대한 결핵 및 호흡기학회 제123차 춘계학술대회

# Microbiome in Airway Diseases

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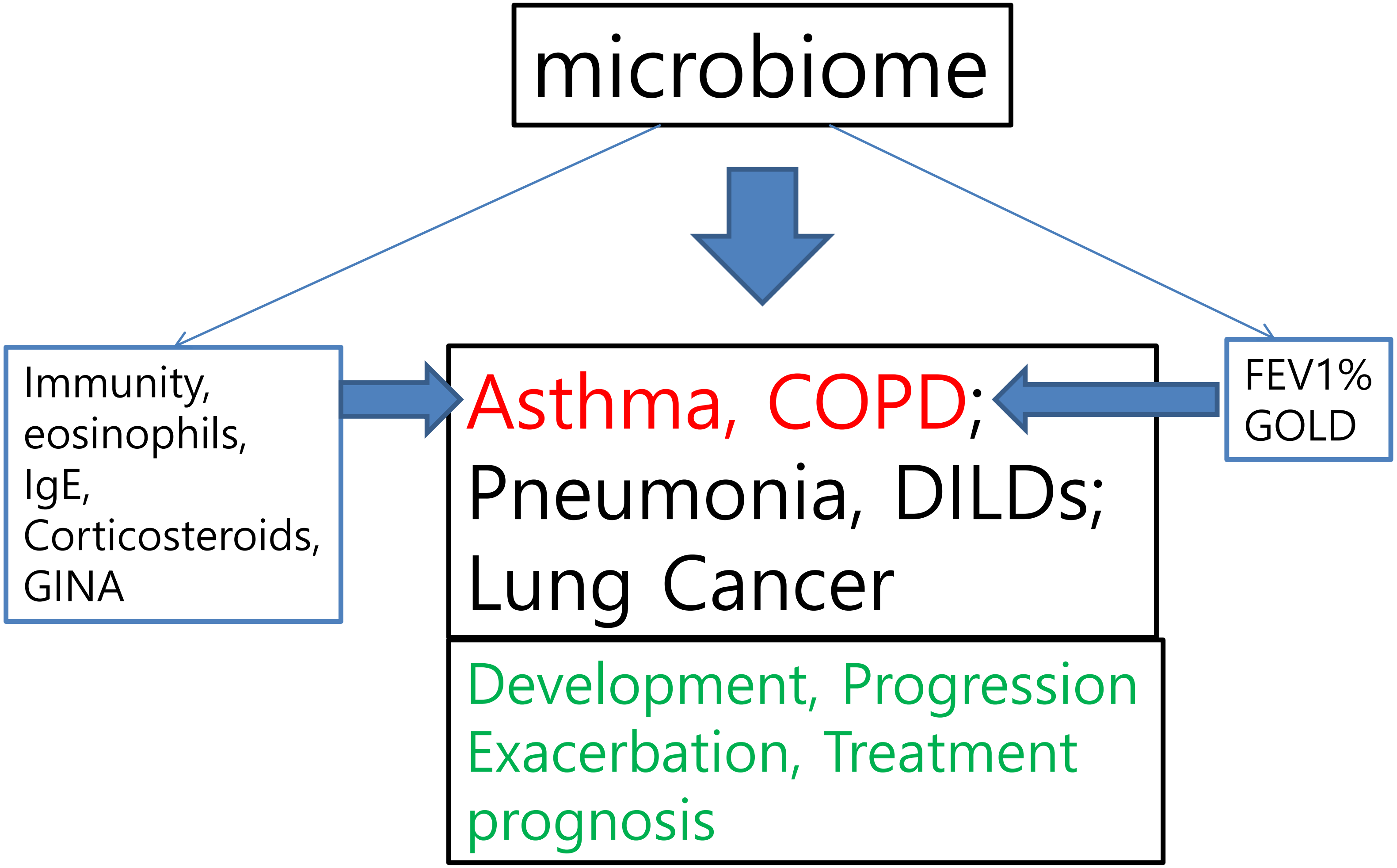
The Korean Academy of
Tuberculosis and Respiratory Diseases

# Clinical Respiratory Microbiome



microbiome

Immunity,
eosinophils,
IgE,
Corticosteroids,
GINA

Asthma, COPD;
Pneumonia, DILDs;
Lung Cancer

FEV1%
GOLD

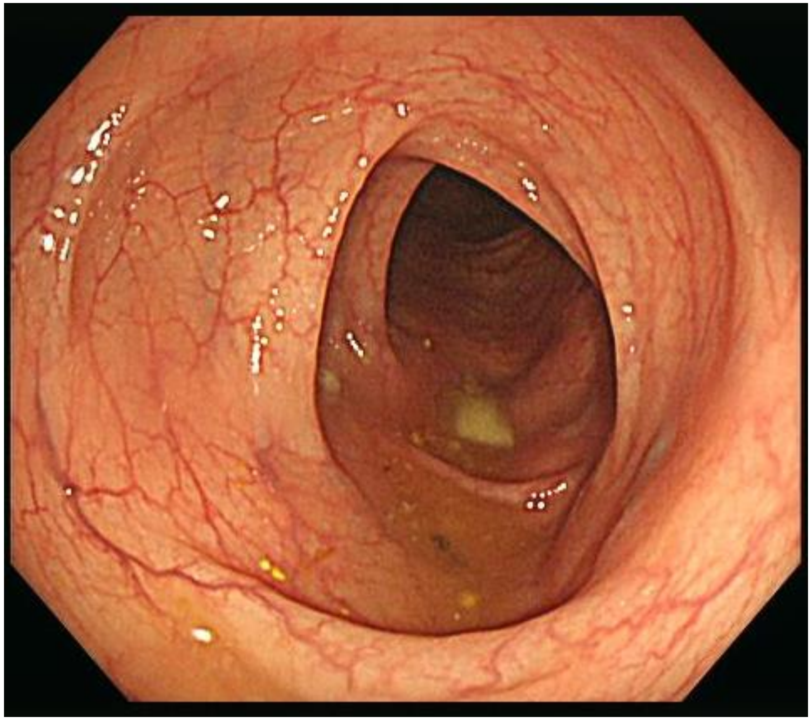
Development, Progression
Exacerbation, Treatment
prognosis

# Key Story

1. Next generation – sequencing and statistical bioinformatics
2. Modeling
3. Asthma, COPD, Pneumonia, IPF, Cancer
4. Sociomicrobiology – quorum sensing and biofilming
5. Integration

# Question 1: What makes the airways sterile?
# Question 2: What makes the concept of sterility in airways?

bronchi



colon

Independent Microbiome

GUT-LUNG AXIS

# General Start-Up

- **Who is there? [Metagenomic Profiling]**
  - Taxonomy diversity
  - Bacteriome, Virome, Mycobiome

- **What are they doing? [Functional Metagenomics]**
  - Functional diversity
  - Community stability: resistance vs. resilience
  - Drivers and passengers

# Q & A in Lungs

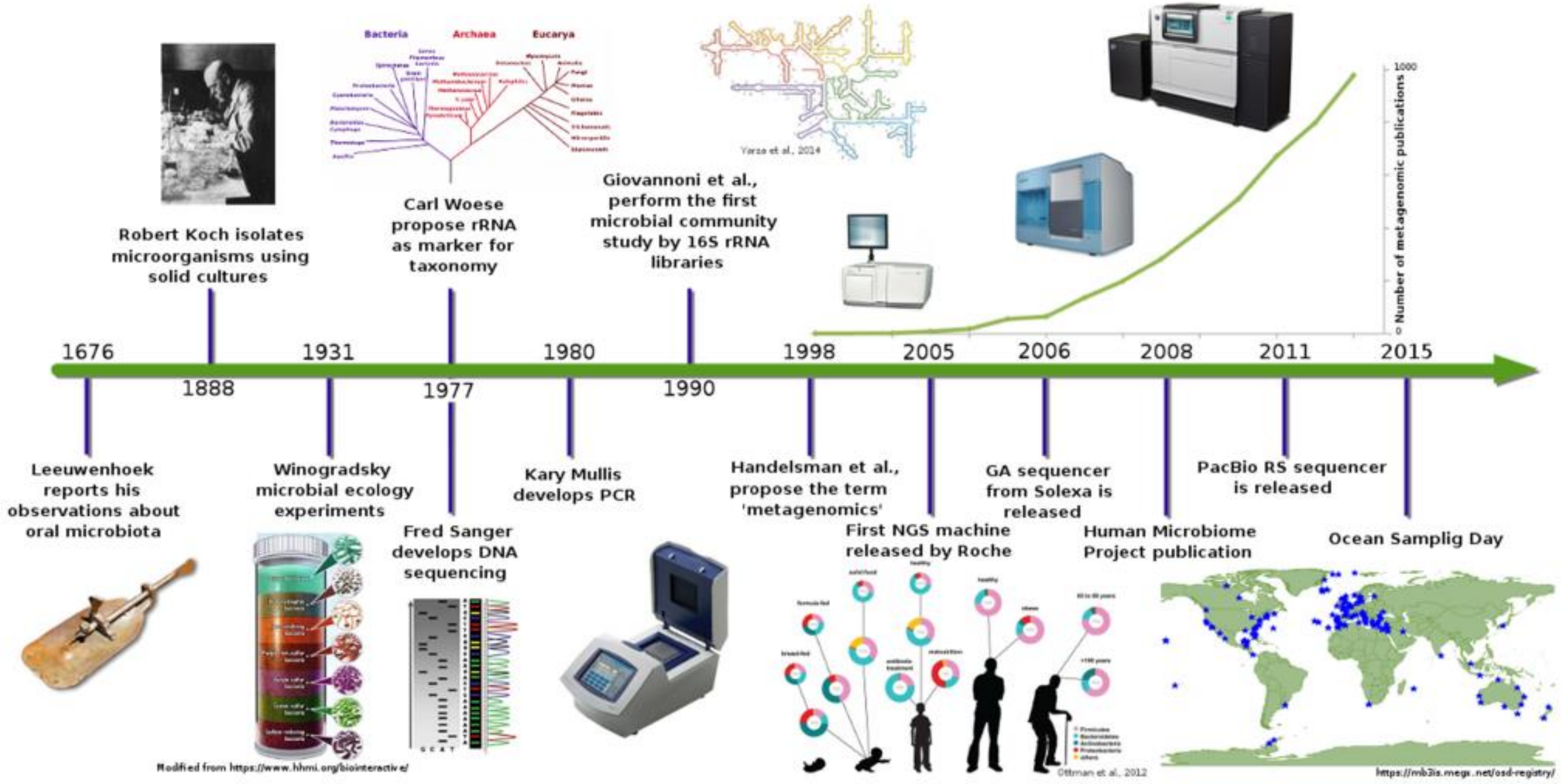
- Spatial and temporal heterogeneity
- Diversity and severity of illness
- Identification of novel pathogens
- Pulmotypes (vs. enterotypes)

- **How are/do lung microbial communities influenced by/influence asthma, COPD, Pneumonia, IPF, Cancer, bronchiectasis?**

