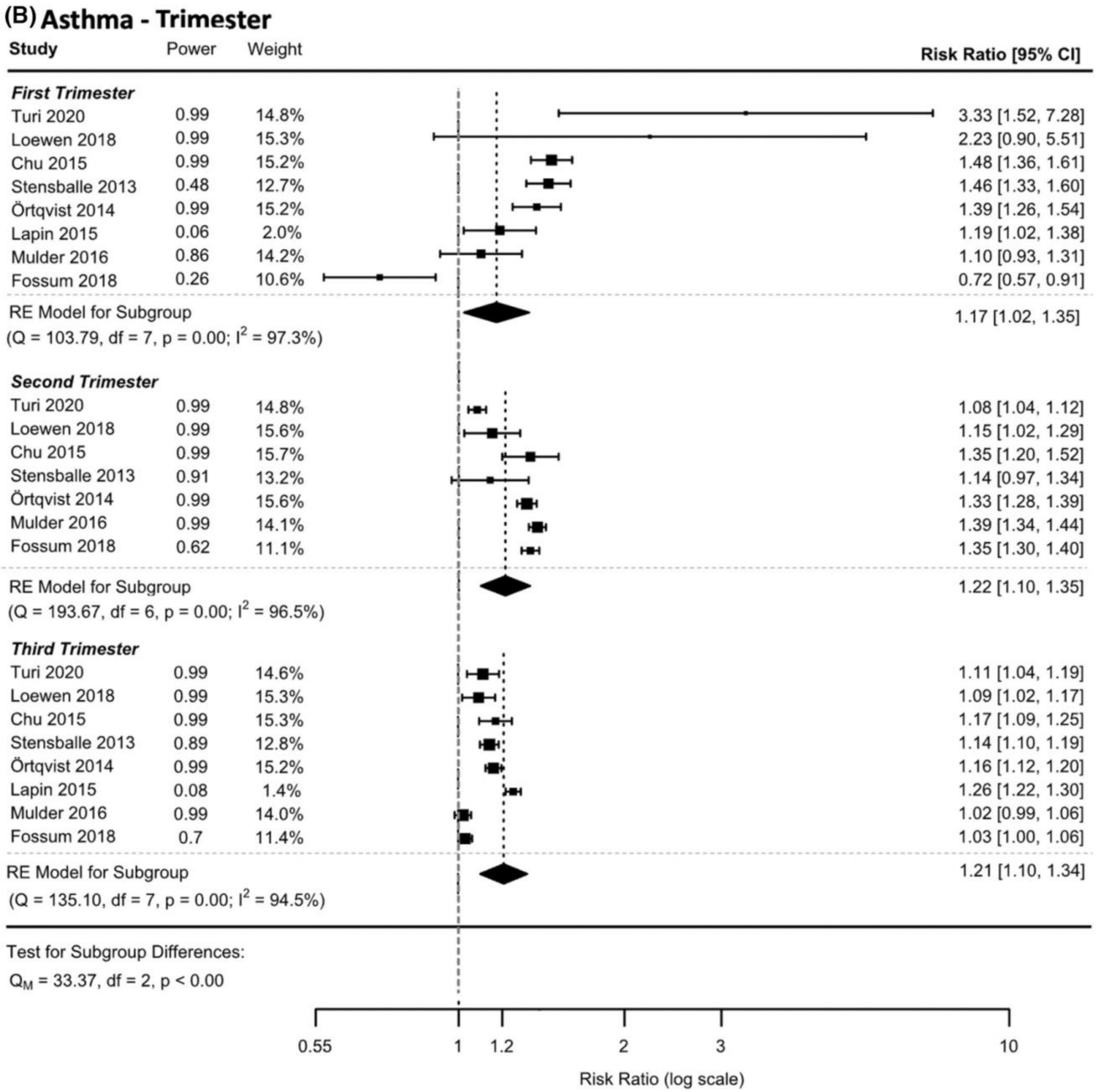


Kamada N, et al. Nat Immunol 2013;14(7):685-690.

Prenatal antibiotic exposure and asthma risk

- ➢ Meta-analysis from 27 studies for antibiotic use during pregnancy and asthma risk in children

Antibiotic use according to trimesters	Asthma development in children Risk ratio (95% CI)
First trimester	1.17 (1.02-1.35)
Second trimester	1.22 (1.10-1.35)
Third trimester	1.21 (1.10-1.34)

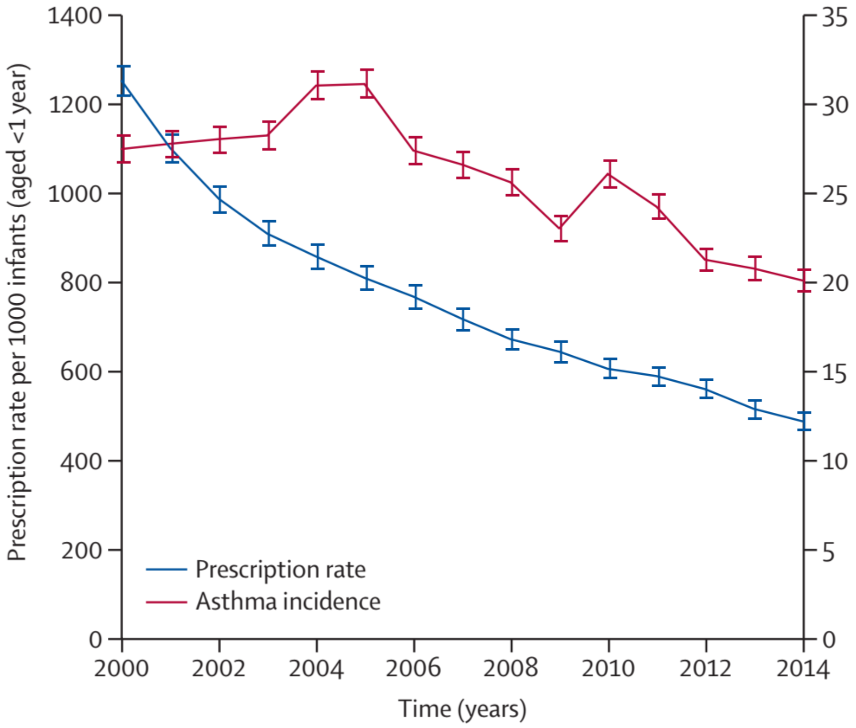


Study	Power	Weight	Risk Ratio [95% CI]
First Trimester			
Turi 2020	0.99	14.8%	3.33 [1.52, 7.28]
Loewen 2018	0.99	15.3%	2.23 [0.90, 5.51]
Chu 2015	0.99	15.2%	1.48 [1.36, 1.61]
Stensballe 2013	0.48	12.7%	1.46 [1.33, 1.60]
Örtqvist 2014	0.99	15.2%	1.39 [1.26, 1.54]
Lapin 2015	0.06	2.0%	1.19 [1.02, 1.38]
Mulder 2016	0.86	14.2%	1.10 [0.93, 1.31]
Fossum 2018	0.26	10.6%	0.72 [0.57, 0.91]
RE Model for Subgroup (Q = 103.79, df = 7, p = 0.00; I^2 = 97.3%)			1.17 [1.02, 1.35]
Second Trimester			
Turi 2020	0.99	14.8%	1.08 [1.04, 1.12]
Loewen 2018	0.99	15.6%	1.15 [1.02, 1.29]
Chu 2015	0.99	15.7%	1.35 [1.20, 1.52]
Stensballe 2013	0.91	13.2%	1.14 [0.97, 1.34]
Örtqvist 2014	0.99	15.6%	1.33 [1.28, 1.39]
Mulder 2016	0.99	14.1%	1.39 [1.34, 1.44]
Fossum 2018	0.62	11.1%	1.35 [1.30, 1.40]
RE Model for Subgroup (Q = 193.67, df = 6, p = 0.00; I^2 = 96.5%)			1.22 [1.10, 1.35]
Third Trimester			
Turi 2020	0.99	14.6%	1.11 [1.04, 1.19]
Loewen 2018	0.99	15.3%	1.09 [1.02, 1.17]
Chu 2015	0.99	15.3%	1.17 [1.09, 1.25]
Stensballe 2013	0.89	12.8%	1.14 [1.10, 1.19]
Örtqvist 2014	0.99	15.2%	1.16 [1.12, 1.20]
Lapin 2015	0.08	1.4%	1.26 [1.22, 1.30]
Mulder 2016	0.99	14.0%	1.02 [0.99, 1.06]
Fossum 2018	0.7	11.4%	1.03 [1.00, 1.06]
RE Model for Subgroup (Q = 135.10, df = 7, p = 0.00; I^2 = 94.5%)			1.21 [1.10, 1.34]

Test for Subgroup Differences:
Q_M = 33.37, df = 2, p < 0.00

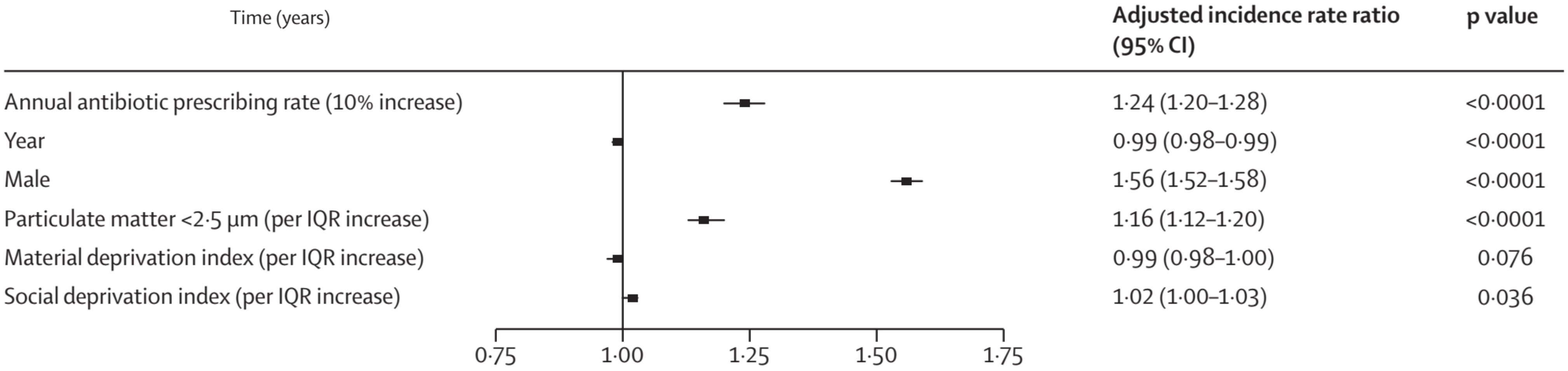
Cait A, et al. Allergy 2022 Jun 11. doi:10.1111/all.15404.

Antibiotic use and asthma incidence



- ➢ 2644 children in a longitudinal birth cohort from Canada (CHILD study)
- ➢ Subsamples of 917 16S rRNA gene sequencing data from fecal samples collected at 3 and 12 months

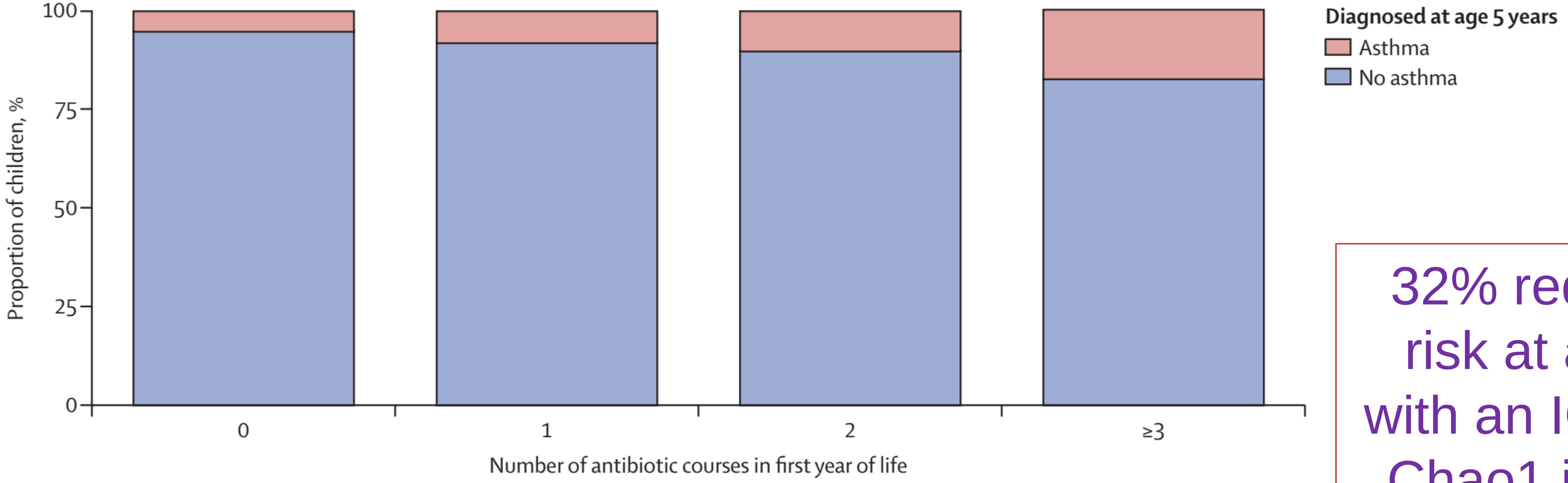
Multivariable Poisson regression of factors associated with asthma incidence



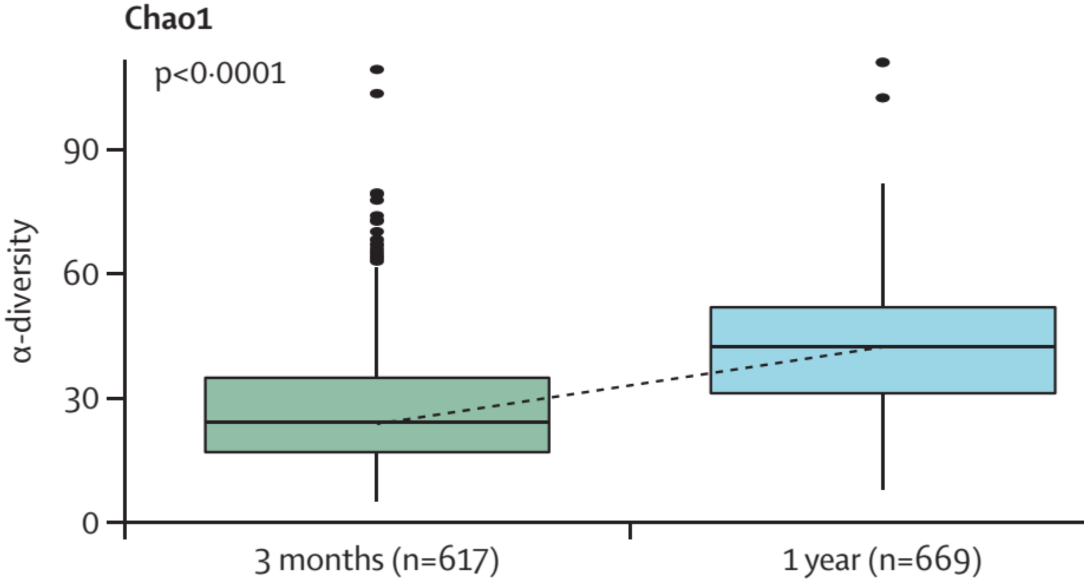
	Adjusted incidence rate ratio (95% CI)	p value
Annual antibiotic prescribing rate (10% increase)	1·24 (1·20–1·28)	<0·0001
Year	0·99 (0·98–0·99)	<0·0001
Male	1·56 (1·52–1·58)	<0·0001
Particulate matter <2·5 μm (per IQR increase)	1·16 (1·12–1·20)	<0·0001
Material deprivation index (per IQR increase)	0·99 (0·98–1·00)	0·076
Social deprivation index (per IQR increase)	1·02 (1·00–1·03)	0·036

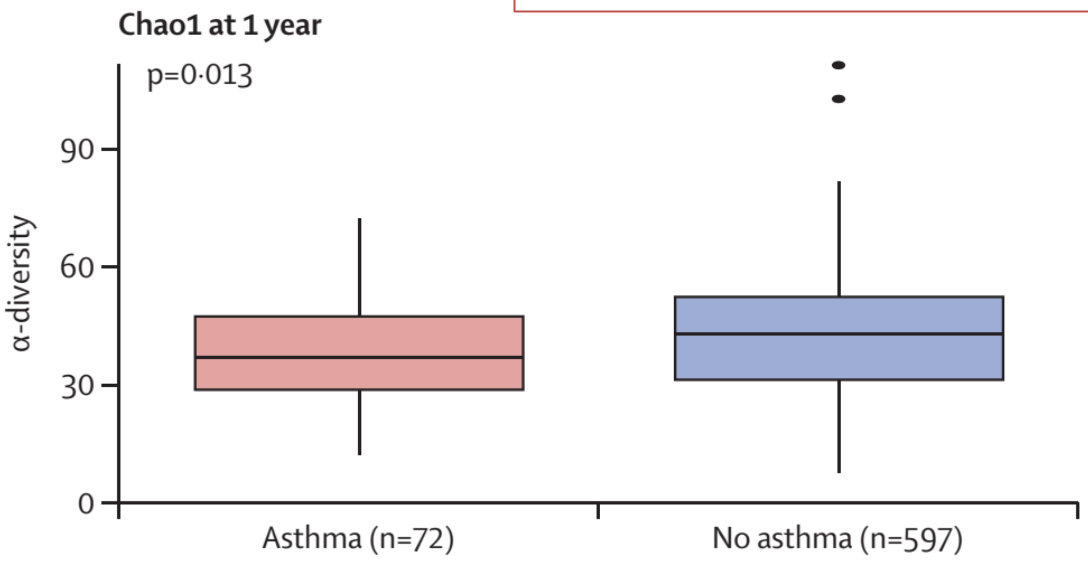
Patrick DM, et al. Lancet Respir Med 2020;8(11):1094-1105.

Antibiotic use and gut microbiota with asthma risk



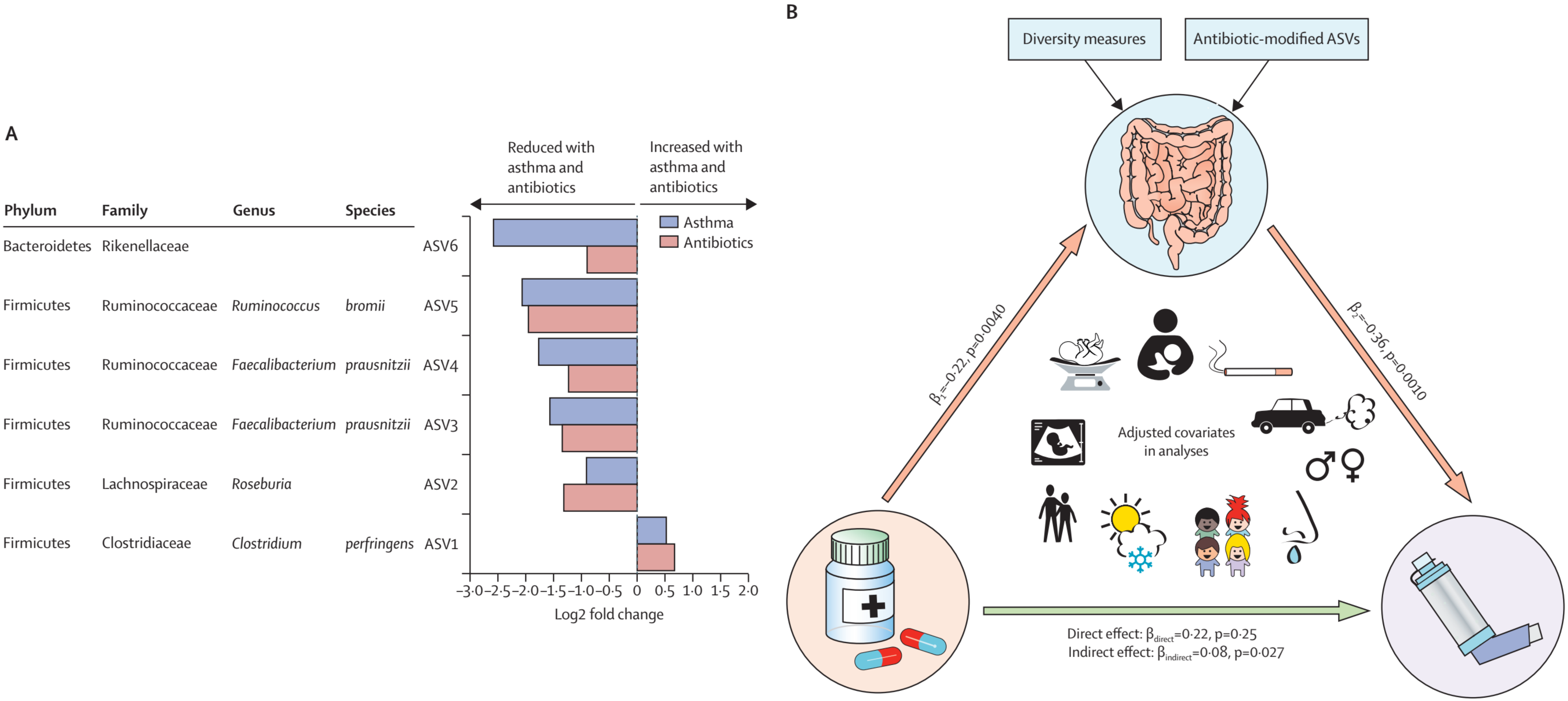
32% reduction in asthma risk at age 5 associated with an IQR increase in the Chao1 index (α-diversity)





Patrick DM, et al. Lancet Respir Med 2020;8(11):1094-1105.

Gut microbiota as a link between antibiotic exposure and asthma



Patrick DM, et al. Lancet Respir Med 2020;8(11):1094-1105.

Asthma risk with nasopharyngeal microbiome during infancy

- COAST birth cohort study

- Analysis for respiratory bacteria and viruses in nasopharyngeal mucus specimens collected from children under 2 sets of conditions

  1) Multiple scheduled visits during periods of good health through 24 months of age
     - 2, 4, 6, 9, 12, 18, and 24 months of age
  2) Acute respiratory illnesses

- 16S rRNA amplicon sequencing of nasopharyngeal samples (swab or aspirate)

Tang HHF, et al. J Allergy Clin Immunol 2021;147(5):1683-1691.

Longitudinal trajectories of nasopharyngeal microbiome

Trajectory A.
Dolosigranulum.dd2e
Corynebactrium.cb50

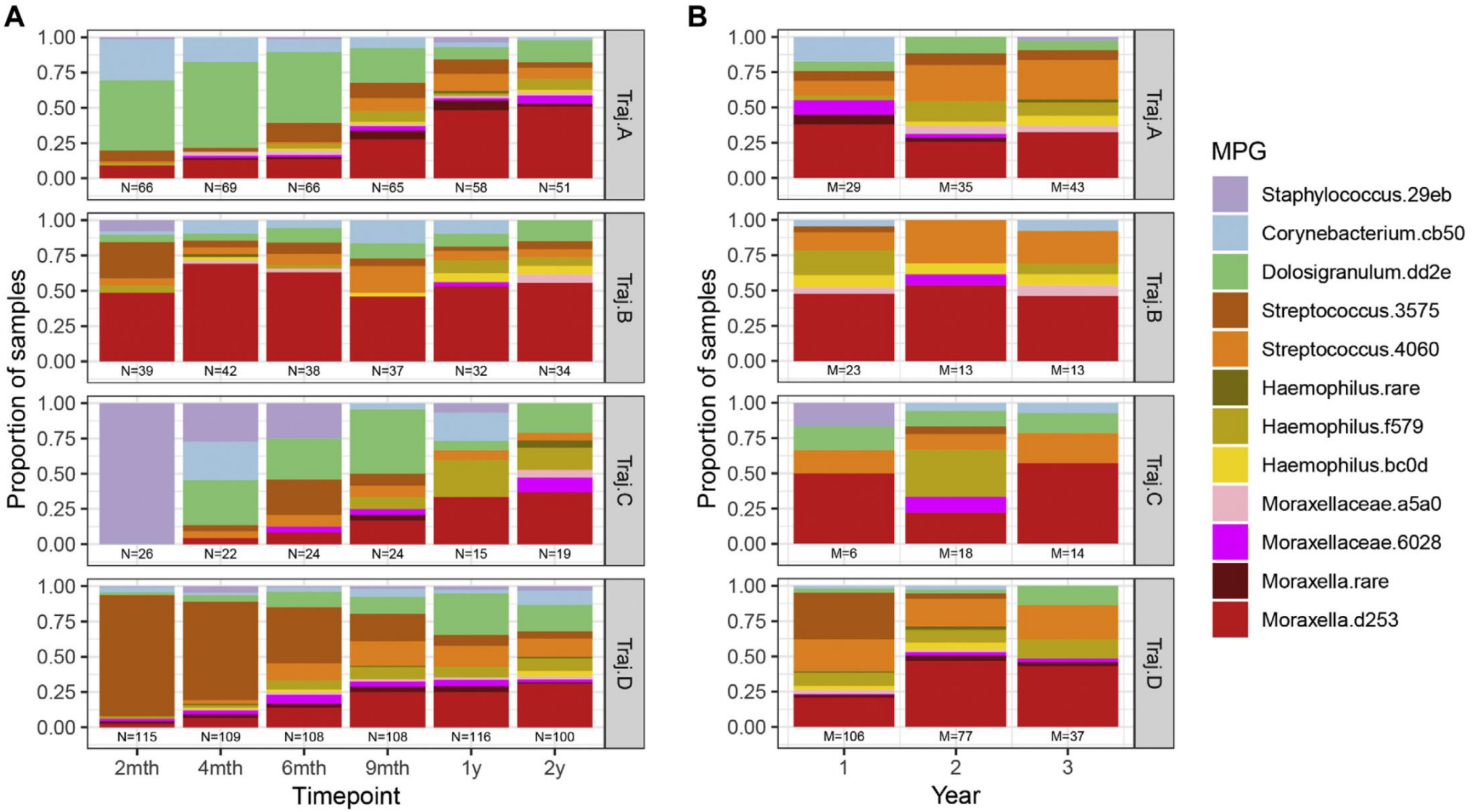
Trajectory B.
Moraxella.d253

Trajectory. C.
Staphylococcus.29eb

Trajectory D.
Streptoccus.3575

A. During routine visit

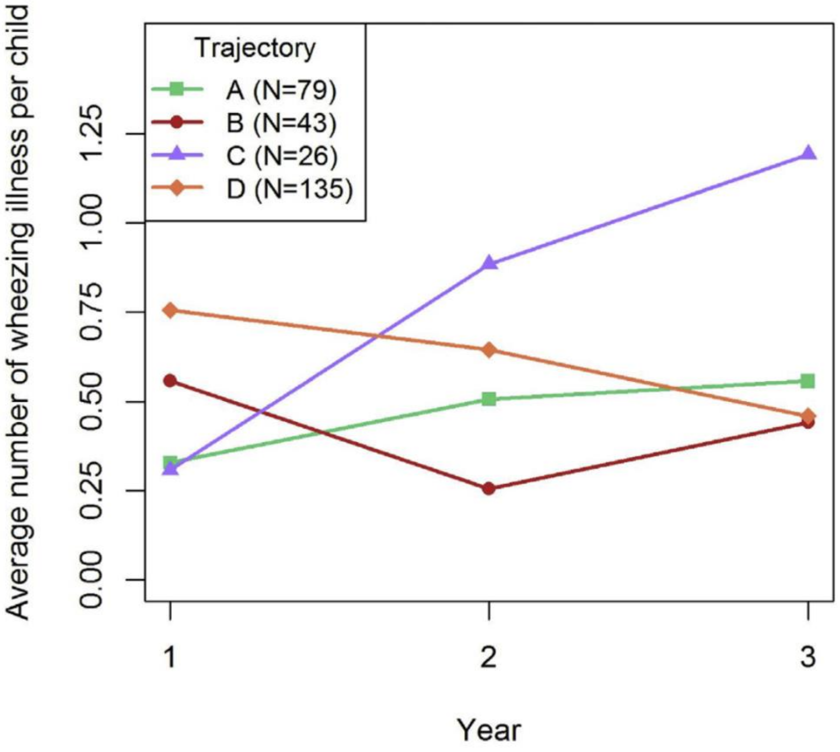
B. During acute illness

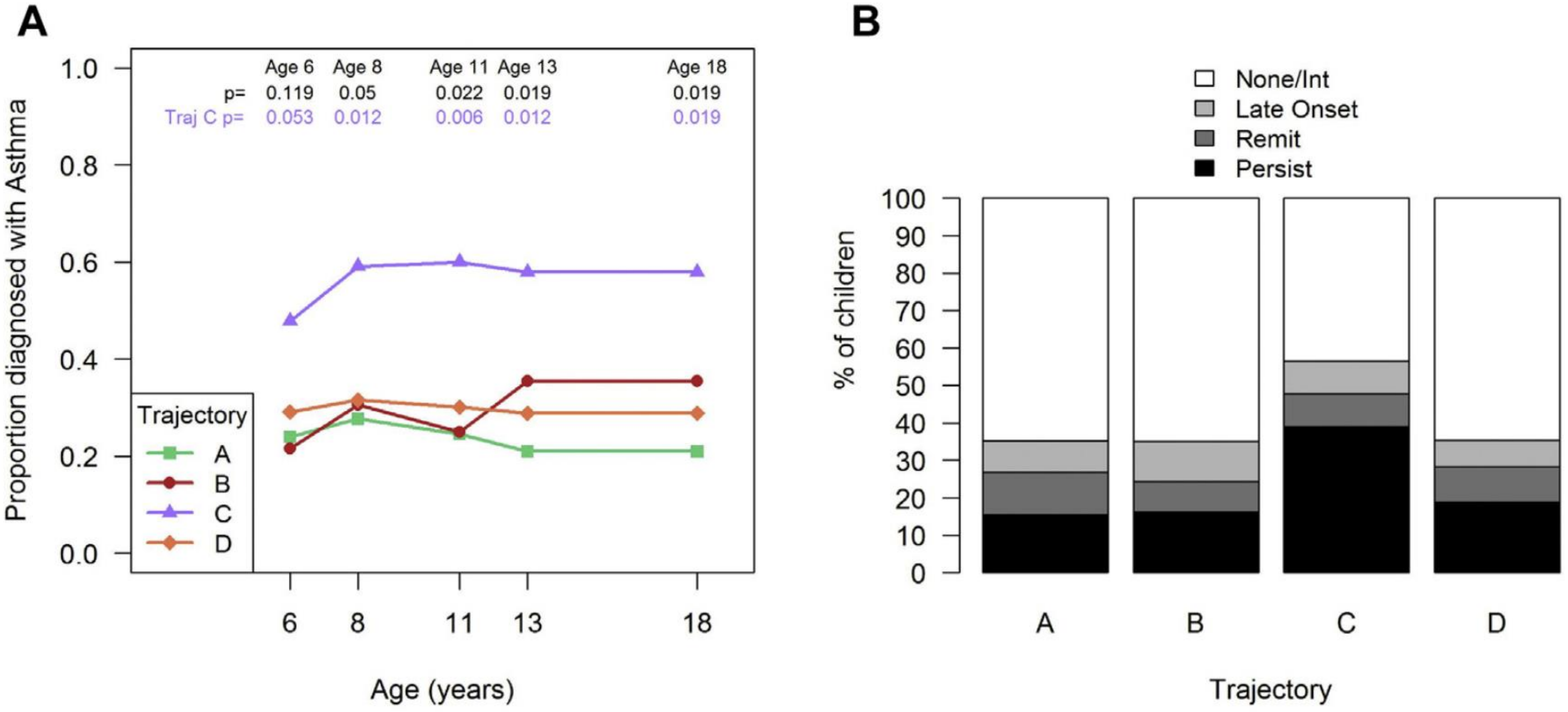


Tang HHF, et al. J Allergy Clin Immunol 2021;147(5):1683-1691.

Association of microbiome trajectory with wheezing/asthma

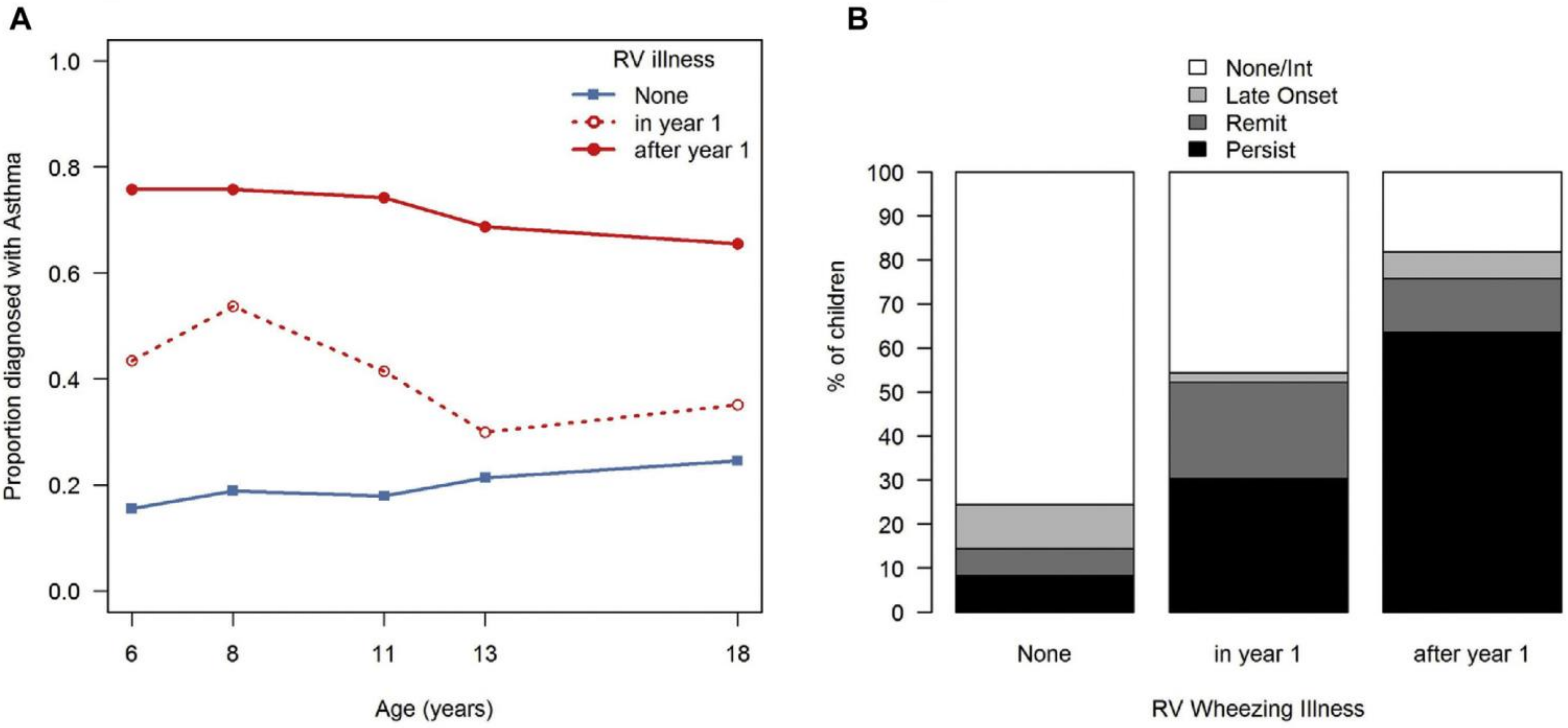
- ➢ Nasal microbiome <u>trajectory C</u> dominated by early Staphylococcus.29eb
  - ✓ Increased risk of recurrent wheezing by age 3 years and persistent asthma phenotype





Tang HHF, et al. J Allergy Clin Immunol 2021;147(5):1683-1691.