- **Would it be the microbiome biomarkers or therapeutic targets?**

  **(Prebiotics, Probiotics)**

# Metagenomics timeline and milestones



1676 Leeuwenhoek reports his observations about oral microbiota

1888 Robert Koch isolates microorganisms using solid cultures

1931 Winogradsky microbial ecology experiments

1977 Carl Woese propose rRNA as marker for taxonomy

1977 Fred Sanger develops DNA sequencing

1980 Kary Mullis develops PCR

1990 Giovannoni et al., perform the first microbial community study by 16S rRNA libraries

1998 Handelsman et al., propose the term 'metagenomics'

2005 First NGS machine released by Roche

2006 GA sequencer from Solexa is released

2008 Human Microbiome Project publication

2011 PacBio RS sequencer is released

2015 Ocean Samplig Day

Modified from https://www.hhmi.org/biointeractive/

Yarza et al., 2014

Ottman et al., 2012

https://mb3is.megx.net/osd-registry/

Frontiers in Genetics. 2015;6:article 348

# PubMed results by year for "microbiome" and "lung-microbiome"

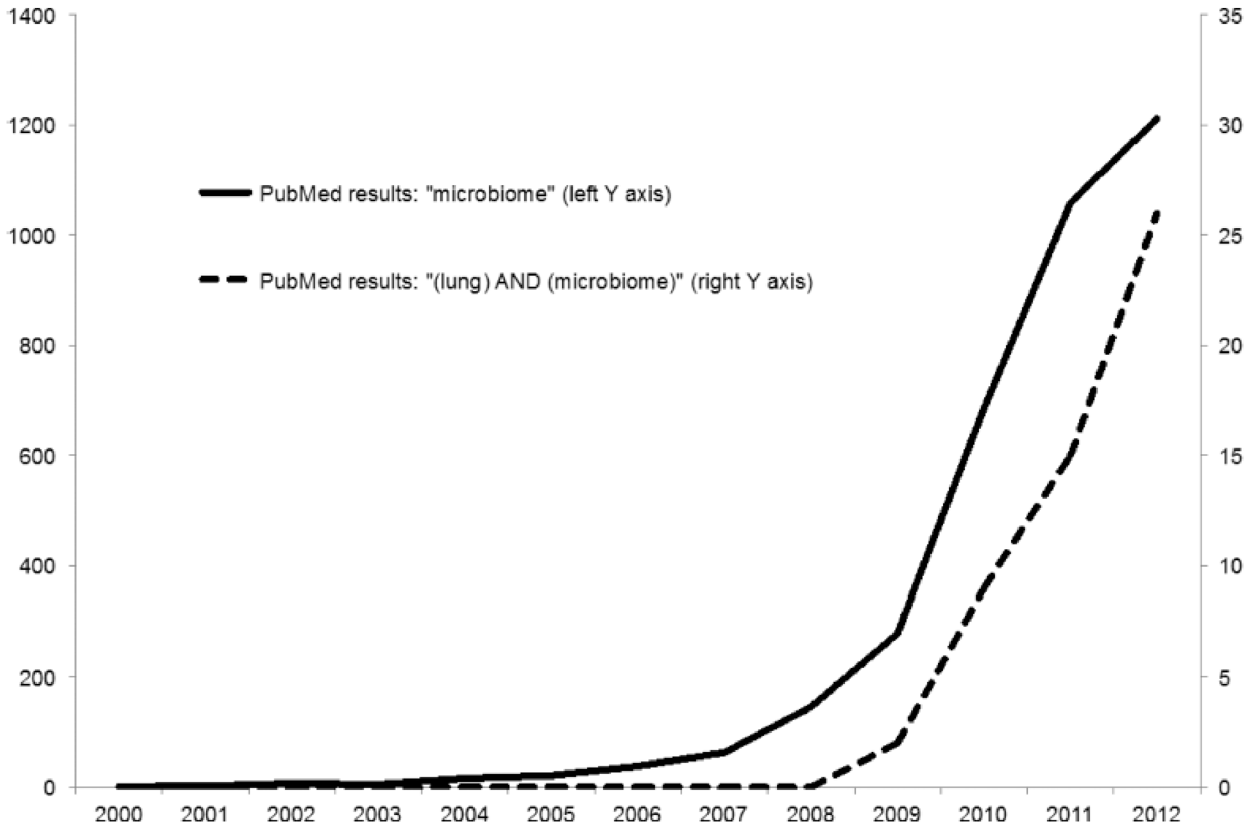
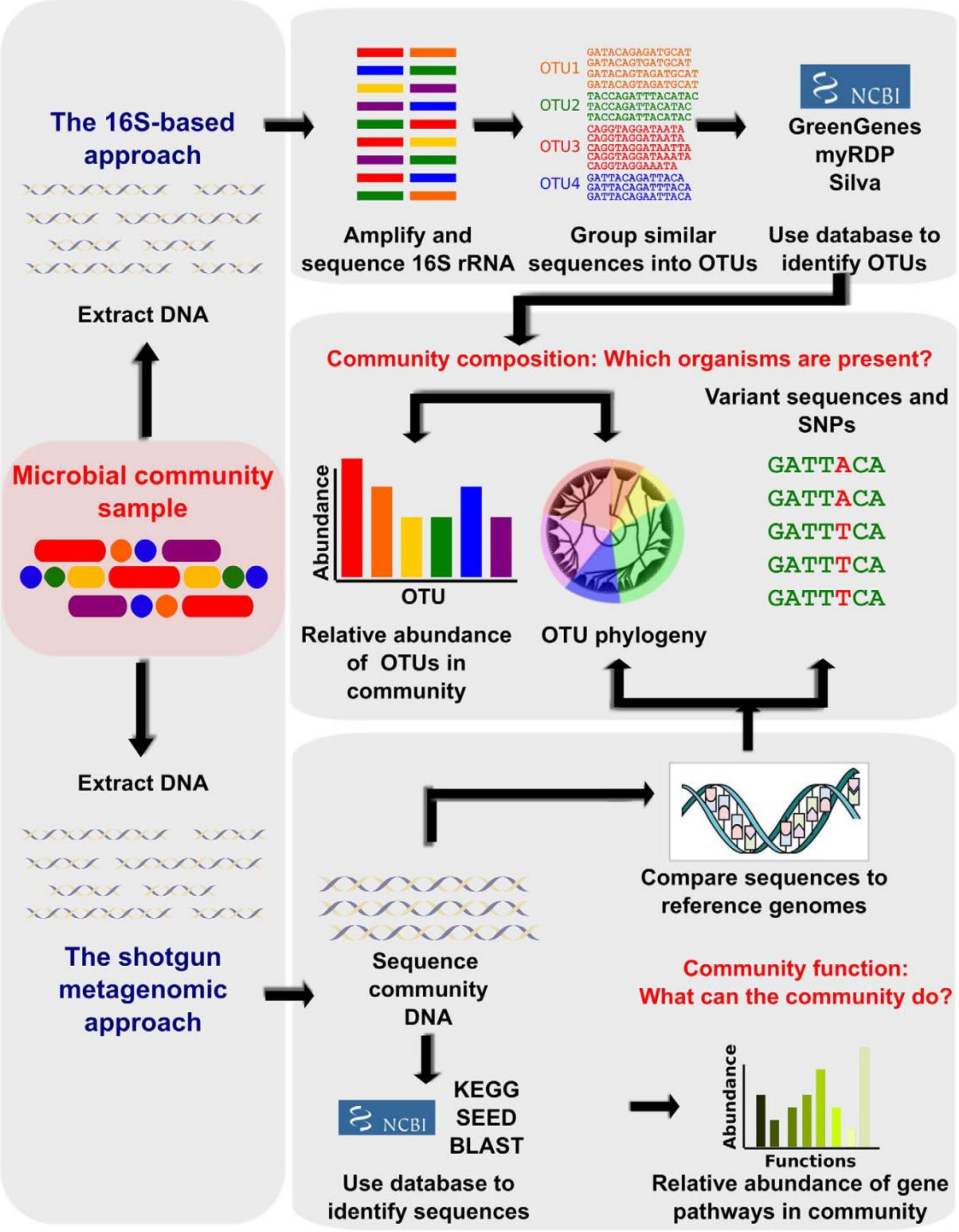


**Figure 1.**

PubMed results by year for "microbiome" (left Y axis) and "(lung) AND (microbiome)" (right Y axis)