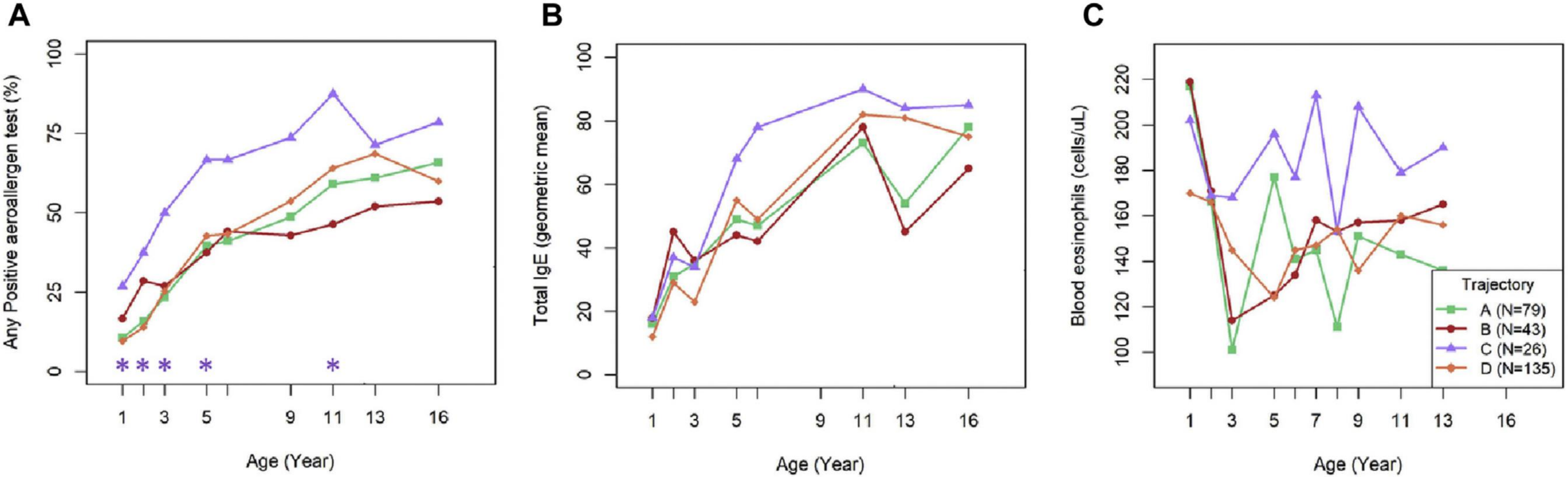
Microbial pathogen detection during illness and asthma risk



Tang HHF, et al. J Allergy Clin Immunol 2021;147(5):1683-1691.

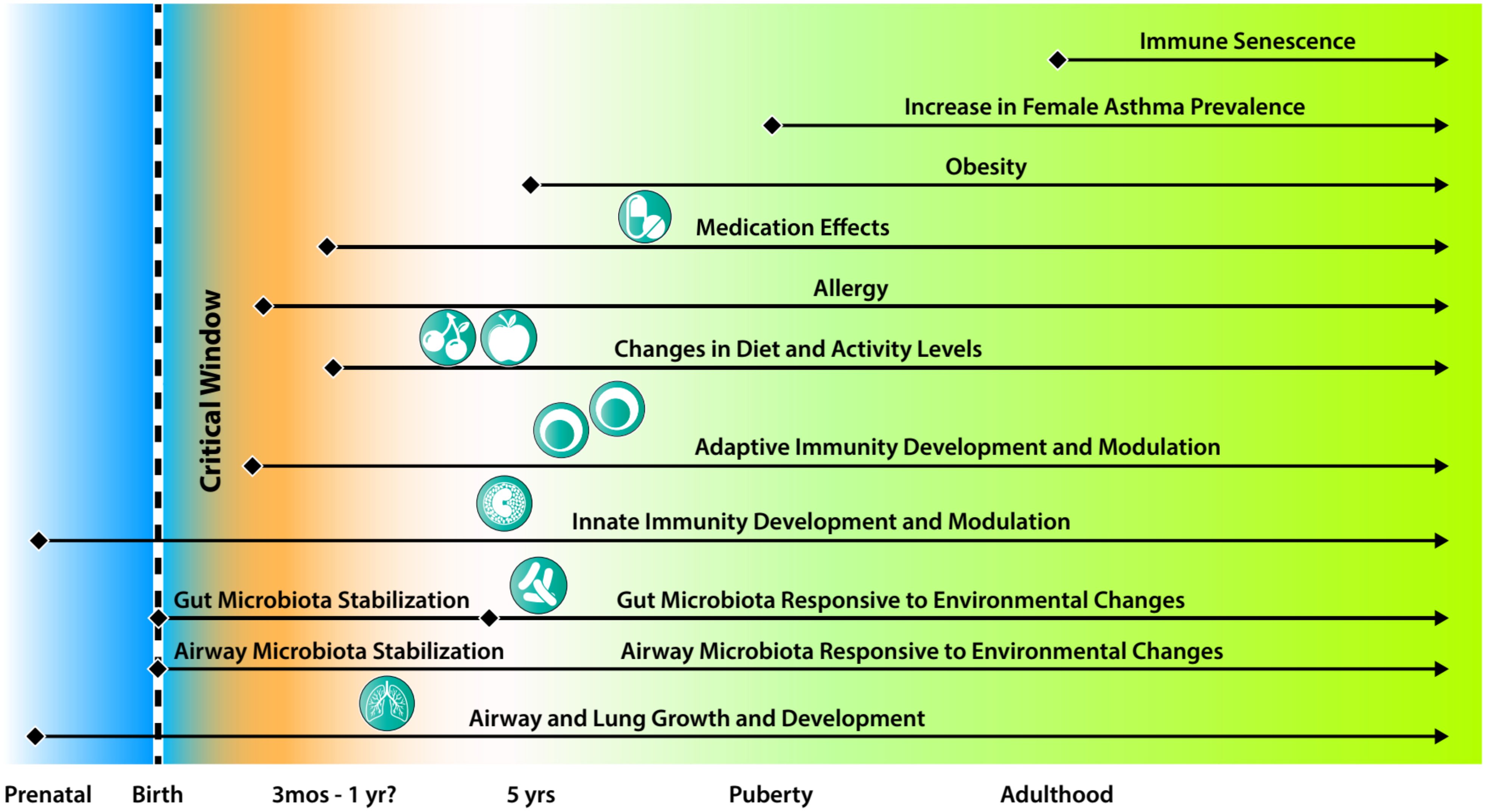
Microbiome trajectory and atopy indicator

- Nasal microbiome trajectory C
  - ✓ Consistently higher proportion of children who were sensitized to at least 1 aeroallergen
  - ✓ Similar nonsignificant trends for total IgE and blood eosinophil levels



Tang HHF, et al. J Allergy Clin Immunol 2021;147(5):1683-1691.

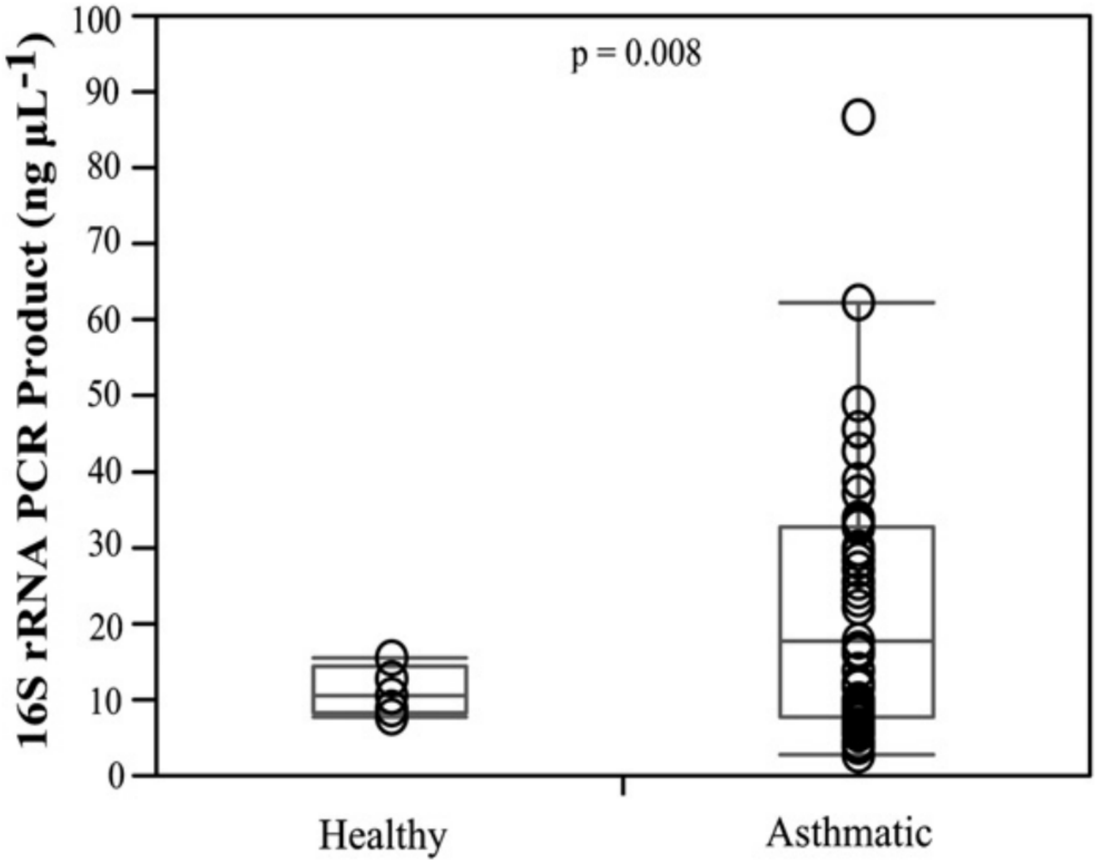
Influences on asthma pathogenesis and phenotype

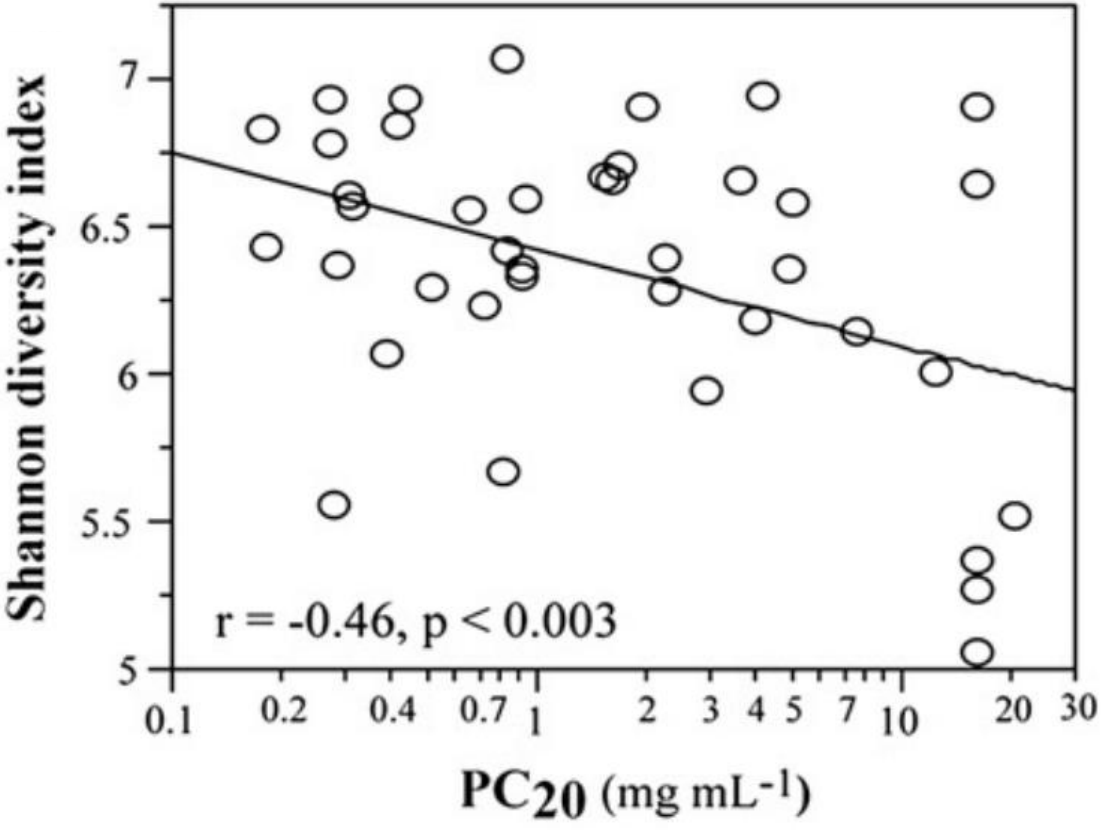


Kozik AJ, et al. Ann Allergy Asthma Immunol 2019;122:270-275.

Airway microbiota and bronchial hyperresponsiveness

- Adults with suboptimally controlled asthma vs. health control
- Bronchial epithelial brushings for 16S ribosomal RNA microarray and parallel clone library-sequencing analysis



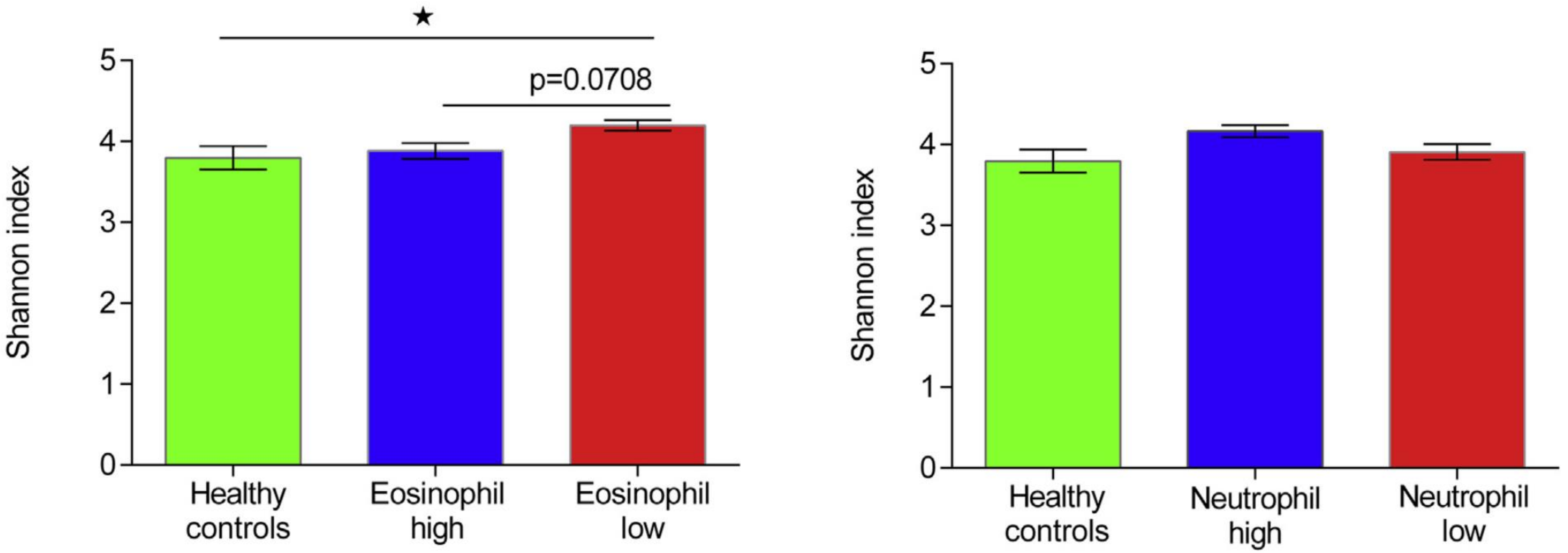


Huang YJ, et al. J Allergy Clin Immunol 2011;127(2):372-381.