**The 16S-based approach**

Extract DNA

**Microbial community sample**

Extract DNA

**The shotgun metagenomic approach**

Amplify and sequence 16S rRNA

Group similar sequences into OTUs

GreenGenes
myRDP
Silva

Use database to identify OTUs

**Community composition: Which organisms are present?**

Relative abundance of OTUs in community

OTU phylogeny

Variant sequences and SNPs

Compare sequences to reference genomes

Sequence community DNA

KEGG
SEED
BLAST

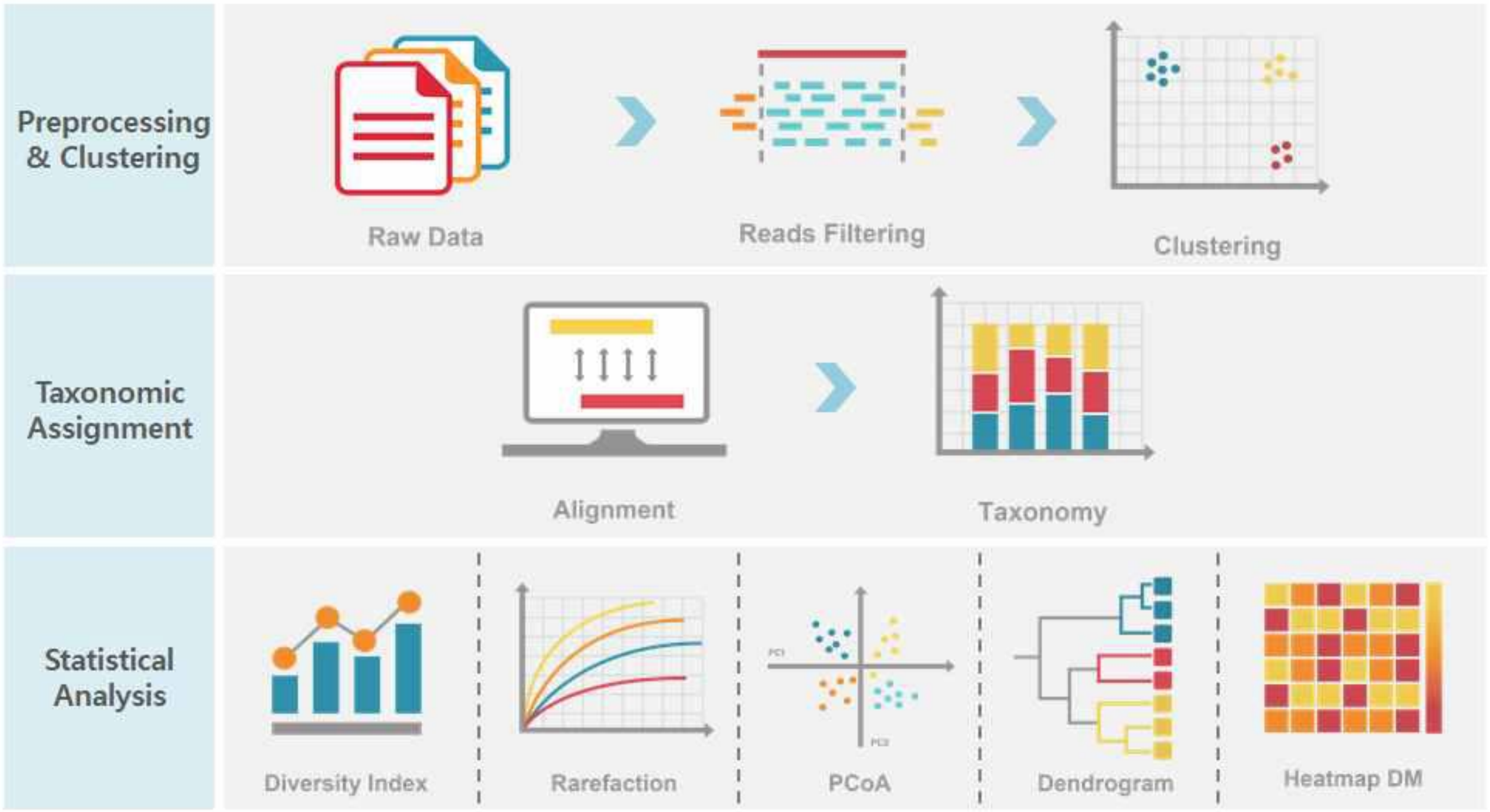
Use database to identify sequences

**Community function: What can the community do?**

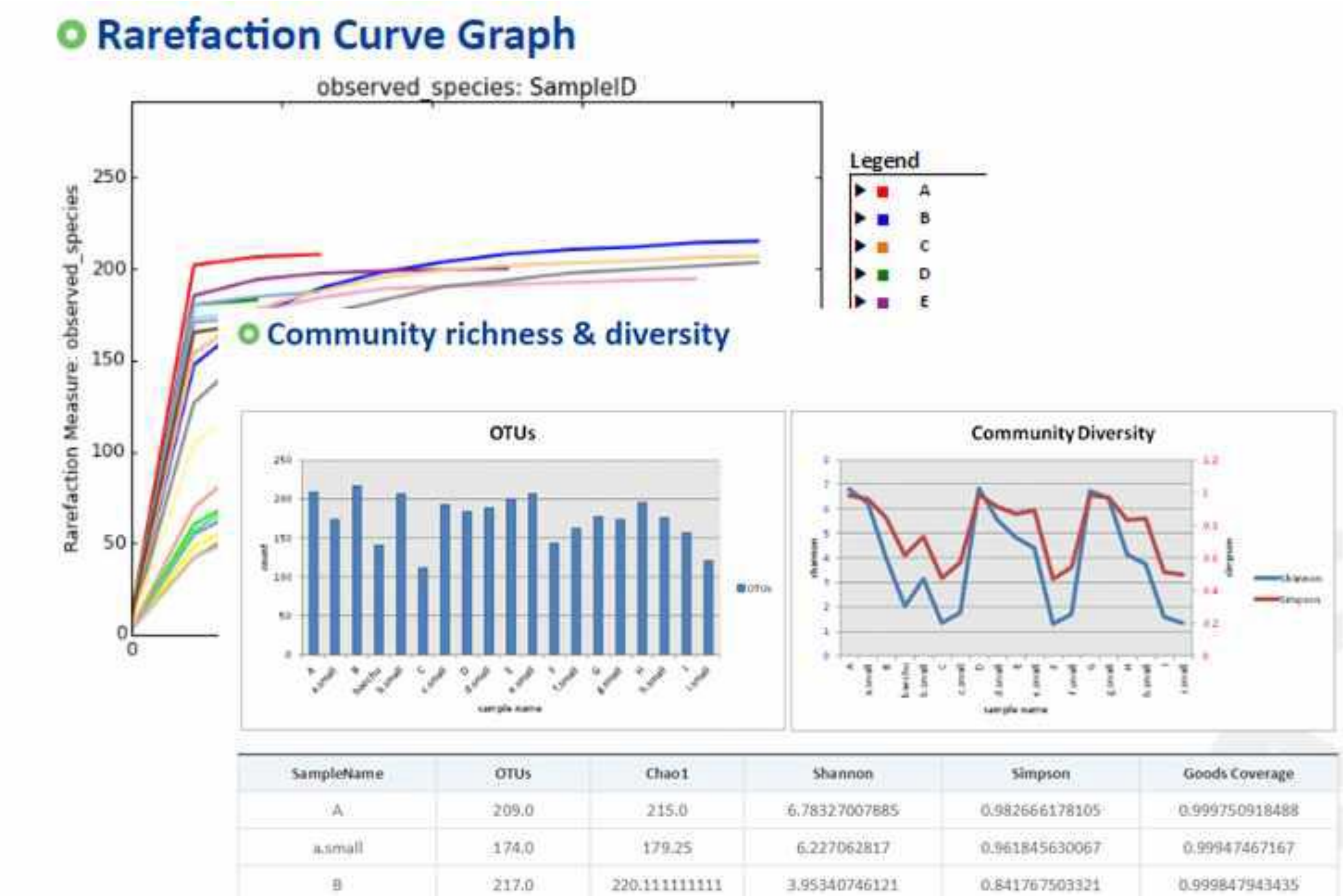
Relative abundance of gene pathways in community

PLOS Computational Biology. 2012;8(2):e1002808

# Data Analysis workflow



# Alpha diversity
# (Diversity Index, Rarefaction curve)

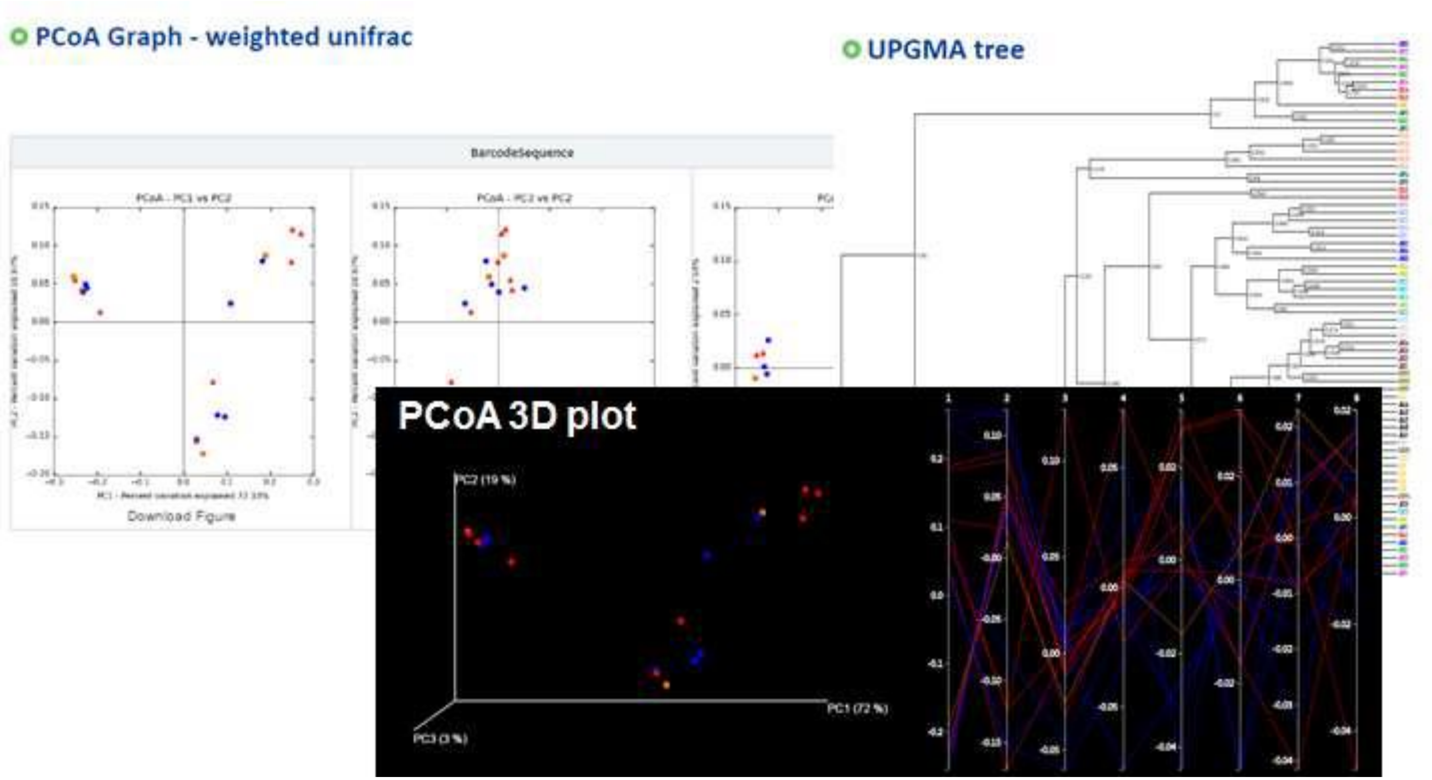


Rarefaction Curve Graph

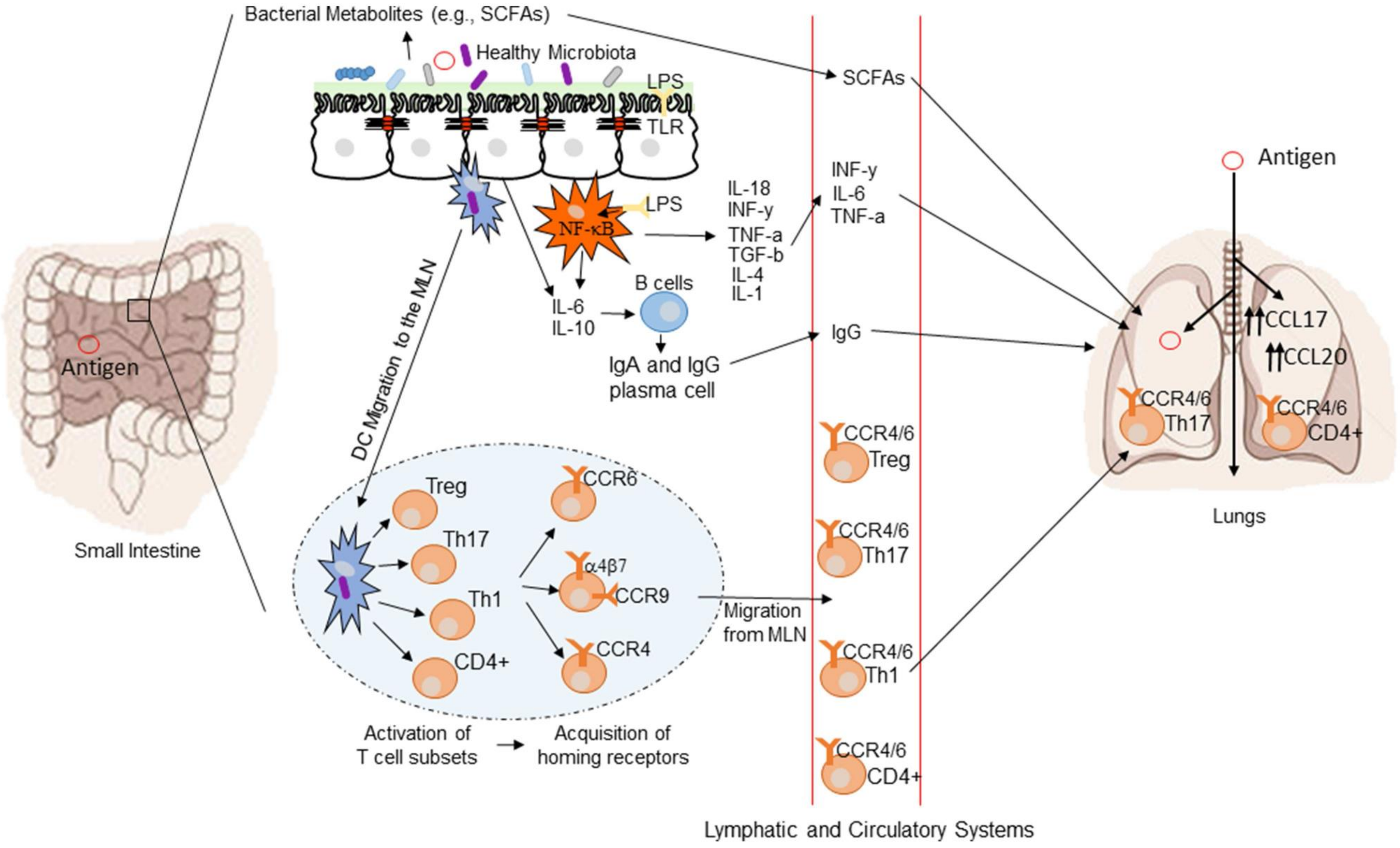
Community richness & diversity

| SampleName | OTUs | Chao1 | Shannon | Simpson | Goods Coverage |
|---|---|---|---|---|---|
| A | 209.0 | 215.0 | 6.78327007885 | 0.982666178105 | 0.999750918488 |
| a.small | 174.0 | 179.25 | 6.227062817 | 0.961845630067 | 0.99947467167 |
| B | 217.0 | 220.111111111 | 3.95340746121 | 0.841767503321 | 0.999847943435 |