Eosinophilic inflammation with an altered airway microbiome

- Adults with steroid-free nonsmoking asthma vs. healthy control
- Bacterial DNA extraction from BAL fluid; eosinophils/neutrophils in submucosa

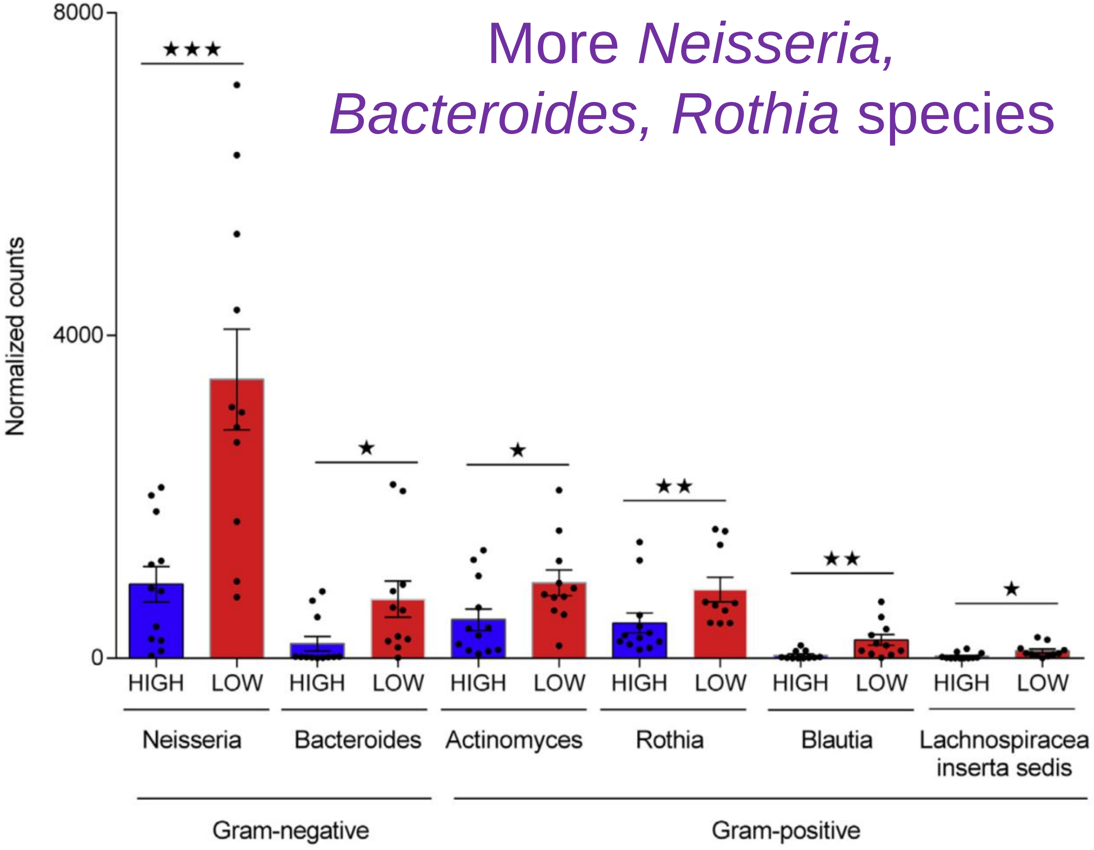
α-diversity



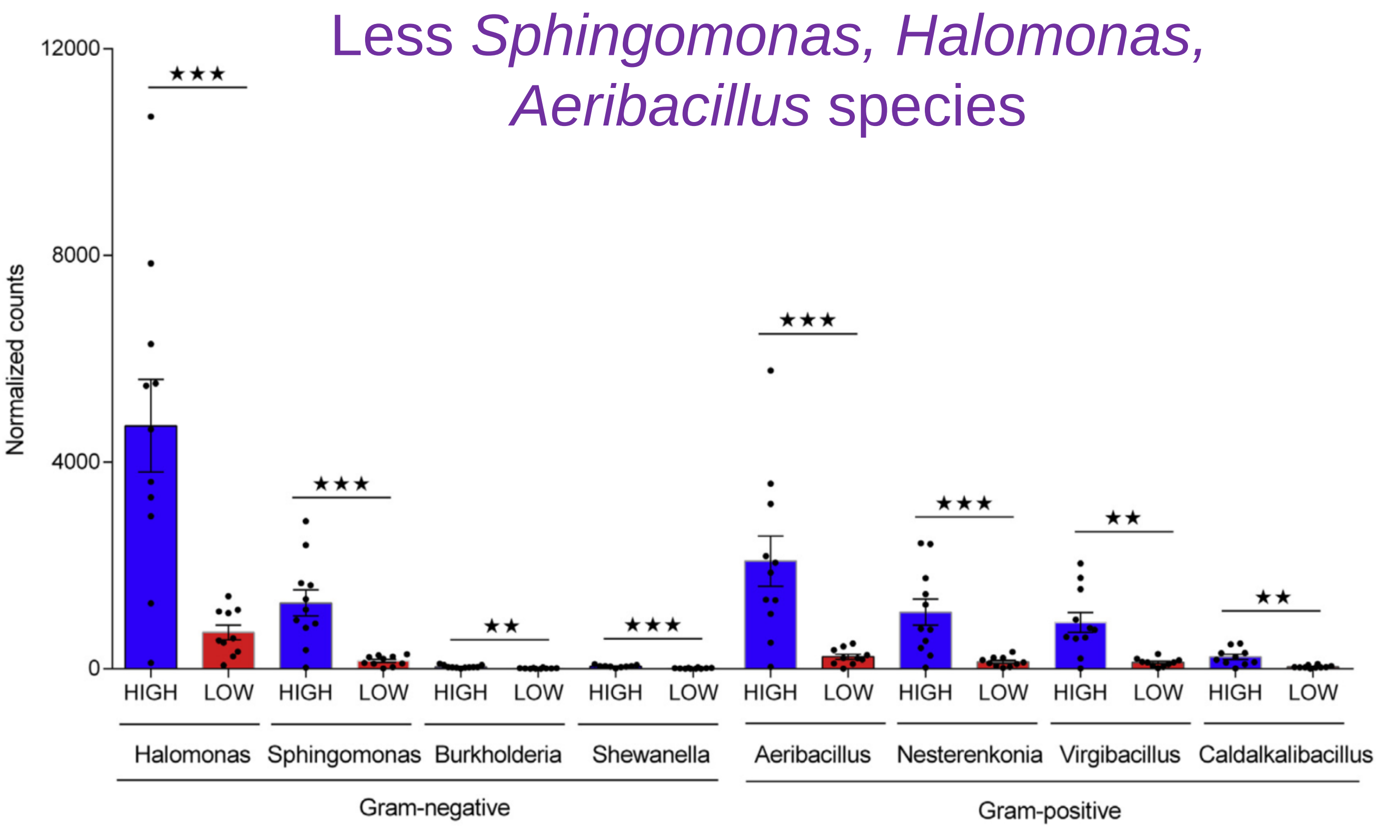
Sverrild A, et al. J Allergy Clin Immunol 2017;140(2):407-417.

Eosinophilic inflammation with an altered airway microbiome

- **Asthma with eosinophil low** vs. eosinophil high
  - ✓ Altered bacterial abundance profile



More *Neisseria, Bacteroides, Rothia* species



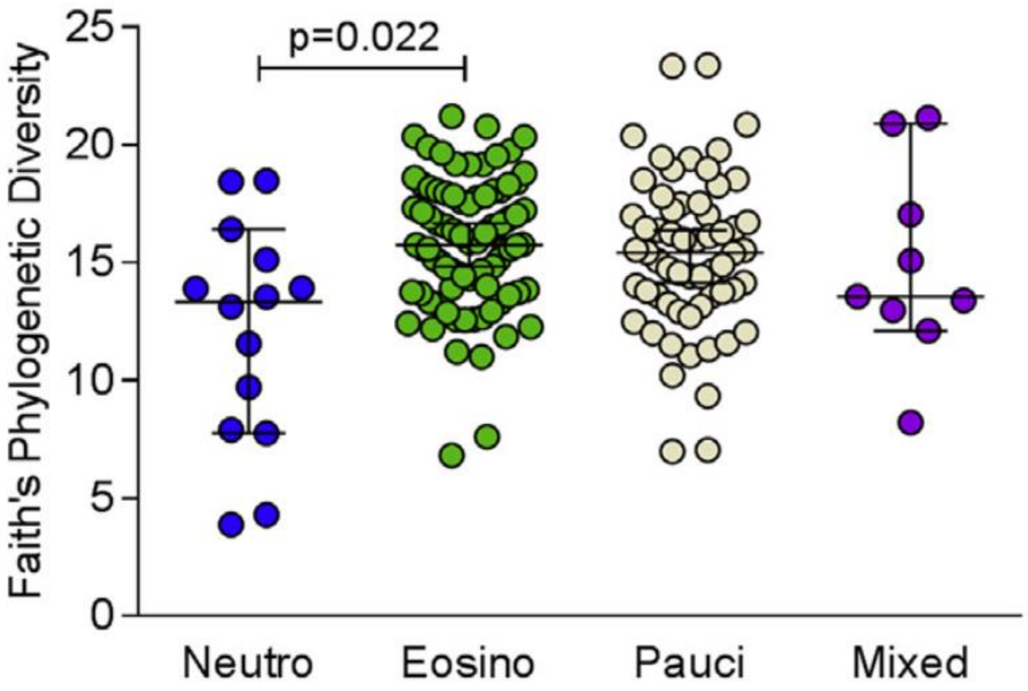
Less *Sphingomonas, Halomonas, Aeribacillus* species

Sverrild A, et al. J Allergy Clin Immunol 2017;140(2):407-417.

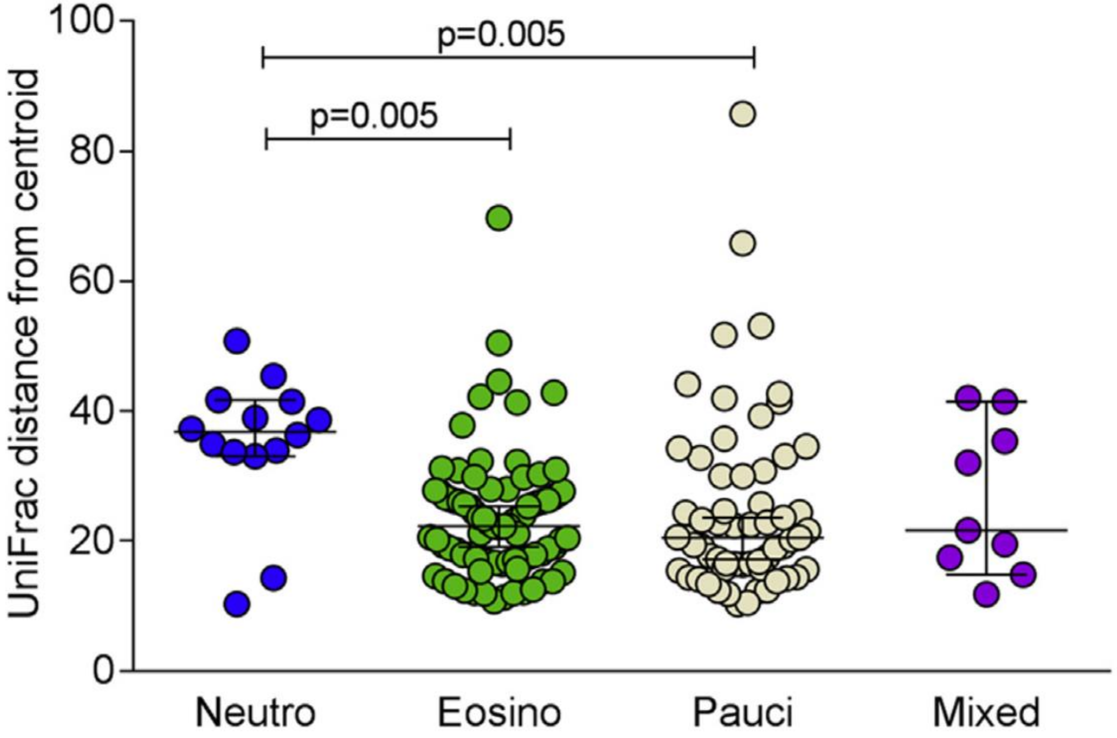
Asthma phenotype with distinct airway microbiome

- 167 patients with symptomatic stable asthma
- Induced sputum specimens, 16S rRNA gene sequencing

α-diversity

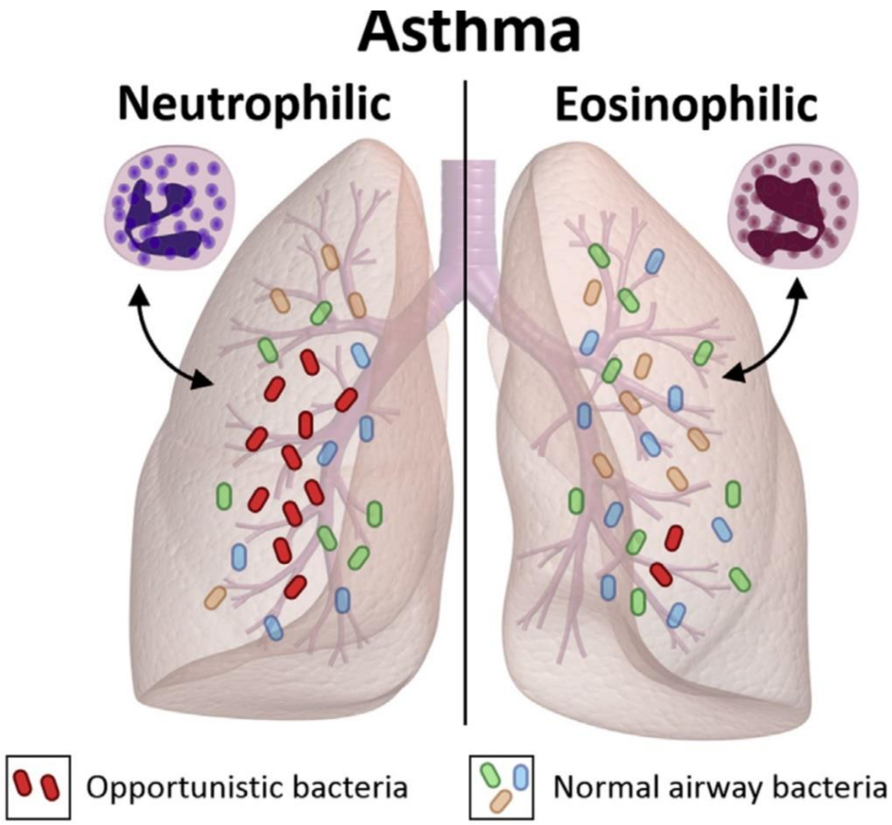


β-diversity



Taylor SL, et al. J Allergy Clin Immunol 2018;141(1):94-103.