# Beta diversity
# (2D,3D PCoA plot, UPGMA tree)

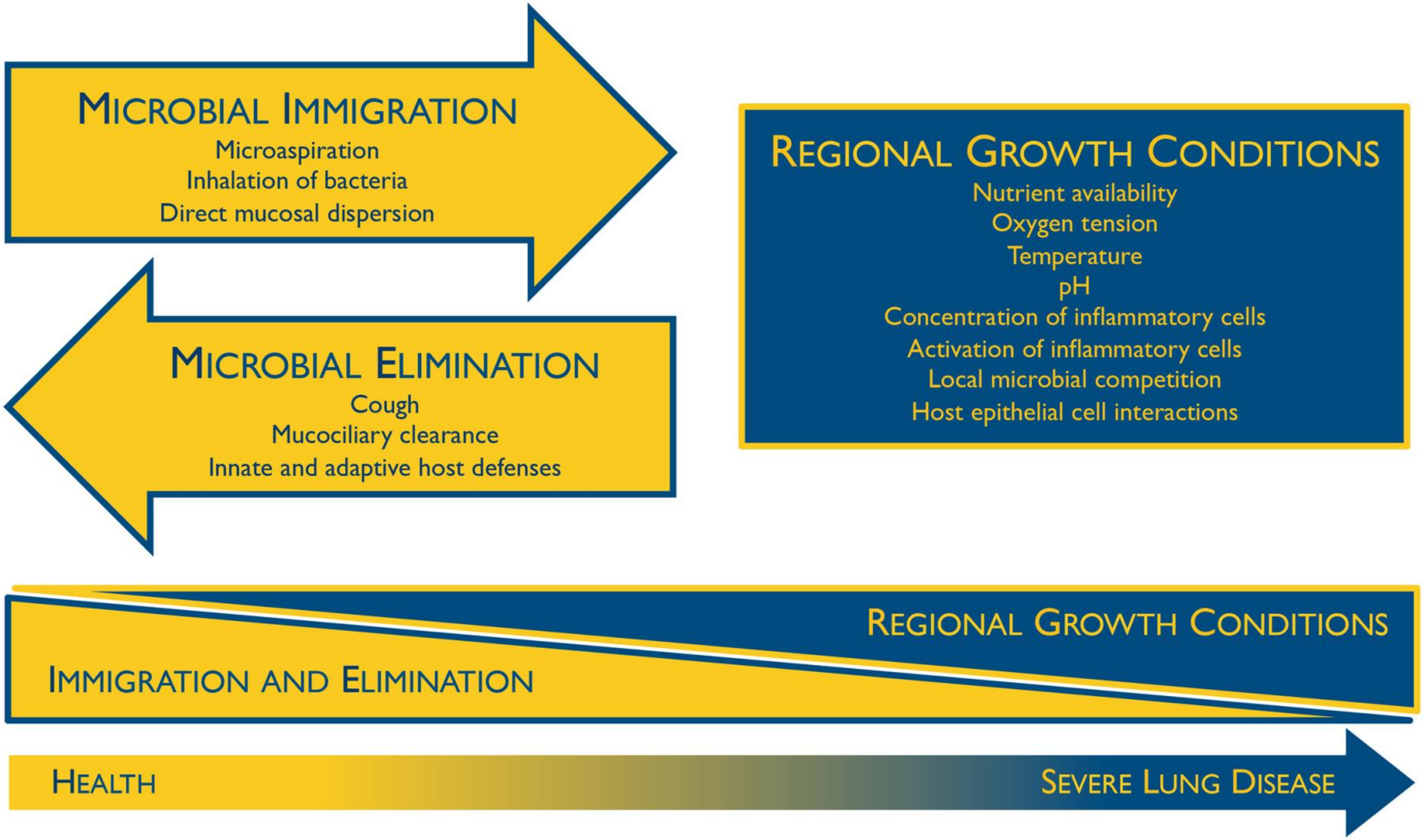


# Gut-Lung Axis: Before Lung Microbiome Period



Samuelson DR, Front Microbiol. 2015

# Adapted Island Model of respiratory microbiome



MICROBIAL IMMIGRATION

Microaspiration

Inhalation of bacteria

Direct mucosal dispersion

MICROBIAL ELIMINATION

Cough

Mucociliary clearance

Innate and adaptive host defenses

REGIONAL GROWTH CONDITIONS

Nutrient availability

Oxygen tension

Temperature

pH

Concentration of inflammatory cells

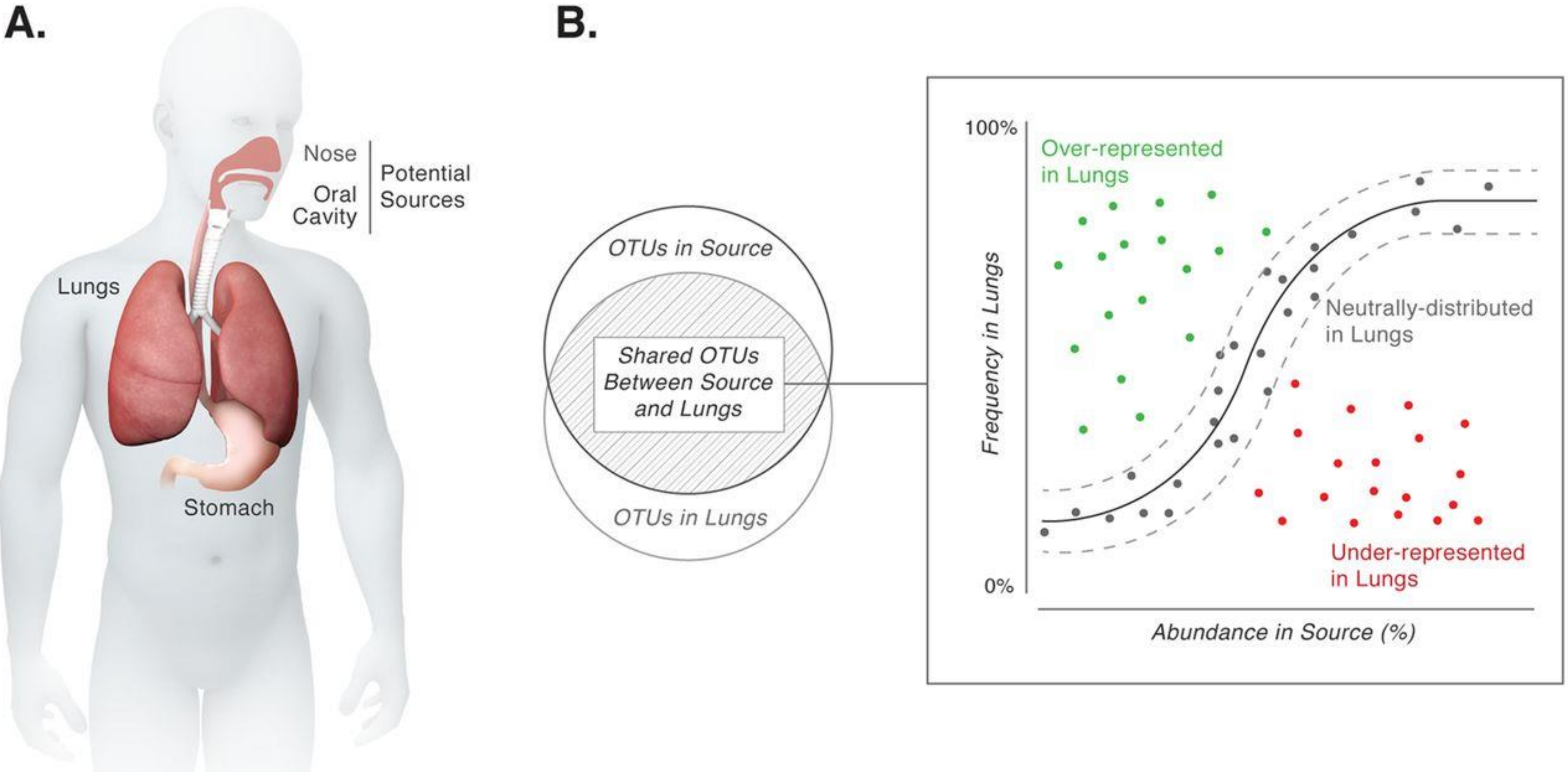
Activation of inflammatory cells

Local microbial competition

Host epithelial cell interactions

PLOS Pathogens | DOI:10.1371/journal.ppat.1004923 July 9, 2015

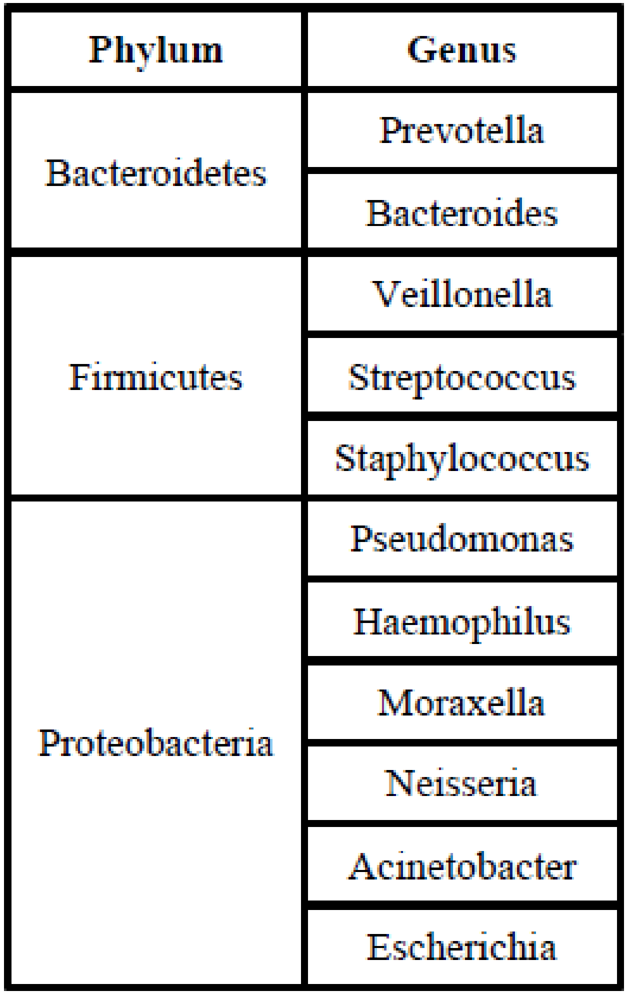
# Neutral model



mBio. 2015;6:e02284-14

# Healthy Lungs

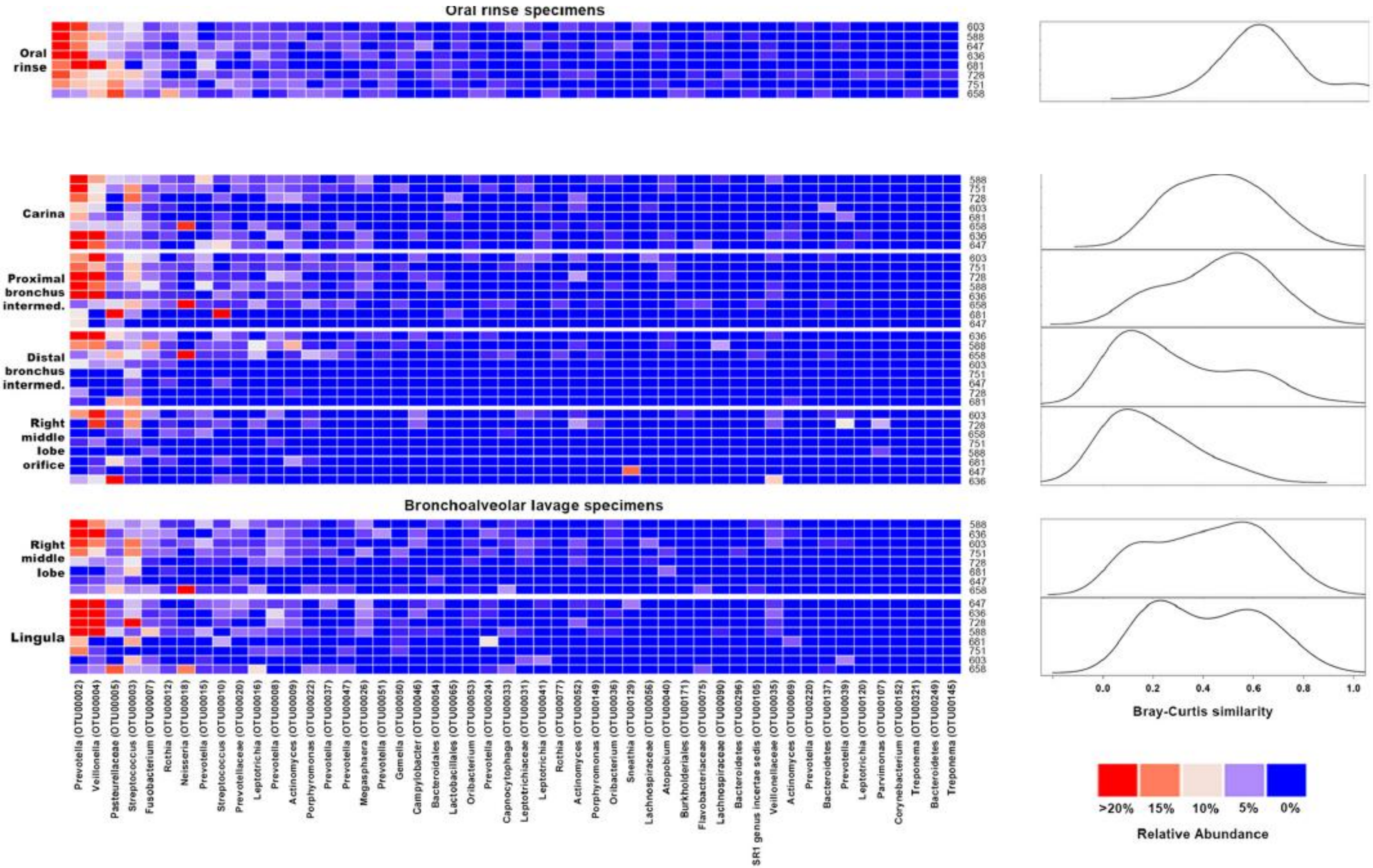
# Taxonomy of phyla and genera



| Phylum | Genus |
|---|---|
| Bacteroidetes | Prevotella |
| | Bacteroides |
| Firmicutes | Veillonella |
| | Streptococcus |
| | Staphylococcus |
| Proteobacteria | Pseudomonas |
| | Haemophilus |
| | Moraxella |
| | Neisseria |
| | Acinetobacter |
| | Escherichia |

*Expert Rev Respir Med*. 2013; 7(3): 245–257.

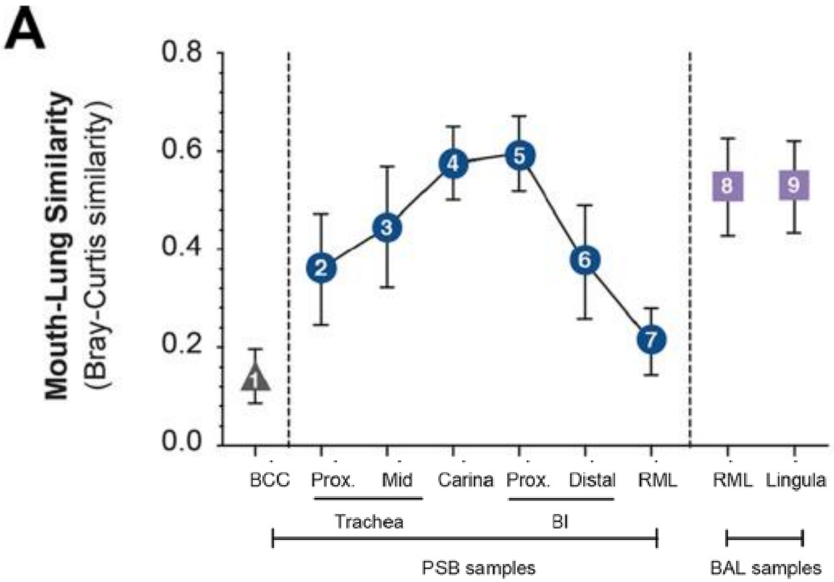
# Bacterial Topography



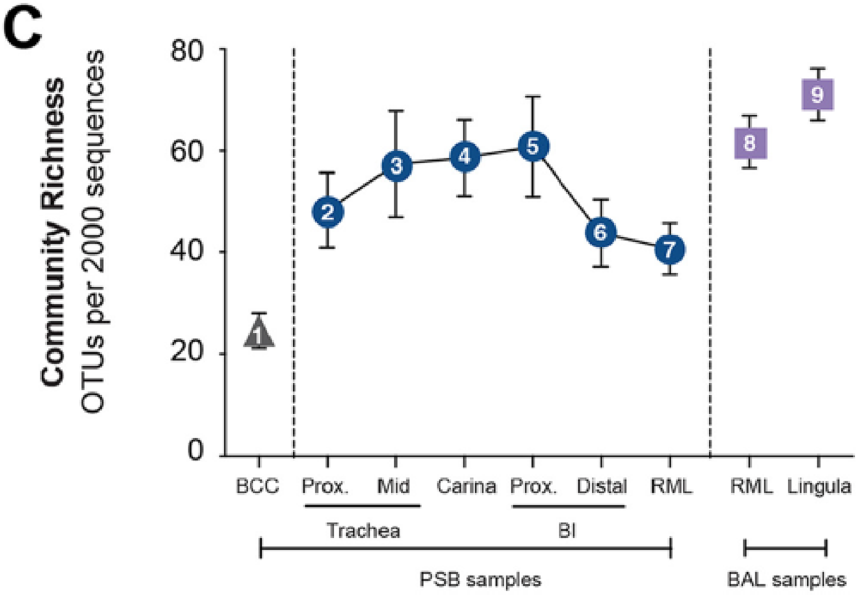
Dickson et al. mBIO. 2017;8(1):e0228-16

# Bacterial topography of healthy LRT

## Mouth-lung bacterial immigration



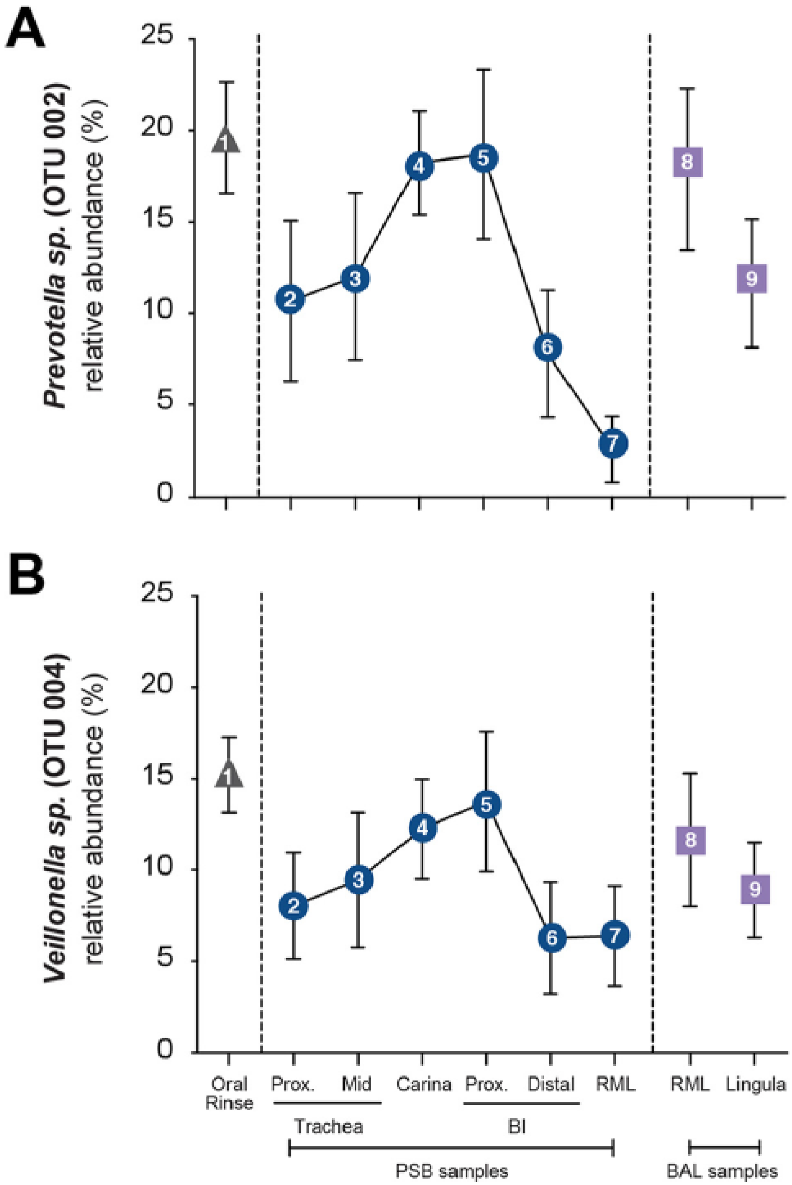
Similarity



Richness

Dickson et al. mBIO. 2017;8(1):e0228-16

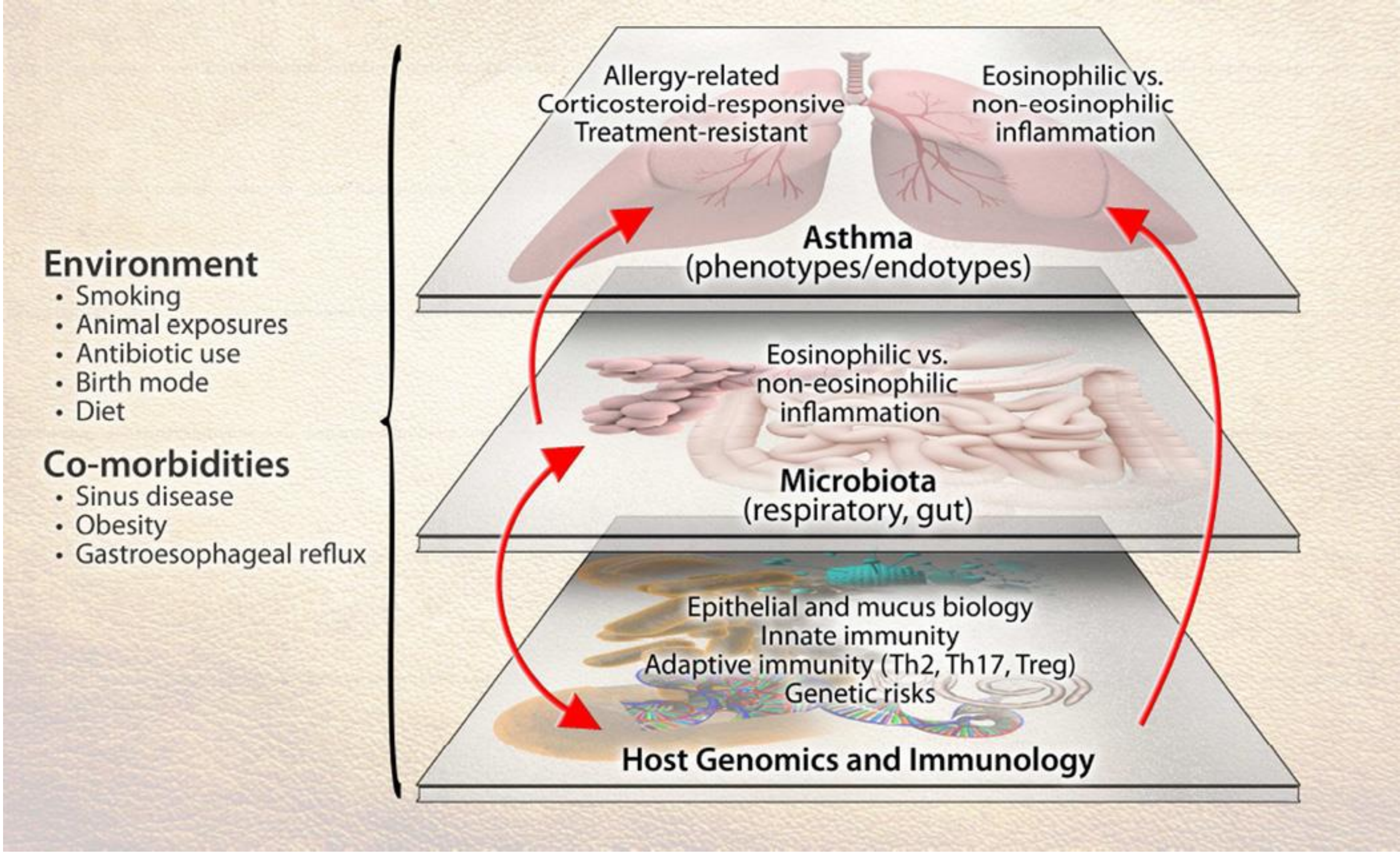
# ABUNDANCE



Dickson et al. mBIO. 2017;8(1):e0228-16

# Asthma
# allergic airway diseases

# Interface of microbiota interactions with other factors in asthma



**Environment**

- Smoking
- Animal exposures
- Antibiotic use
- Birth mode
- Diet

**Co-morbidities**

- Sinus disease
- Obesity
- Gastroesophageal reflux

Allergy-related
Corticosteroid-responsive
Treatment-resistant

Eosinophilic vs.
non-eosinophilic
inflammation

**Asthma**
(phenotypes/endotypes)

Eosinophilic vs.
non-eosinophilic
inflammation

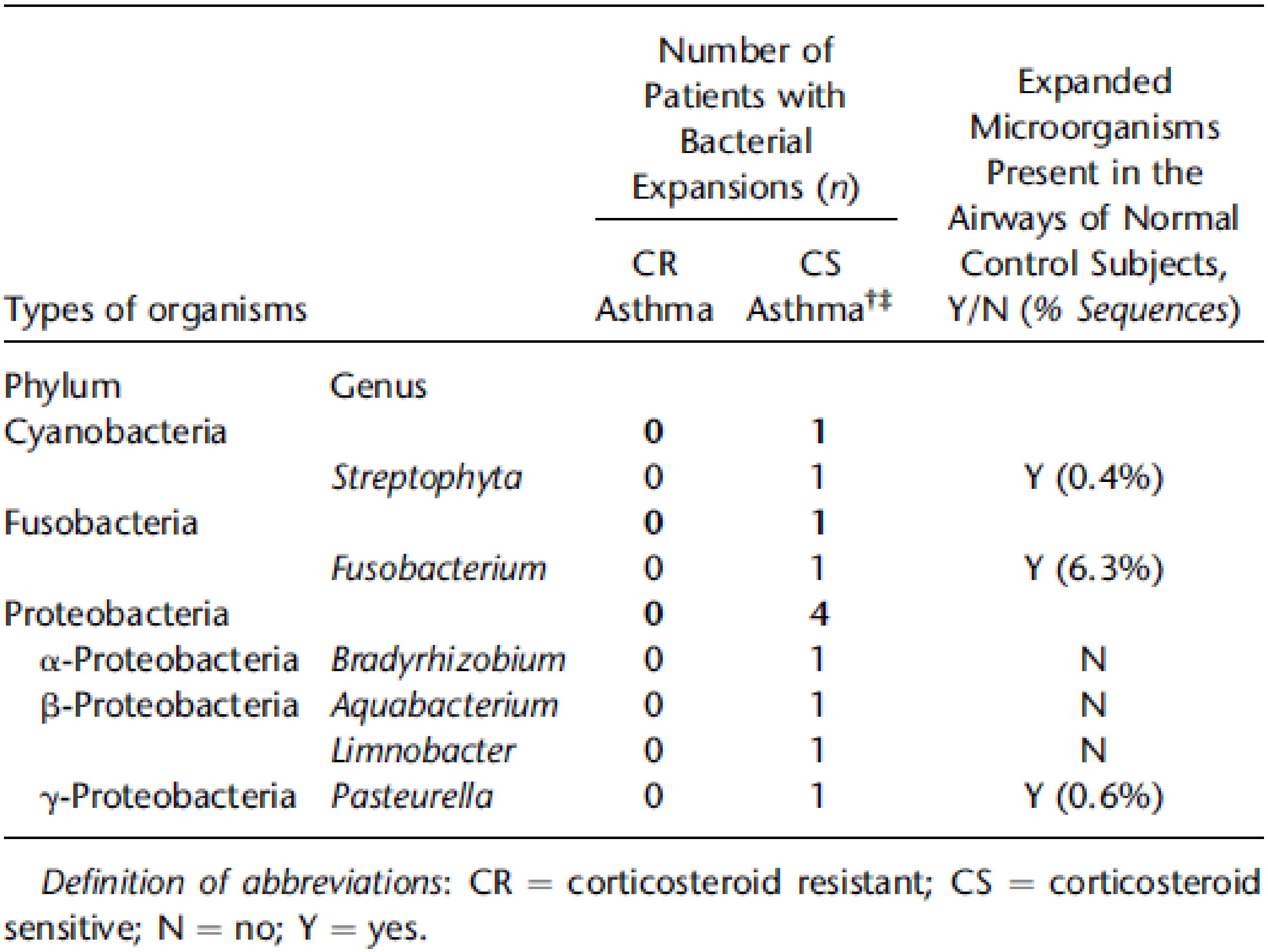
**Microbiota**
(respiratory, gut)

Epithelial and mucus biology