Sputum neutrophil proportion as a predictor of microbiota proportion



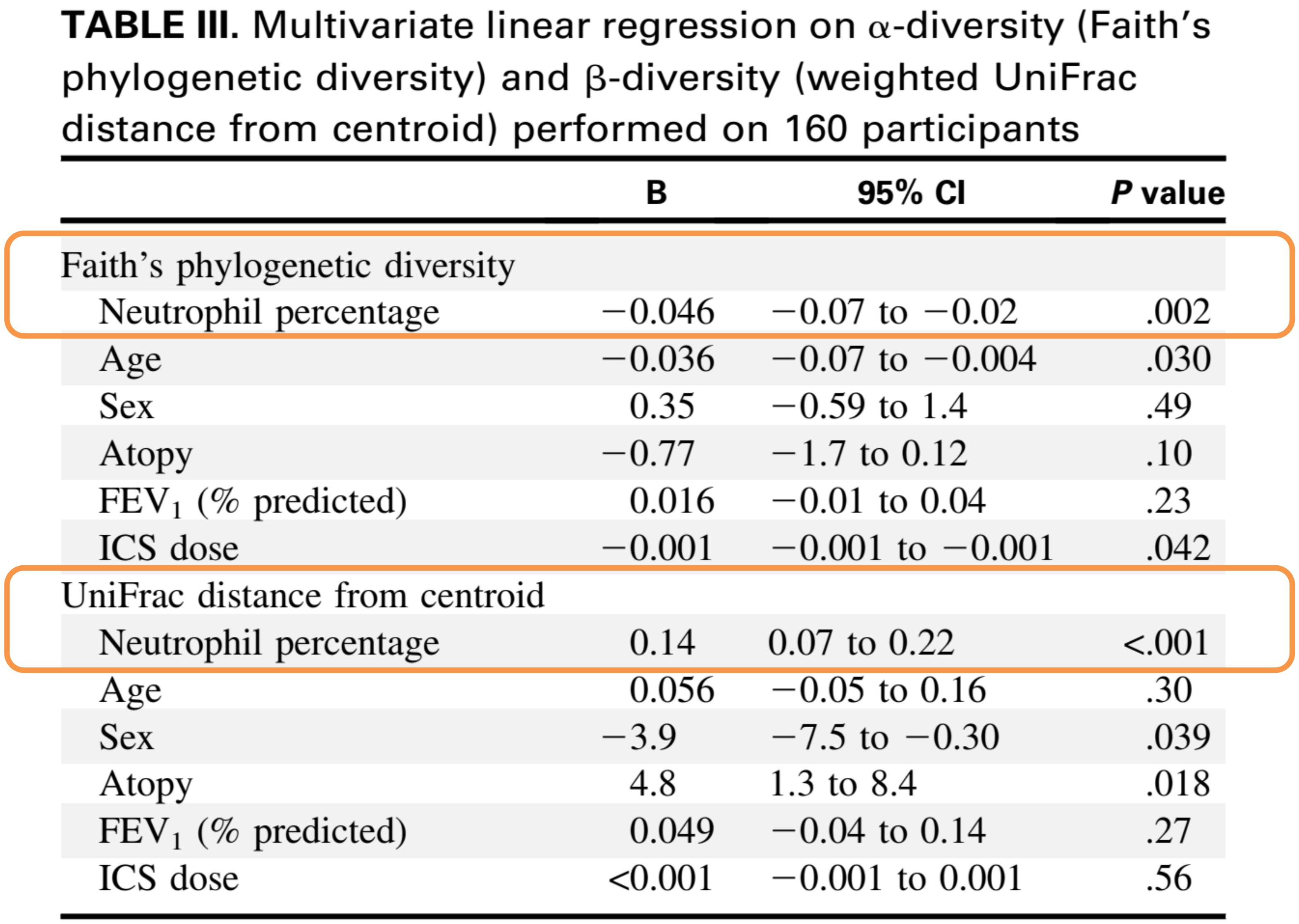


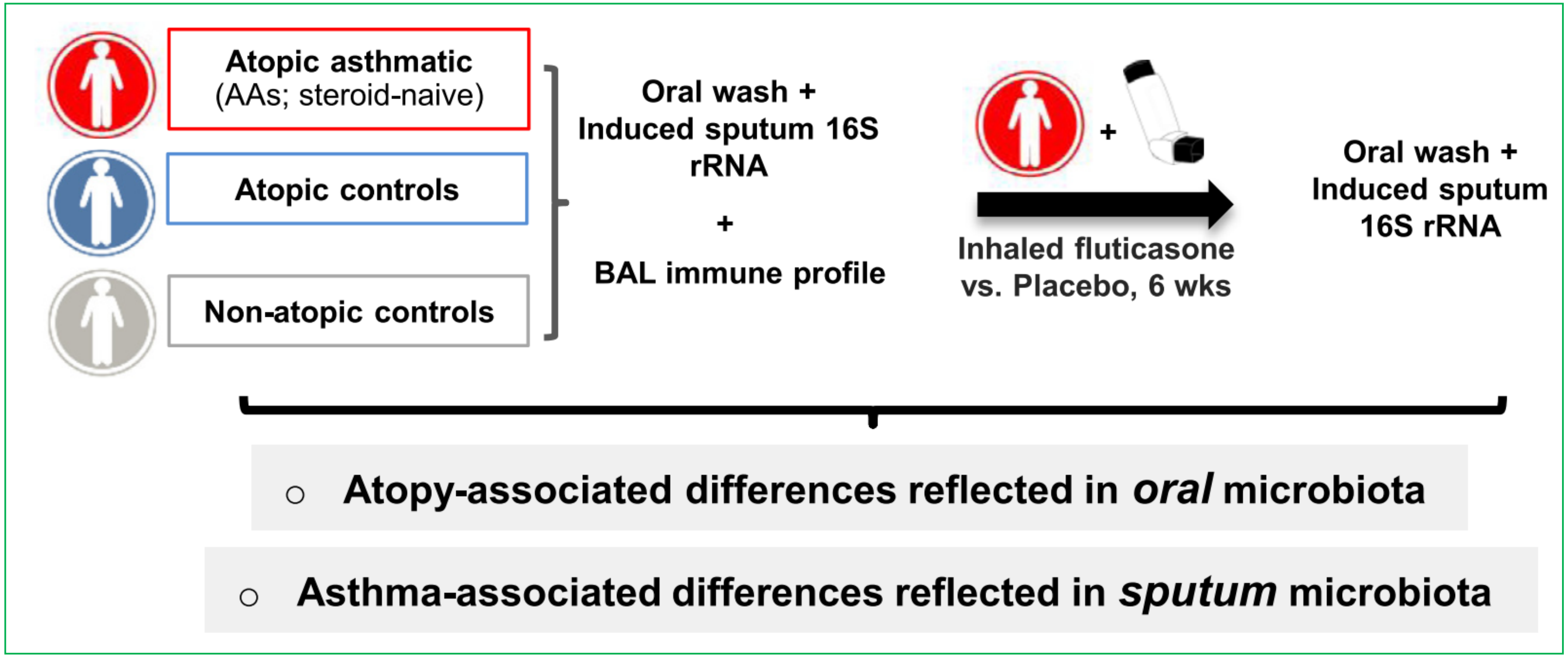
TABLE III. Multivariate linear regression on α-diversity (Faith's phylogenetic diversity) and β-diversity (weighted UniFrac distance from centroid) performed on 160 participants

	B	95% CI	*P* value
Faith's phylogenetic diversity			
Neutrophil percentage	−0.046	−0.07 to −0.02	.002
Age	−0.036	−0.07 to −0.004	.030
Sex	0.35	−0.59 to 1.4	.49
Atopy	−0.77	−1.7 to 0.12	.10
FEV$_1$ (% predicted)	0.016	−0.01 to 0.04	.23
ICS dose	−0.001	−0.001 to −0.001	.042
UniFrac distance from centroid			
Neutrophil percentage	0.14	0.07 to 0.22	<.001
Age	0.056	−0.05 to 0.16	.30
Sex	−3.9	−7.5 to −0.30	.039
Atopy	4.8	1.3 to 8.4	.018
FEV$_1$ (% predicted)	0.049	−0.04 to 0.14	.27
ICS dose	<0.001	−0.001 to 0.001	.56

Tayloer SL, et al. J Allergy Clin Immunol 2018;141(1):94-103.

Microbiome in mild asthma

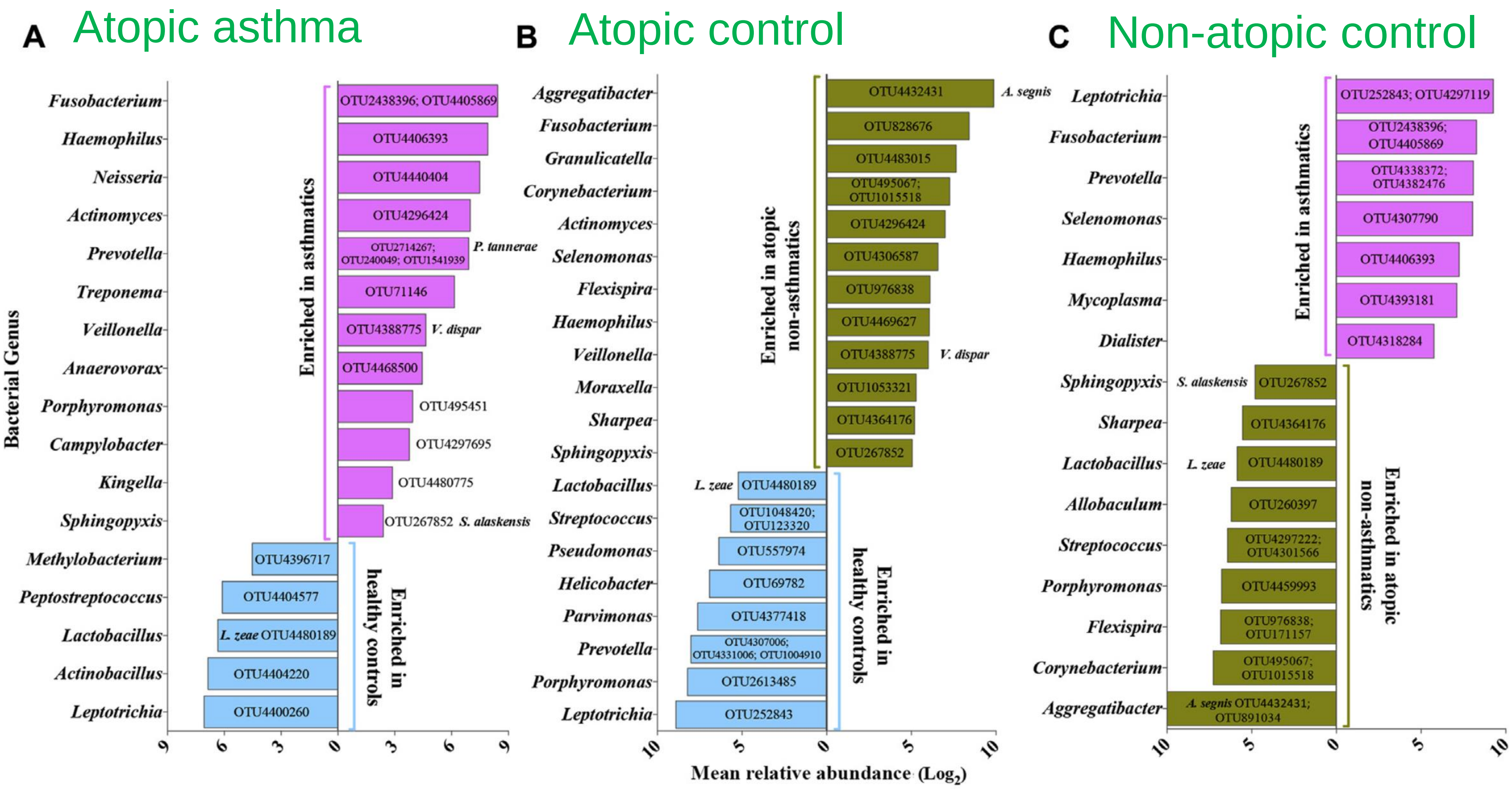
- Atopic asthma adults (AA) vs. atopic control (ANA)/non-atopic control (HC)
  - ✓ Enrolled in clinically stable status for at least 3 months including no use of antibiotics
- **Three-gene mean (TGM) score**: Expression levels of 3 bronchial epithelial genes (CLCA1, SERPINB2, POSTN) induced by IL-13
  - ✓ **T2-high asthma**: TGM score ≥ 1.117



- ○ **Atopy-associated differences reflected in *oral* microbiota**
- ○ **Asthma-associated differences reflected in *sputum* microbiota**

Durack J, et al. J Allergy Clin Immunol 2020;146(5):1016-1026.

Differential features of airway microbiome



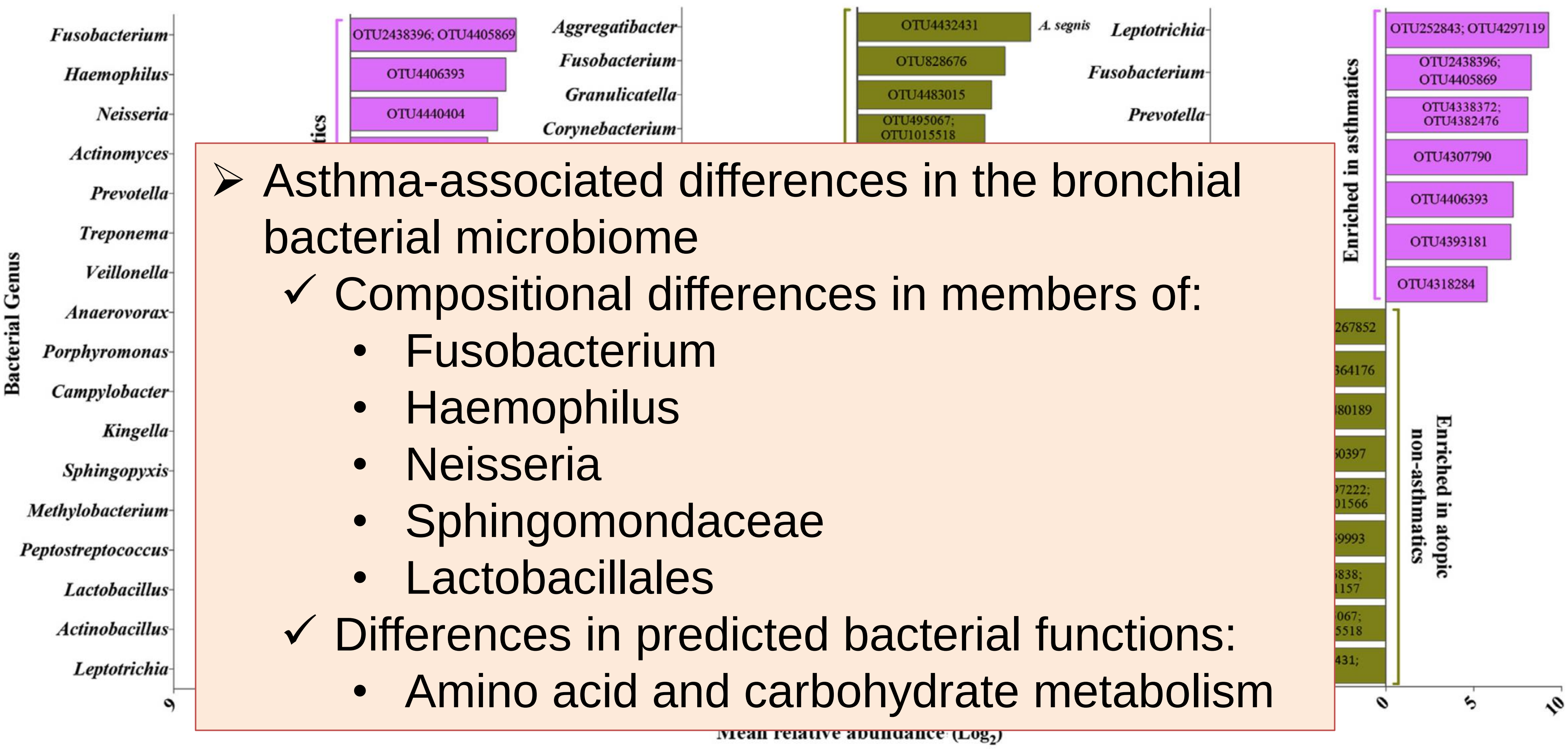
Durack J, et al. J Allergy Clin Immunol 2017;140(1):63-75.

Differential features of airway microbiome

A Atopic asthma

B Atopic control

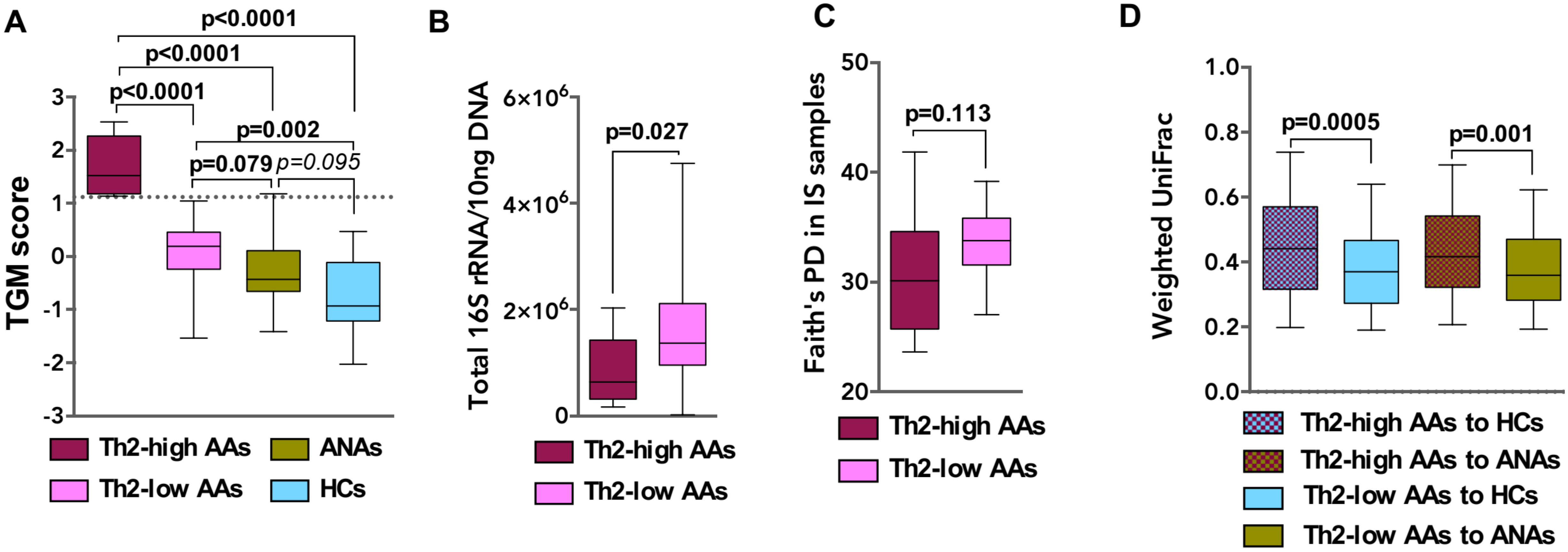
C Non-atopic control



- Asthma-associated differences in the bronchial bacterial microbiome
  - ✓ Compositional differences in members of:
    - Fusobacterium
    - Haemophilus
    - Neisseria
    - Sphingomondaceae
    - Lactobacillales
  - ✓ Differences in predicted bacterial functions:
    - Amino acid and carbohydrate metabolism

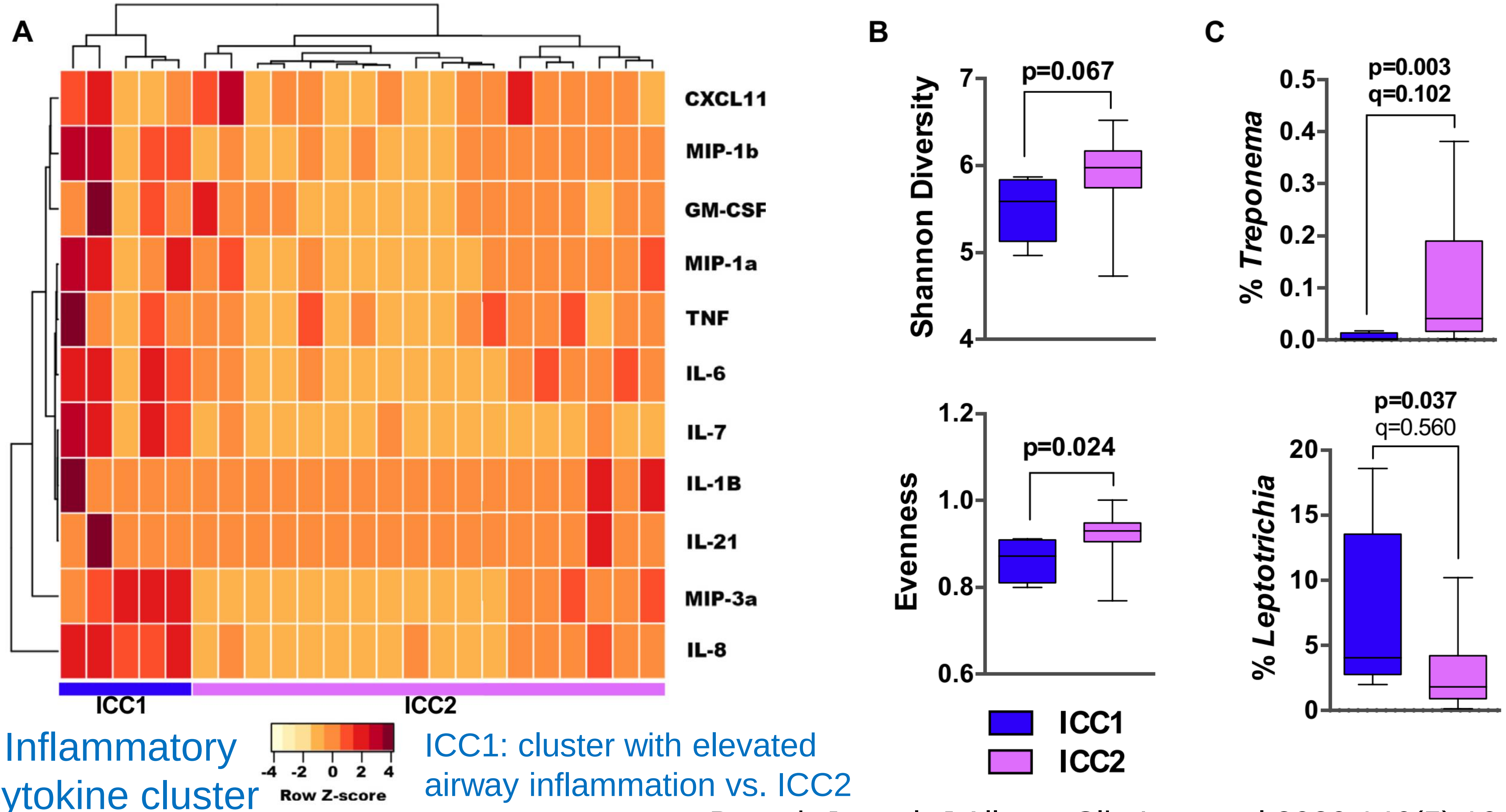
Durack J, et al. J Allergy Clin Immunol 2017;140(1):63-75.