Innate immunity
Adaptive immunity (Th2, Th17, Treg)
Genetic risks

**Host Genomics and Immunology**

J Allergy Clin Immunol. 2015; 135(1): 25–30

# Unique Microorganisms in corticosteroid sensitive asthma

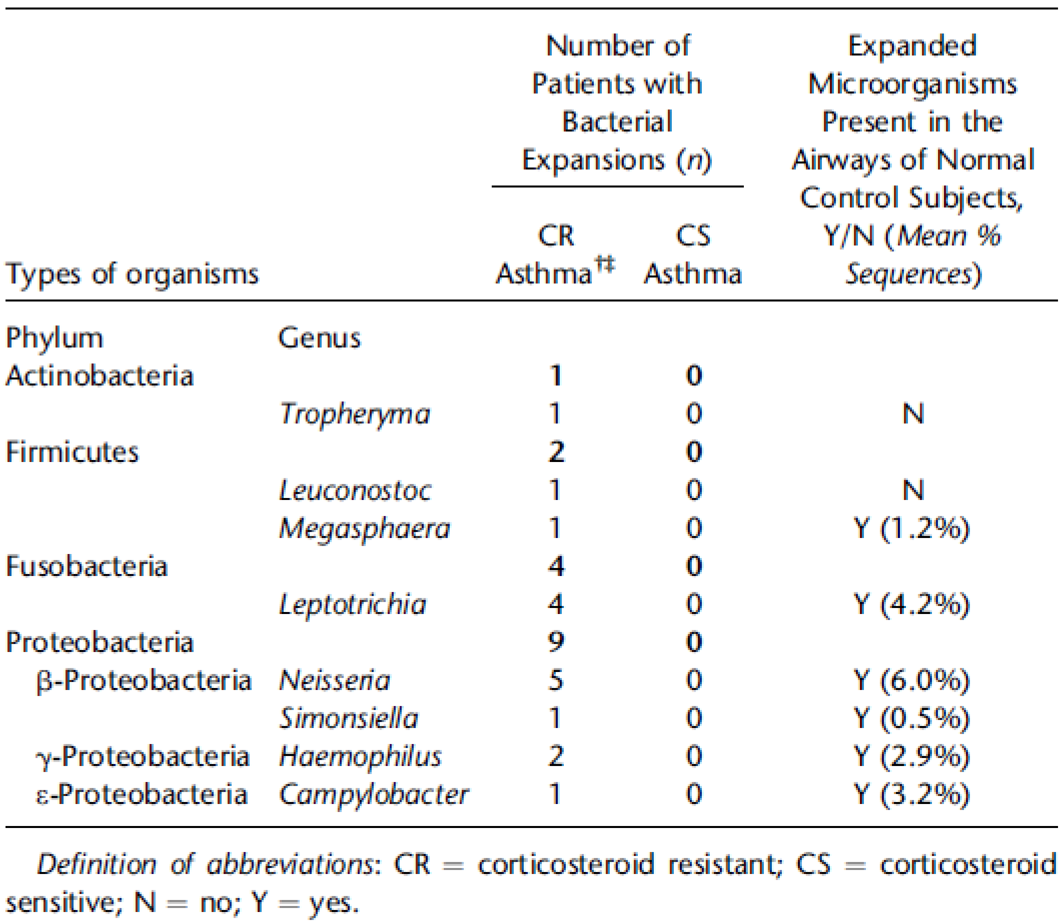


| Types of organisms | | Number of Patients with Bacterial Expansions (*n*) | | Expanded Microorganisms Present in the Airways of Normal Control Subjects, Y/N (% *Sequences*) |
|---|---|---|---|---|
| | | CR Asthma | CS Asthma†‡ | |
| Phylum | Genus | | | |
| Cyanobacteria | | **0** | **1** | |
| | *Streptophyta* | 0 | 1 | Y (0.4%) |
| Fusobacteria | | **0** | **1** | |
| | *Fusobacterium* | 0 | 1 | Y (6.3%) |
| Proteobacteria | | **0** | **4** | |
| α-Proteobacteria | *Bradyrhizobium* | 0 | 1 | N |
| β-Proteobacteria | *Aquabacterium* | 0 | 1 | N |
| | *Limnobacter* | 0 | 1 | N |
| γ-Proteobacteria | *Pasteurella* | 0 | 1 | Y (0.6%) |

*Definition of abbreviations*: CR = corticosteroid resistant; CS = corticosteroid sensitive; N = no; Y = yes.