Microbiome and phenotype in mild asthma



Durack J, et al. J Allergy Clin Immunol 2020;146(5):1016-1026.

Inflammatory cytokines among T2-low asthma

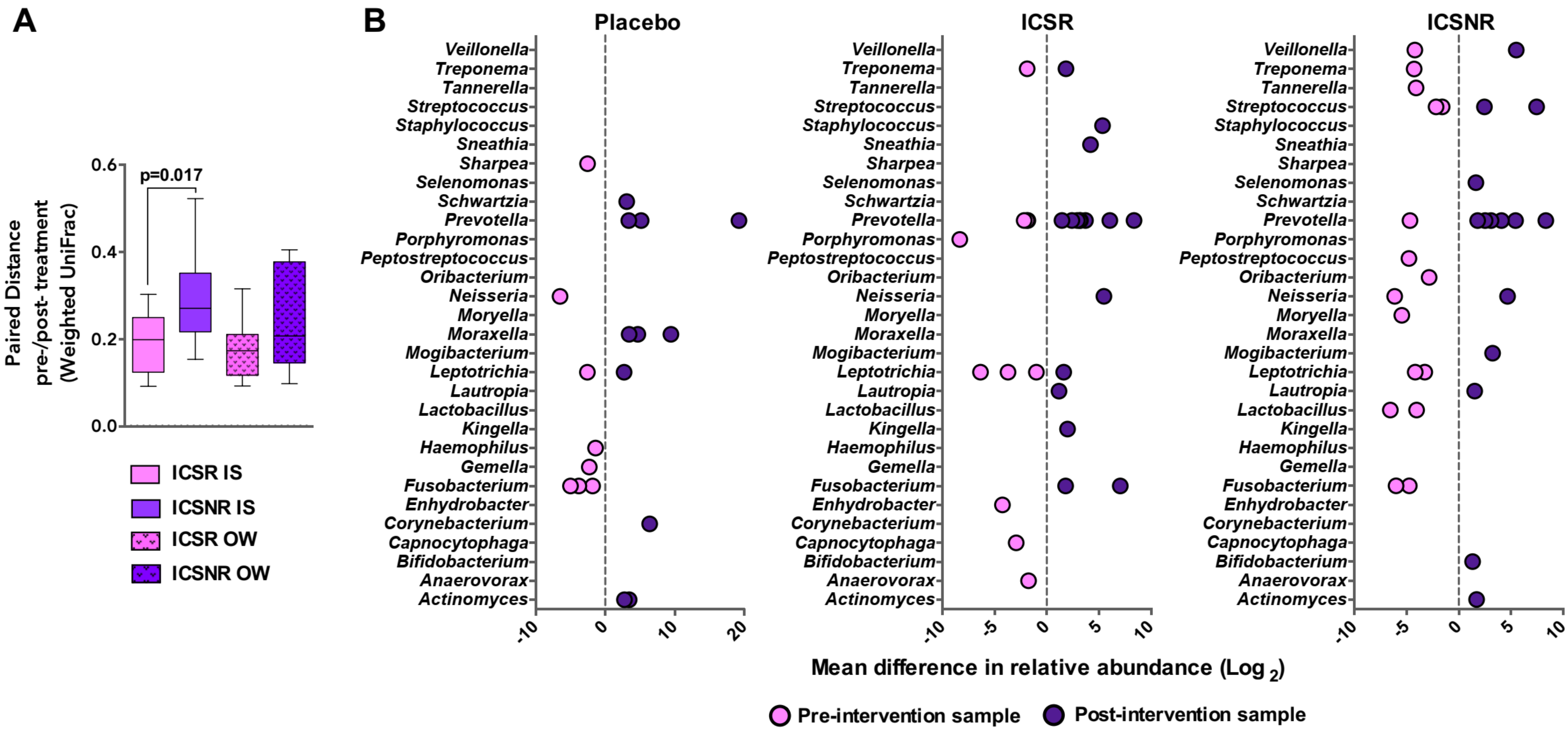


Inflammatory cytokine cluster

ICC1: cluster with elevated airway inflammation vs. ICC2

Durack J, et al. J Allergy Clin Immunol 2020;146(5):1016-1026.

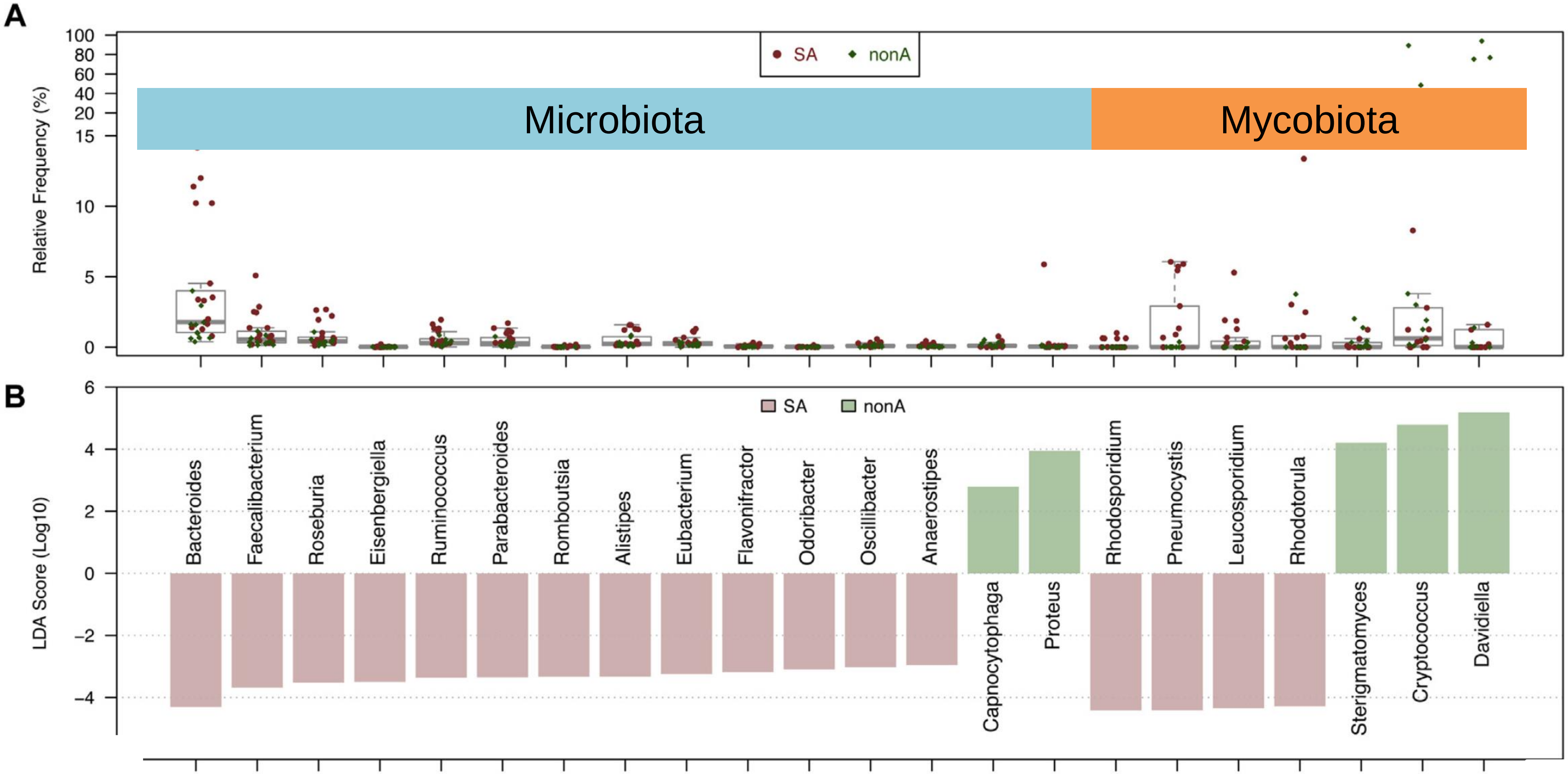
ICS response and change of sputum microbiome



Durack J, et al. J Allergy Clin Immunol 2020;146(5):1016-1026.

Lower airway microbiota/mycobiota in children with severe asthma

- BAL pellets from children who underwent bronchoscopy over a 20-month period

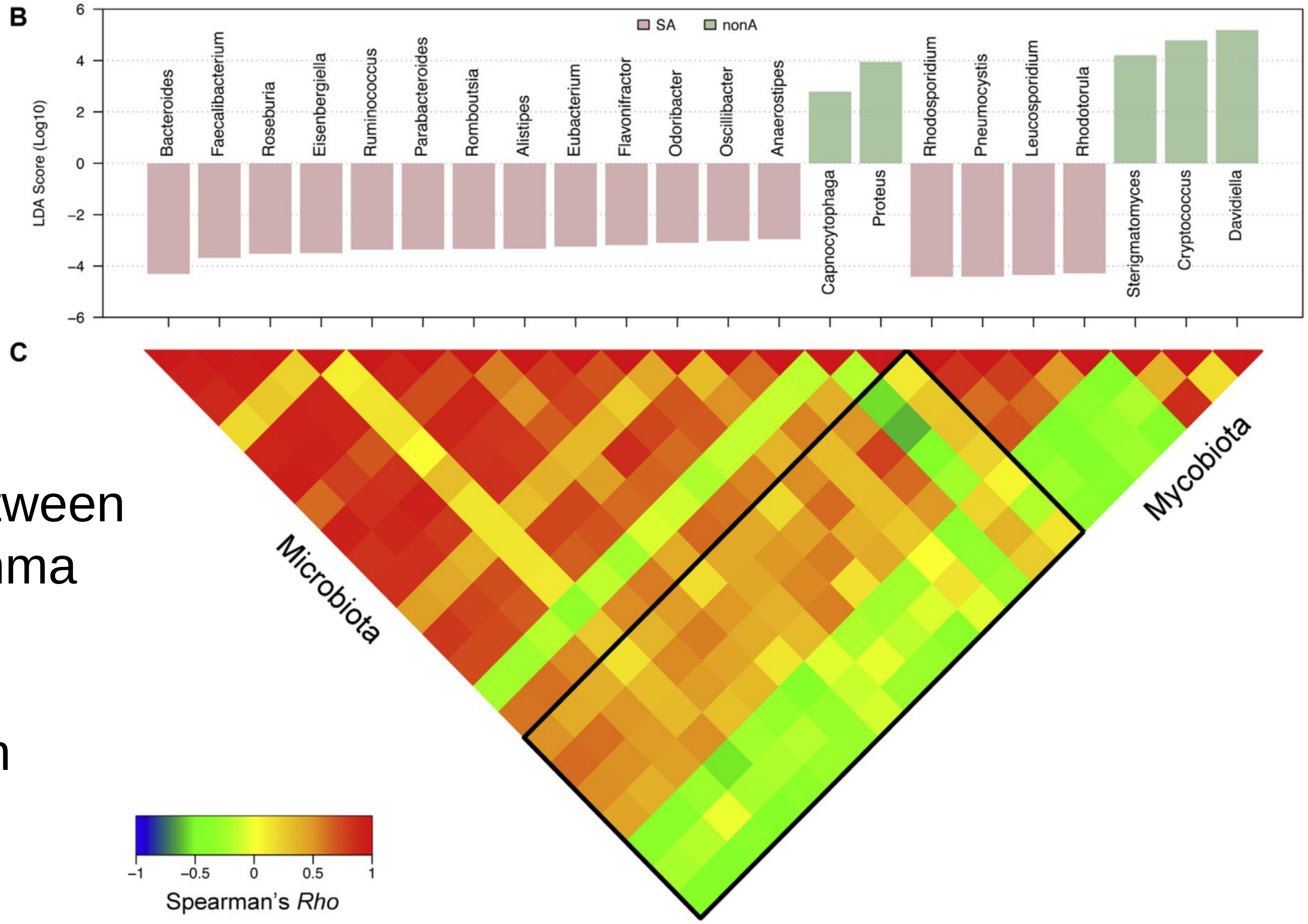


Goldman DL, et al. J Allergy Clin Immunol 2018;141(2):808-811.

Lower airway mycobiota in children with severe asthma

- Bacterial and fungal genera differentially represented between severe asthma and non-asthma cohorts

- Pairwise correlation between bacterial and fungal genera

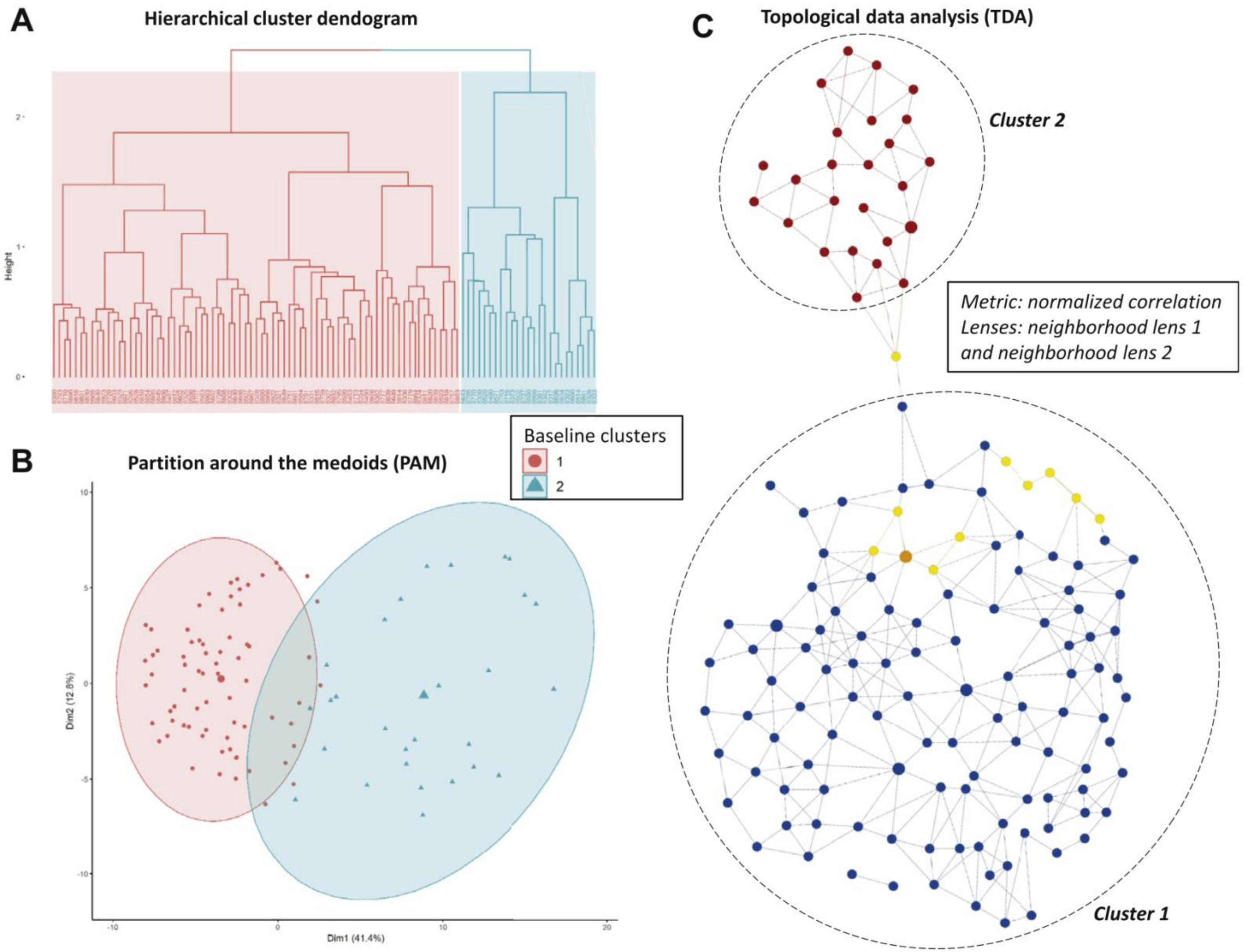


Goldman DL, et al. J Allergy Clin Immunol 2018;141(2):808-811.