Am J Respir Crit Care Med. 2013;188:1193–1201

# Unique Microorganisms in corticosteroid resistant asthma



| Types of organisms | | Number of Patients with Bacterial Expansions (*n*) | | Expanded Microorganisms Present in the Airways of Normal Control Subjects, Y/N (*Mean % Sequences*) |
|---|---|---|---|---|
| | | CR Asthma[†‡] | CS Asthma | |
| Phylum | Genus | | | |
| Actinobacteria | | **1** | **0** | |
| | *Tropheryma* | 1 | 0 | N |
| Firmicutes | | **2** | **0** | |
| | *Leuconostoc* | 1 | 0 | N |
| | *Megasphaera* | 1 | 0 | Y (1.2%) |
| Fusobacteria | | **4** | **0** | |
| | *Leptotrichia* | 4 | 0 | Y (4.2%) |
| Proteobacteria | | **9** | **0** | |
| β-Proteobacteria | *Neisseria* | 5 | 0 | Y (6.0%) |
| | *Simonsiella* | 1 | 0 | Y (0.5%) |
| γ-Proteobacteria | *Haemophilus* | 2 | 0 | Y (2.9%) |
| ε-Proteobacteria | *Campylobacter* | 1 | 0 | Y (3.2%) |

*Definition of abbreviations*: CR = corticosteroid resistant; CS = corticosteroid sensitive; N = no; Y = yes.

Am J Respir Crit Care Med. 2013;188:1193–1201

# Respiratory Microbiome in Severe IgE-mediated Asthma patients

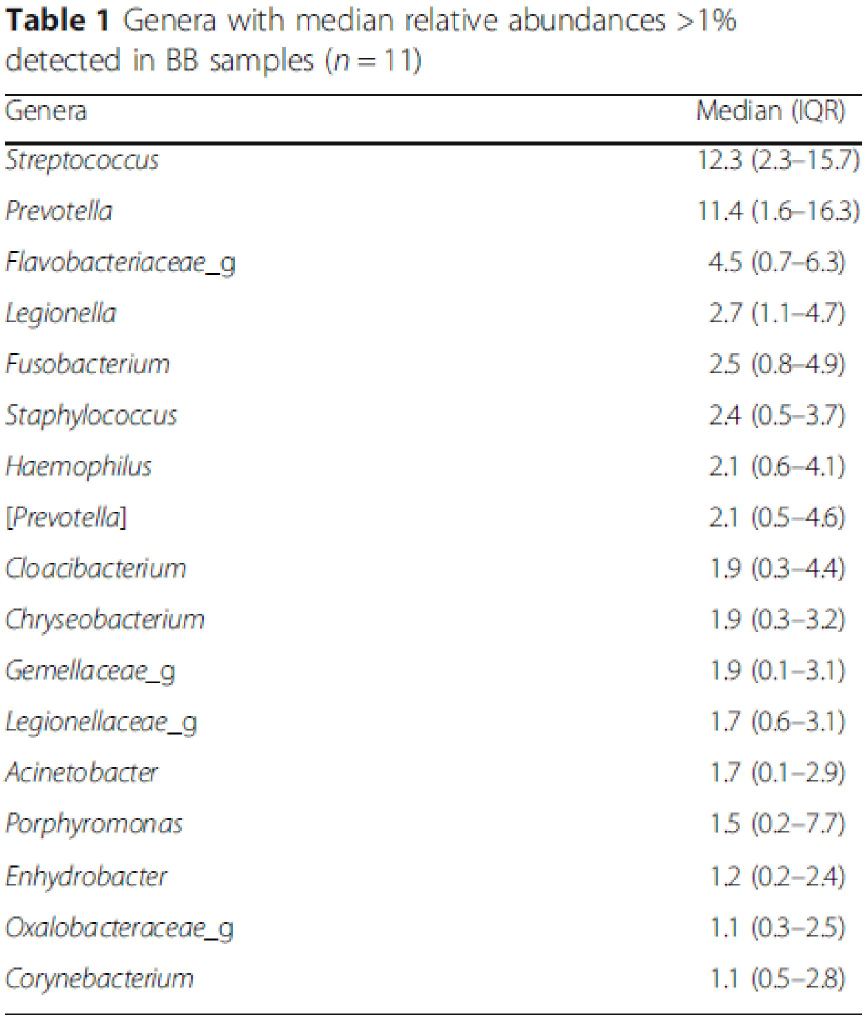


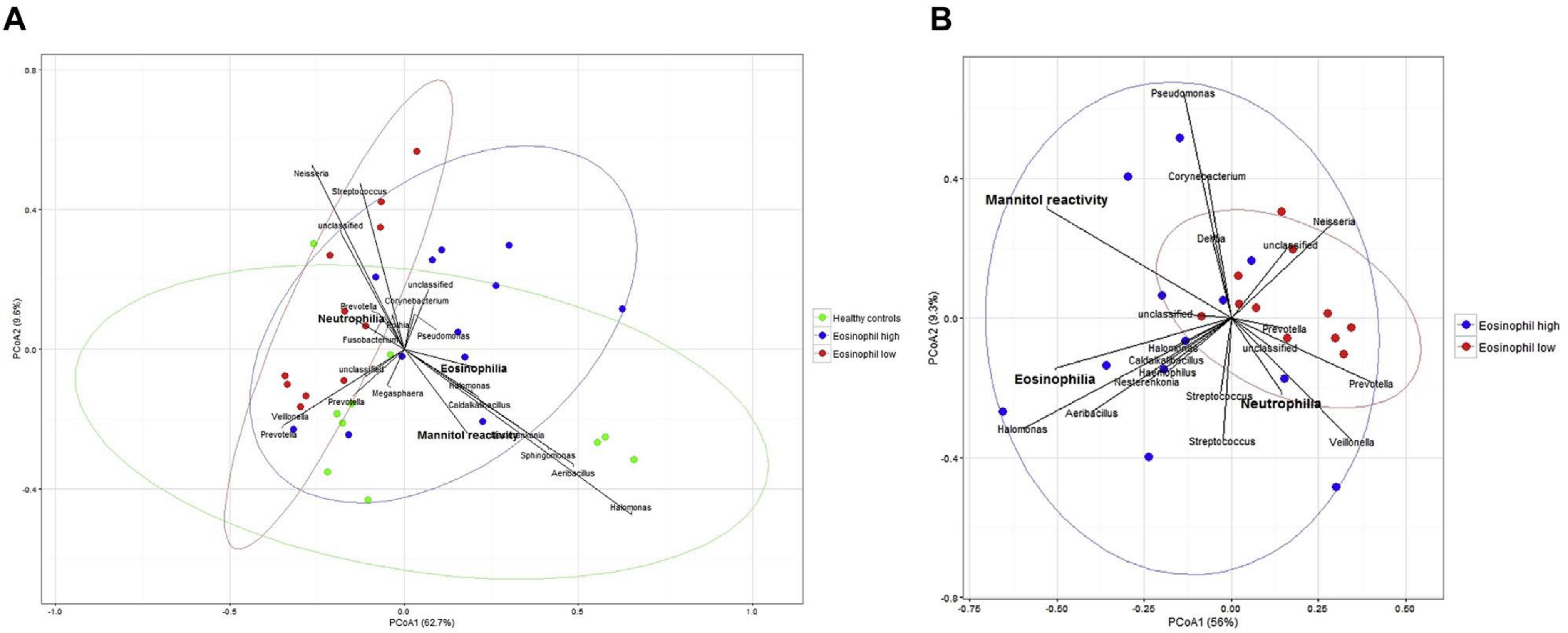
**Table 1** Genera with median relative abundances >1% detected in BB samples ($n = 11$)

| Genera | Median (IQR) |
|---|---|
| *Streptococcus* | 12.3 (2.3–15.7) |
| *Prevotella* | 11.4 (1.6–16.3) |
| *Flavobacteriaceae_g* | 4.5 (0.7–6.3) |
| *Legionella* | 2.7 (1.1–4.7) |
| *Fusobacterium* | 2.5 (0.8–4.9) |
| *Staphylococcus* | 2.4 (0.5–3.7) |
| *Haemophilus* | 2.1 (0.6–4.1) |
| [*Prevotella*] | 2.1 (0.5–4.6) |
| *Cloacibacterium* | 1.9 (0.3–4.4) |
| *Chryseobacterium* | 1.9 (0.3–3.2) |
| *Gemellaceae_g* | 1.9 (0.1–3.1) |
| *Legionellaceae_g* | 1.7 (0.6–3.1) |
| *Acinetobacter* | 1.7 (0.1–2.9) |
| *Porphyromonas* | 1.5 (0.2–7.7) |
| *Enhydrobacter* | 1.2 (0.2–2.4) |
| *Oxalobacteraceae_g* | 1.1 (0.3–2.5) |
| *Corynebacterium* | 1.1 (0.5–2.8) |