Sputum microbiome profile of severe asthma

- ➢ Prospective asthma cohort with 3 subcohorts (U-BIOPRED cohort)
    - ✓ Nonsmoking patients with severe asthma
    - ✓ Previously or currently smoking patients with severe asthma
    - ✓ Nonsmoking patients with mild-to-moderate asthma

- ➢ Induced sputum for microbial profiles at baseline and after 12~18 months



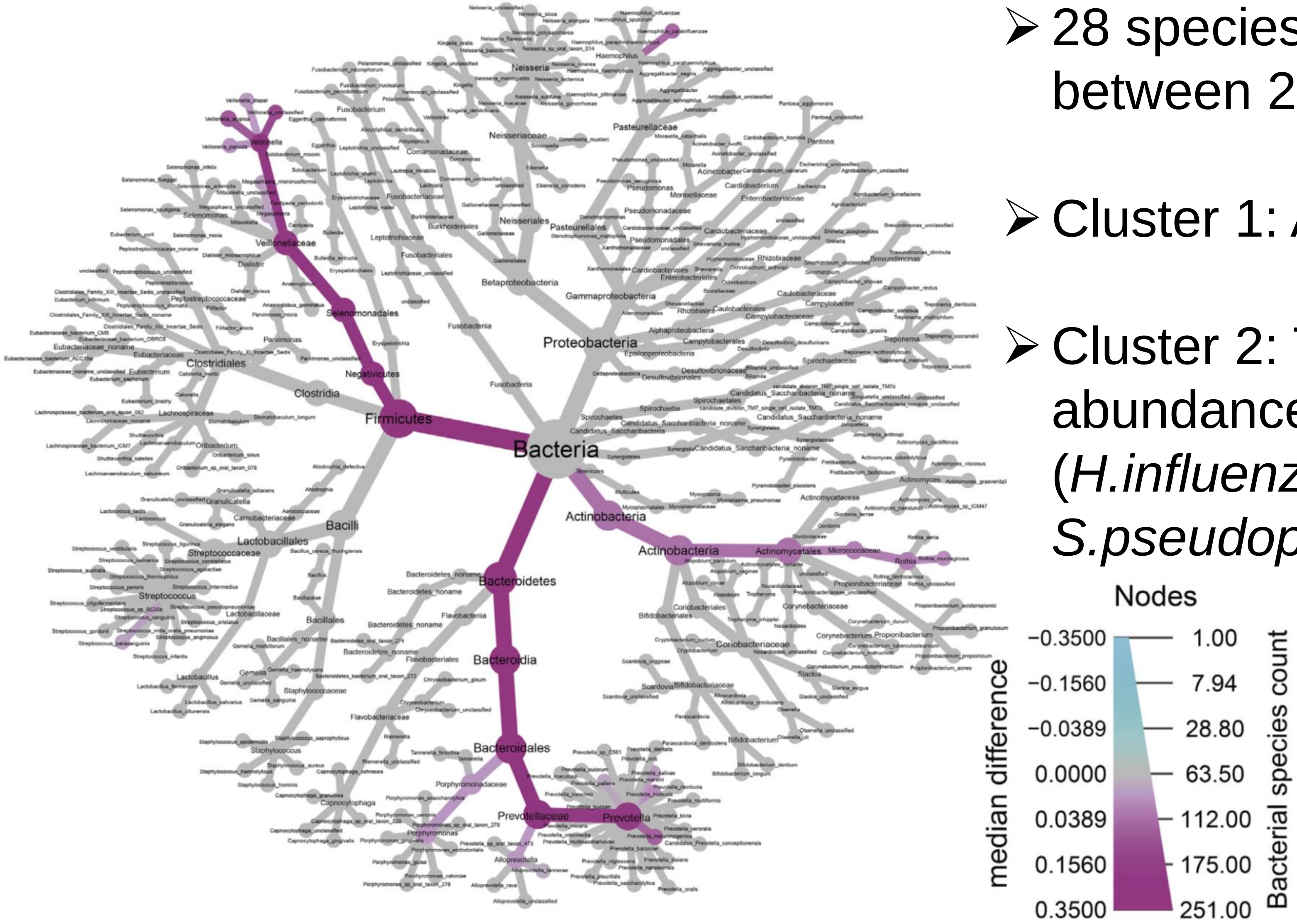
Abdel-Aziz MI, et al. J Allergy Clin Immunol 2021;147(1):123-134.

Microbiome-driven clusters for severe asthma

	Baseline		
Characteristics	**Cluster 1 (n=75)**	**Cluster 2 (n=25)**	**P-value**
Median age of onset, y (IQR)	30.50 (14.00-48.25)	16.00 (5.00-33.50)	0.012
Nonsmoker, n (%)	45 (60.0%)	21 (84.0%)	0.028
Residential location, n (%)			0.009
Rural	21 (28.0%)	5 (20.0%)	
Suburban	13 (17.3%)	12 (48.0%)	
Urban	41 (54.7%)	8 (32.0%)	
Sputum neutrophil % (IQR)	53.40 (32.40-70.74)	86.90 (57.32-92.73)	<0.001
PostBD median FEV1 % predicted (IQR)	74.88 (61.52-86.94)	51.44 (42.90-87.62)	0.009
PostBD median FEV1/FVC % predicted (IQR)	79.25 (67.02-89.11)	68.19 (52.31-83.86)	0.012

Abdel-Aziz MI, et al. J Allergy Clin Immunol 2021;147(1):123-134.

Microbial profiles of different clusters



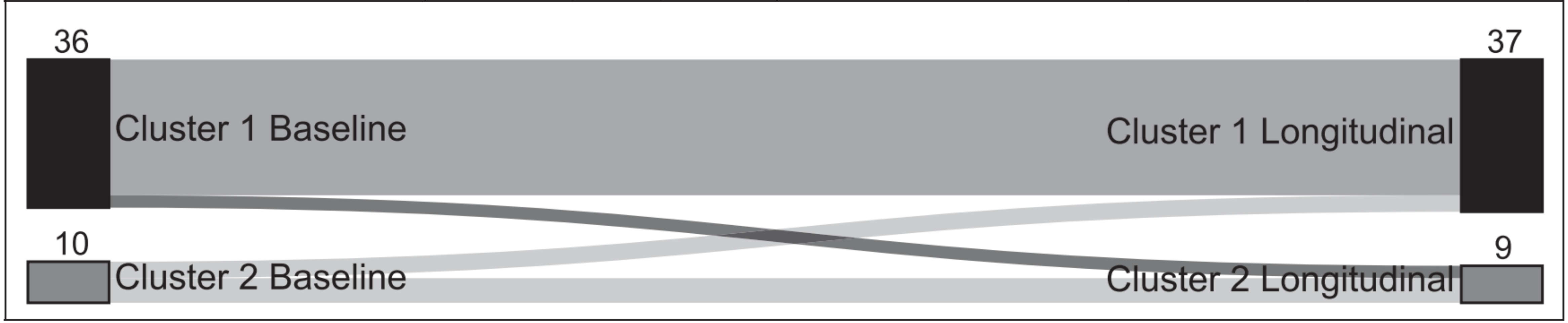
- ➢ 28 species remained significantly different between 2 clusters
- ➢ Cluster 1: All of them were more abundant
- ➢ Cluster 2: Trend toward increased relative abundance of a few pathogenic species (*H.influenzae, M.catarrhalis, S.pseudopneumoniae*)

Abdel-Aziz MI, et al. J Allergy Clin Immunol 2021;147(1):123-134.

Stability of longitudinal clusters

- Longitudinal clusters revealed high relative stability after 12 to 18 months in those with severe asthma
  - ✓ 84.7% remained cluster stable longitudinally

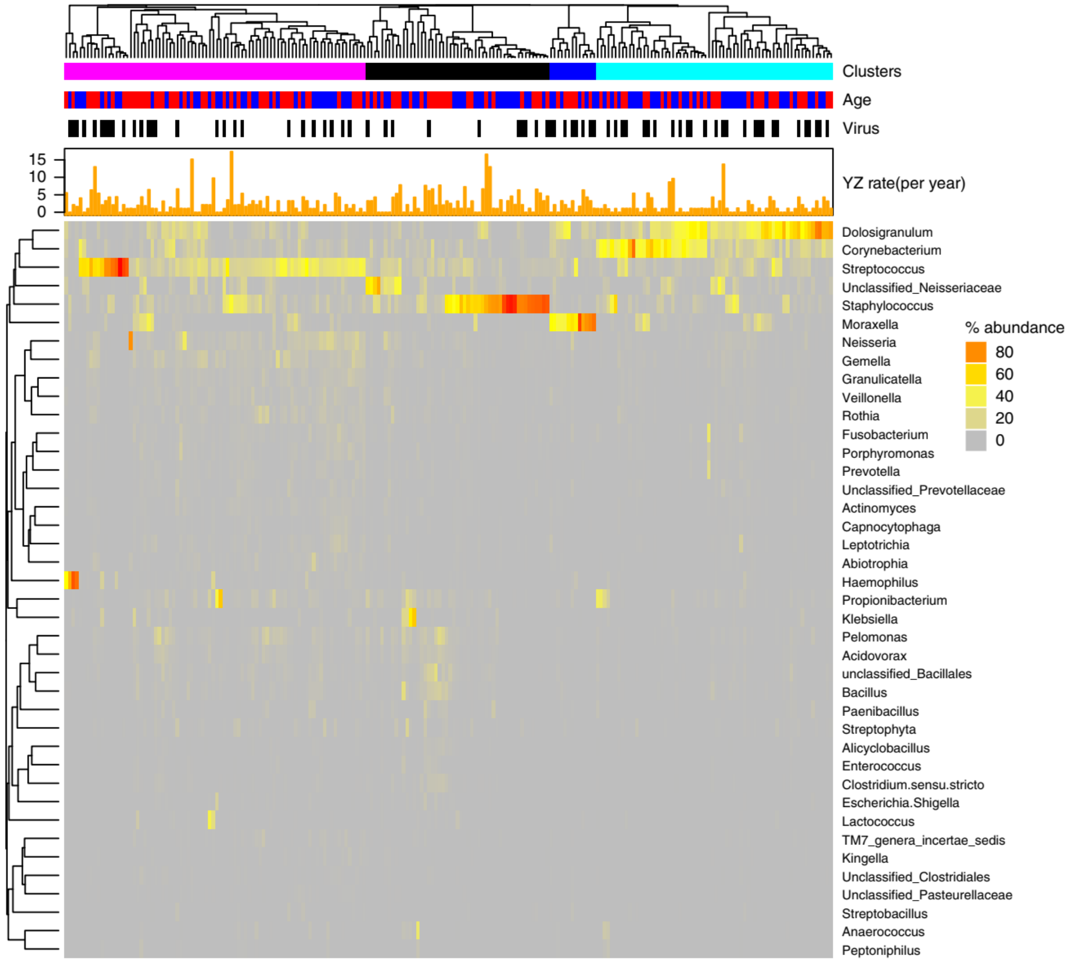
	Cluster 1 Baseline	Cluster 2 Baseline	p-value	Rand index
Cluster 1 Longitudinal	**33 (91.7%)**	4 (40%)	.0014	0.736
Cluster 2 Longitudinal	3 (8.3%)	**6 (60%)**		



Abdel-Aziz MI, et al. J Allergy Clin Immunol 2021;147(1):123-134.

Microbiome and asthma exacerbation

- School-age children with mild-to-moderate persistent asthma treated with ICS
- Nasal blow samples for upper airway microbiota
- **Yellow zone**: early signs of loss of asthma control



Baseline airway microbiota clusters

Clusters

🟦 Corynebacterium+Dolosigranulum

⬛ Staphylococcus

🟪 Streptococcus

🟦 Moraxella

Age

🟥 5–7 years

🟦 8–11 years

Virus

Negative

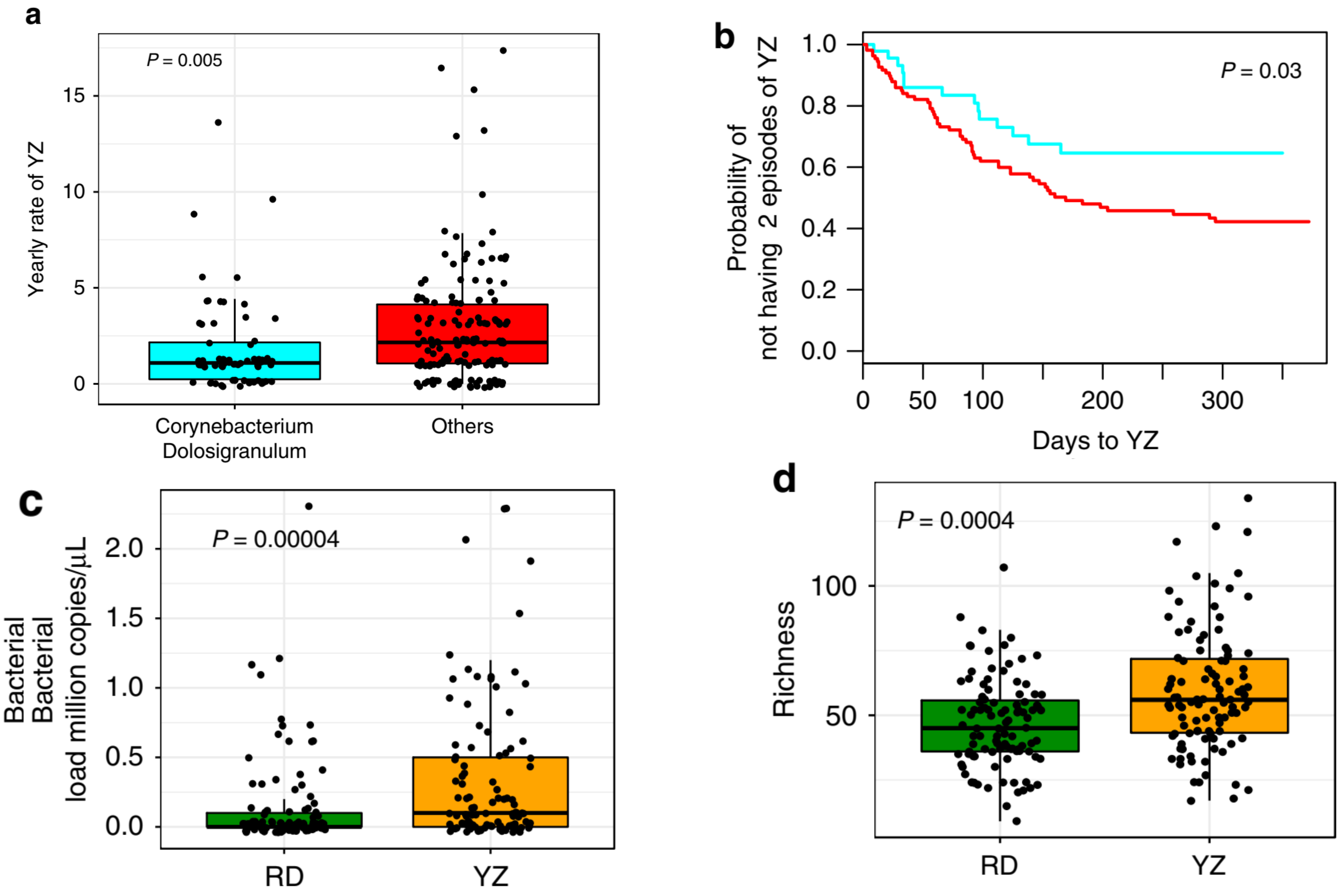
⬛ Positive

Zhou Y, et al. Nat Commun 2019;10(1):5714.

Microbiome clutering and exacerbation

Yearly rate of exacerbation in respiratory clusters at baseline

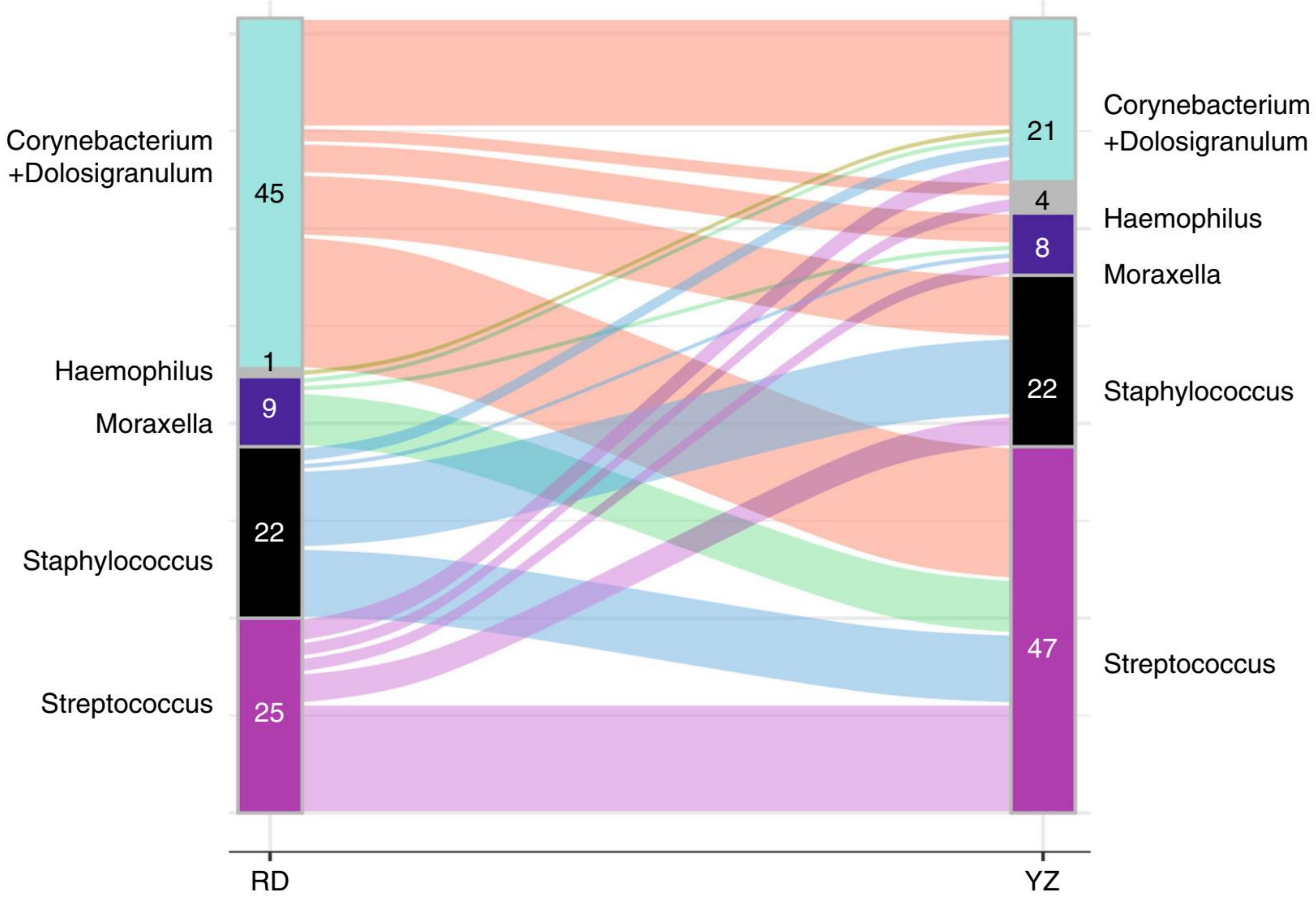
Bacterial microbiome comparison between baseline and exacerbation



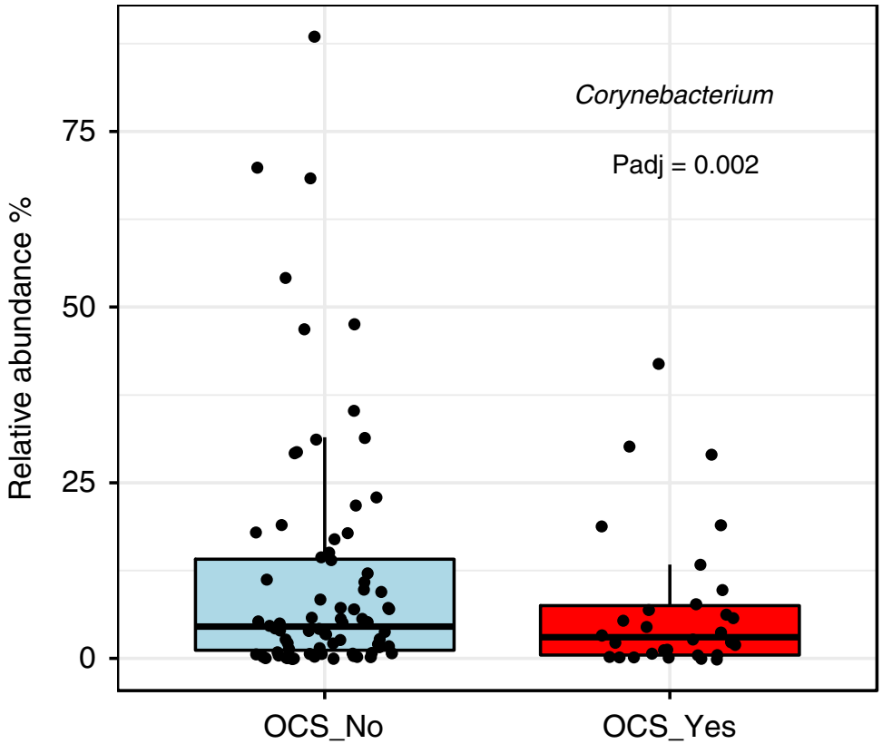
Zhou Y, et al. Nat Commun 2019;10(1):5714.

Change of microbiome and asthma exacerbation

Changes in bacterial clusters from baseline to exacerbation

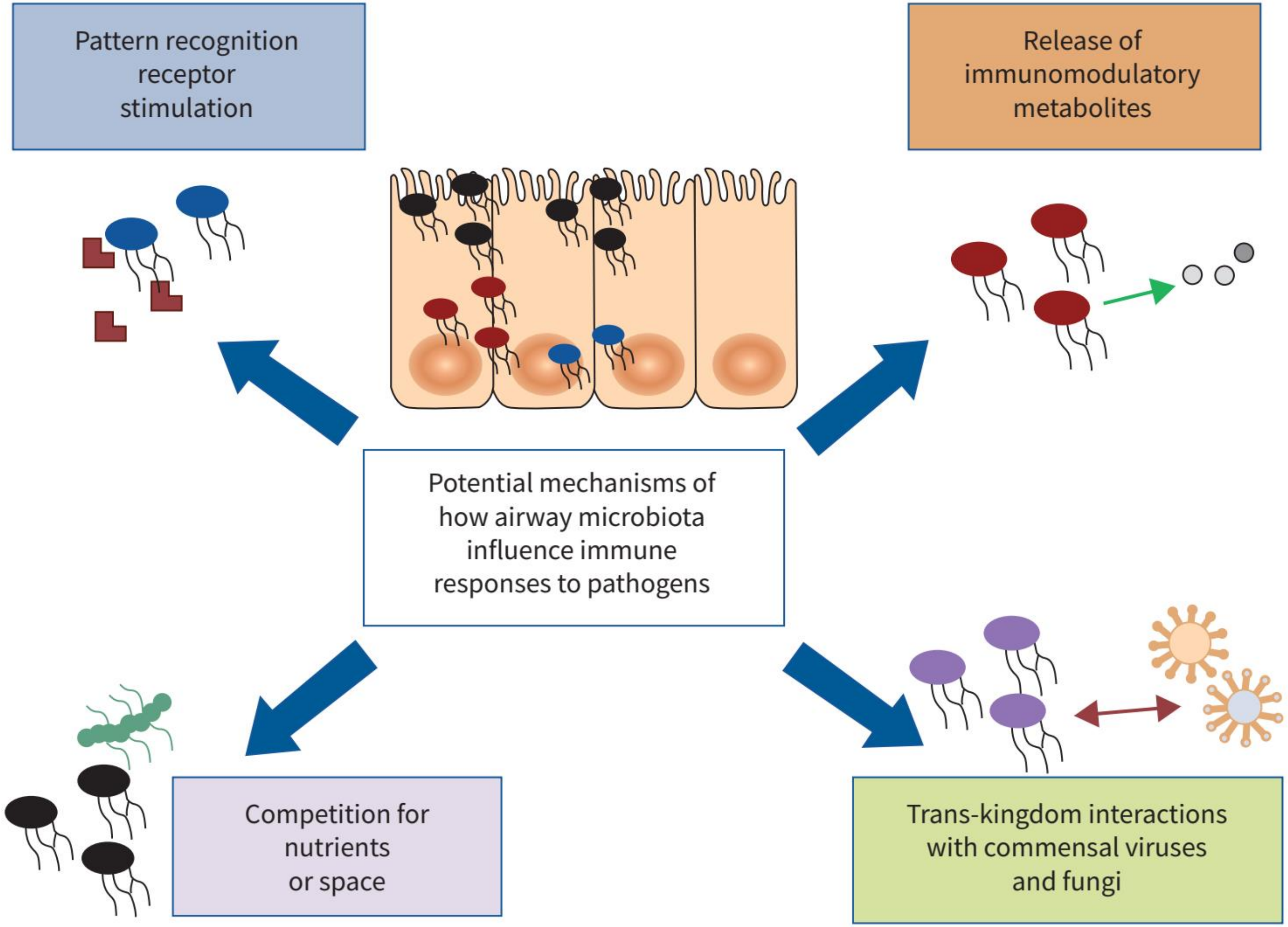


Corynebacterium's relative abundance at exacerbation
: Inverse association with severe exacerbation



Zhou Y, et al. Nat Commun 2019;10(1):5714.

Potential molecular mechanisms of airway microbiota regulation of immune responses to pathogen



Pattern recognition receptor stimulation

Release of immunomodulatory metabolites

Potential mechanisms of how airway microbiota influence immune responses to pathogens

Competition for nutrients or space

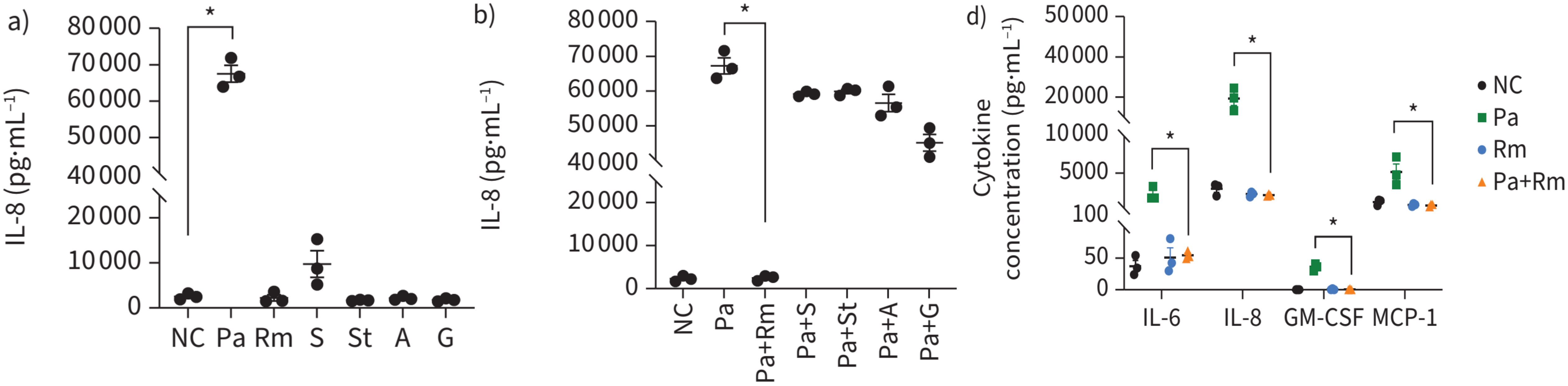
Trans-kingdom interactions with commensal viruses and fungi

Morton R, et al. Eur Respir 2022;59(5):2103079.

Rothia mucilaginosa **as anti-inflammatory bacterium**

- *Rothia mucilaginosa*
  - ✓ Gram-positive cocccus, common commensal of the oral cavity
  - ✓ Frequently detected in the lungs of patients of chronic lung disease (cystic fibrosis, asthma, COPD)

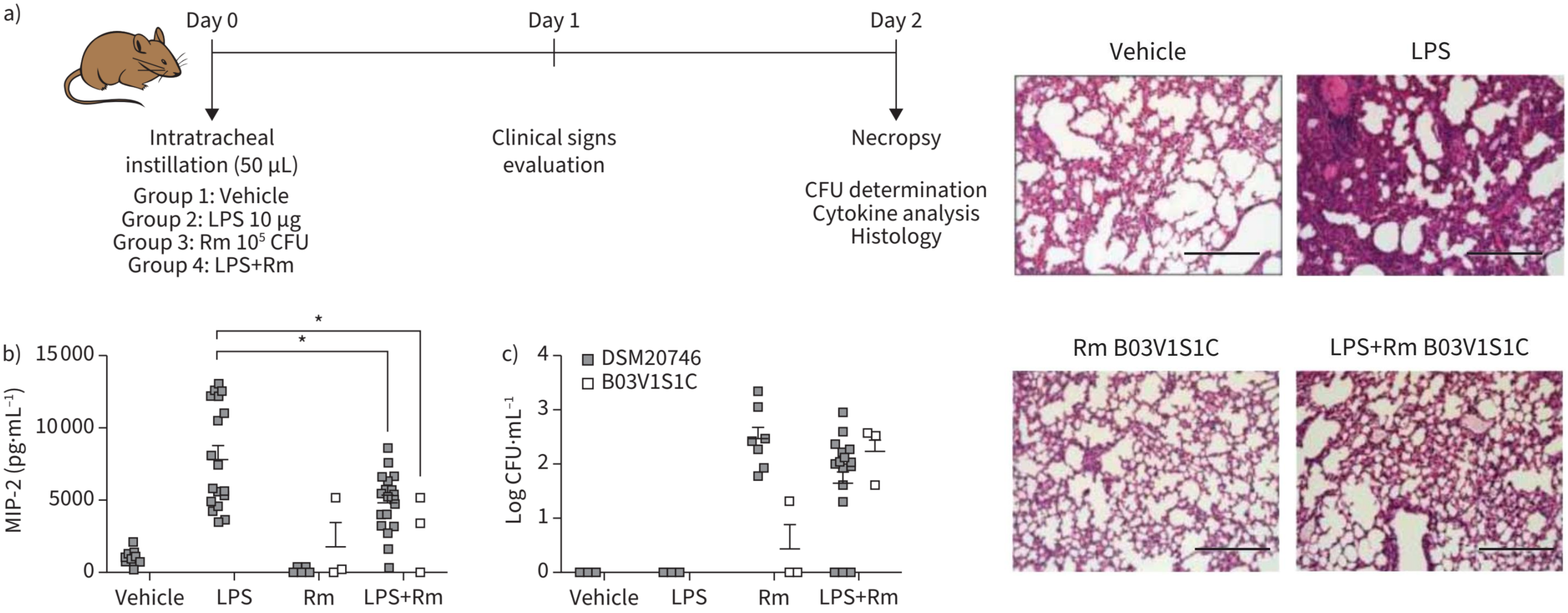
Alveloar epithelial model to *P.aeruginosa*-induced inflammation



Pa, Pseudomonas aeruginosa PAO1; **Rm**, Rothia mucilaginosa DSM20746;
S, Staphylococcus aureus SP123; **St**, Streptococcus anginosus LMG14696;
A, Achromobacter xylosoxidans LMG26680; **G**, Gemella haemolysans LMG18984

Rigauts C, et al. Eur Respir 2022;59(5):2101293.

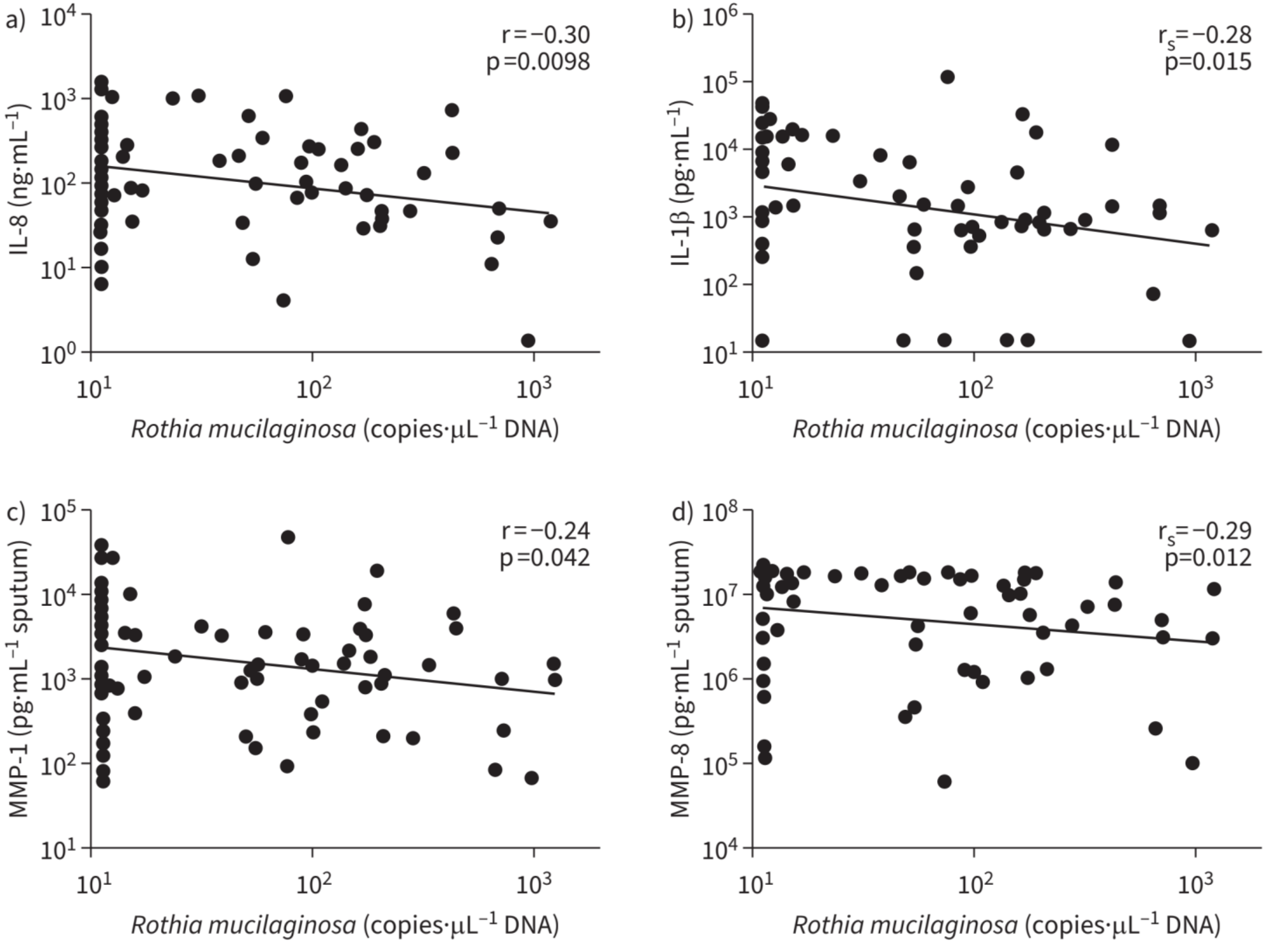
Anti-inflammatory effect in vivo model



Rigauts C, et al. Eur Respir 2022;59(5):2101293.

Rothia species in patients with neutrophilic airway disease

- Correlation of absolute load of *Rothia mucilaginosa* with pro-inflammatory parameters in induced sputum samples from bronchiectasis patients



Rigauts C, et al. Eur Respir 2022;59(5):2101293.

Summary

- **Gut/airway microbiome in asthma**
    - Pathogenesis
    - Phenotype/ICS response
    - Severity
    - Disease control/exacerbation
    - Potential treatment method

Thank You for Your Attention