Millares et al. BMC Microbiology. 2017;17:20

# PCoA plots in BAL microbiome

## weighted UniFrac distances



**Healthy control**
**(n = 10)**

**eosinophil-high asthma**
**(n = 11)**

**eosinophil-low asthma**
**(n = 12)**

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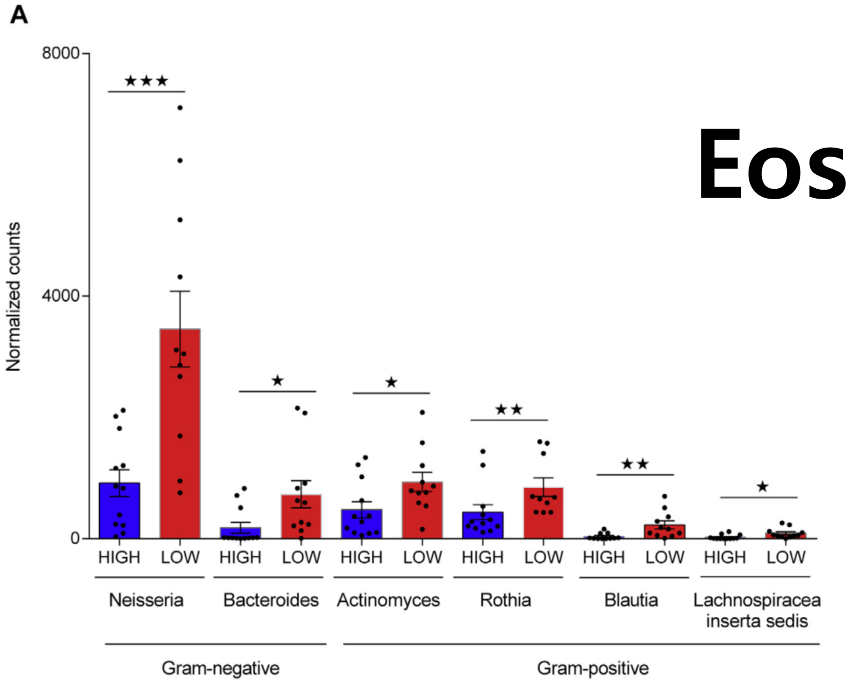
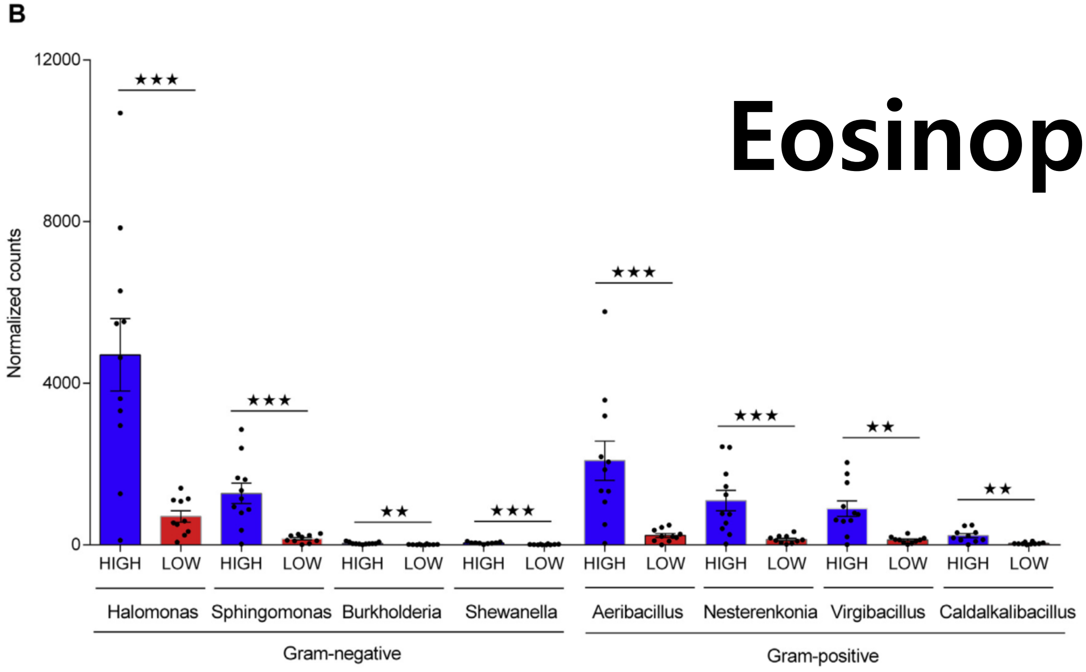


# Eosinophil-low



# Eosinophil-high

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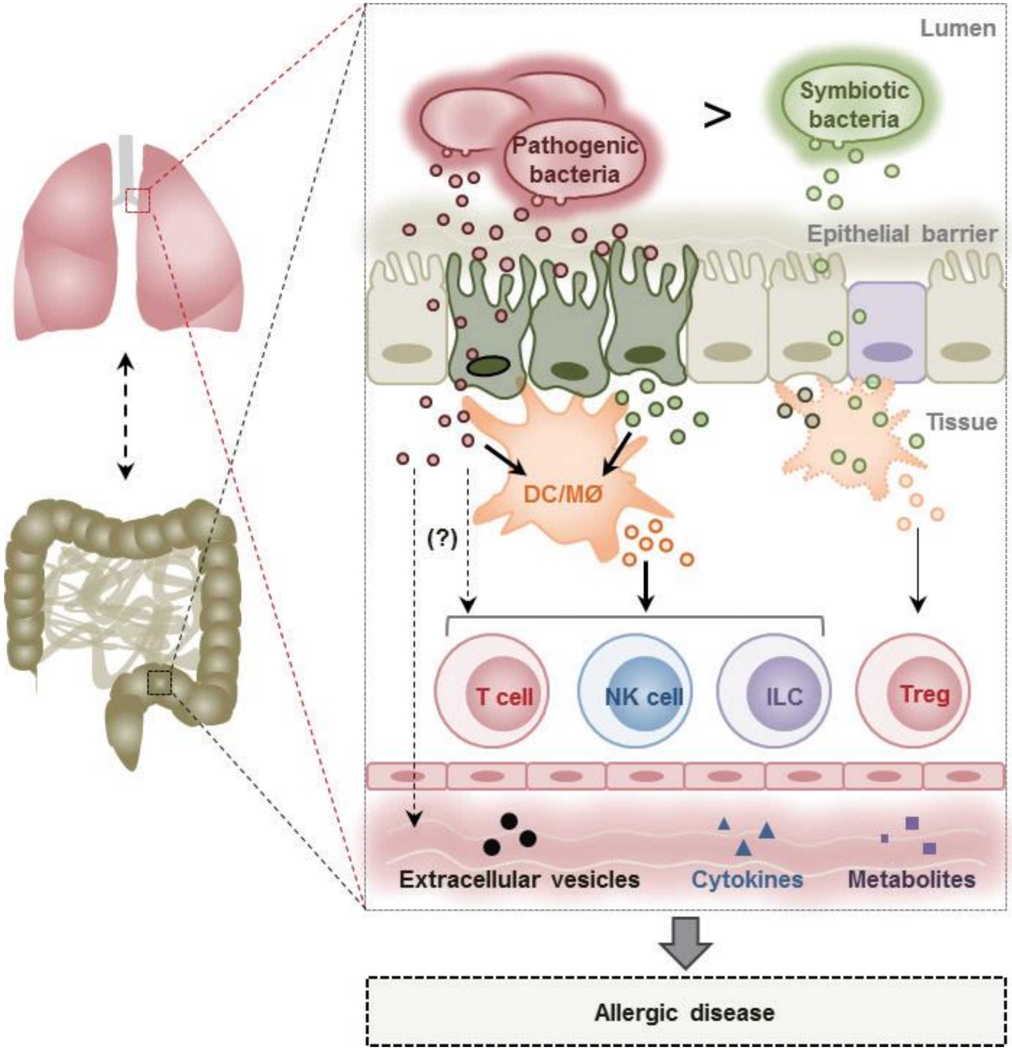
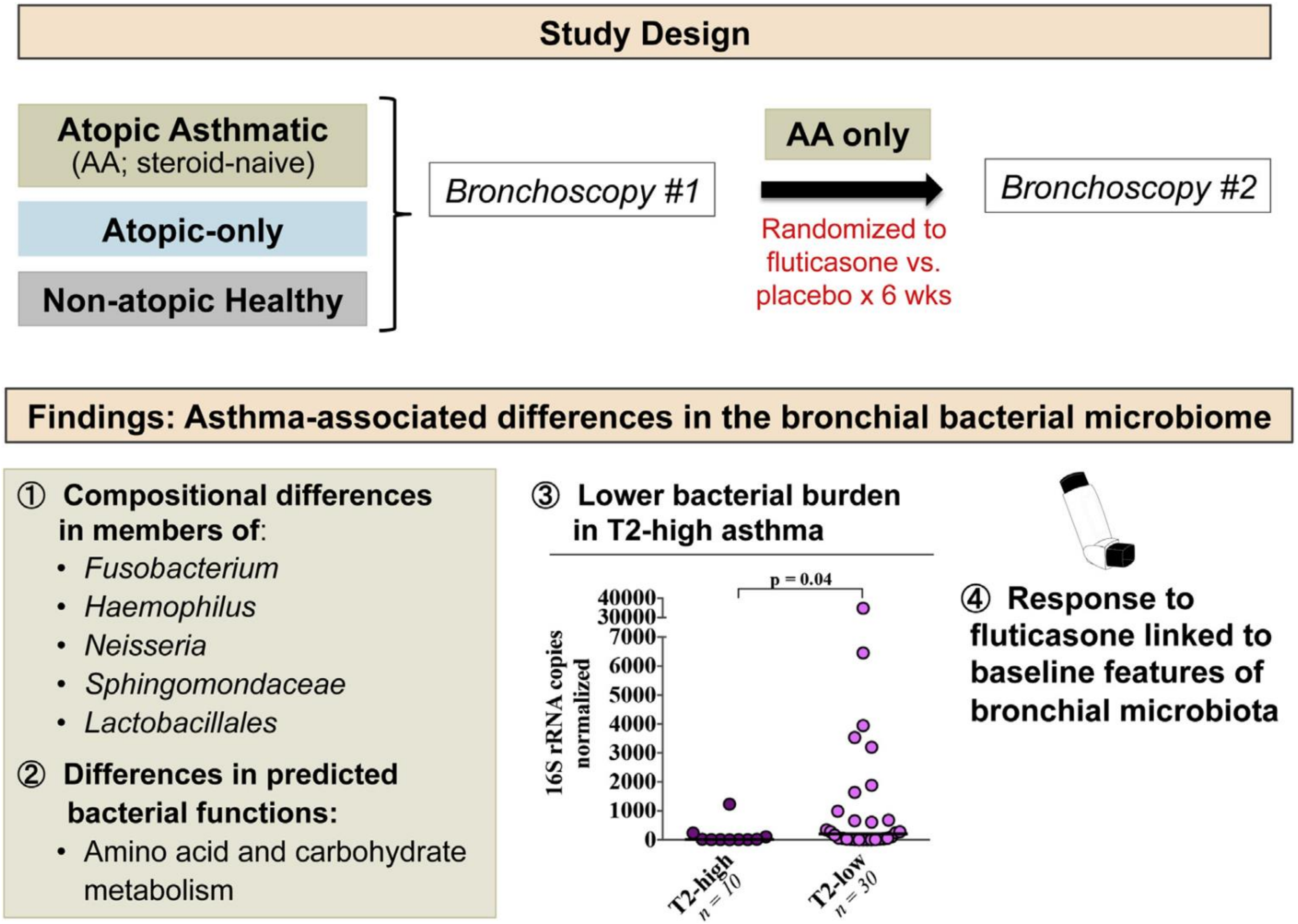
# lung immunity and gut immunity



Allergy Asthma Immunol Res. 2017 March;9(2):101-106.

# AsthmaNet(NHBLI)



**Study Design**

**Atopic Asthmatic**
(AA; steroid-naive)

**Atopic-only**

**Non-atopic Healthy**

*Bronchoscopy #1*

**AA only**

Randomized to fluticasone vs. placebo x 6 wks

*Bronchoscopy #2*

**Findings: Asthma-associated differences in the bronchial bacterial microbiome**

① **Compositional differences in members of**:
- *Fusobacterium*
- *Haemophilus*
- *Neisseria*
- *Sphingomondaceae*
- *Lactobacillales*

② **Differences in predicted bacterial functions:**
- Amino acid and carbohydrate metabolism

③ **Lower bacterial burden in T2-high asthma**

④ **Response to fluticasone linked to baseline features of bronchial microbiota**

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- **Atopic Asthma(AA)**
- **Atopy without Asthma(ANA)**
- **Healthy Control(HC)**

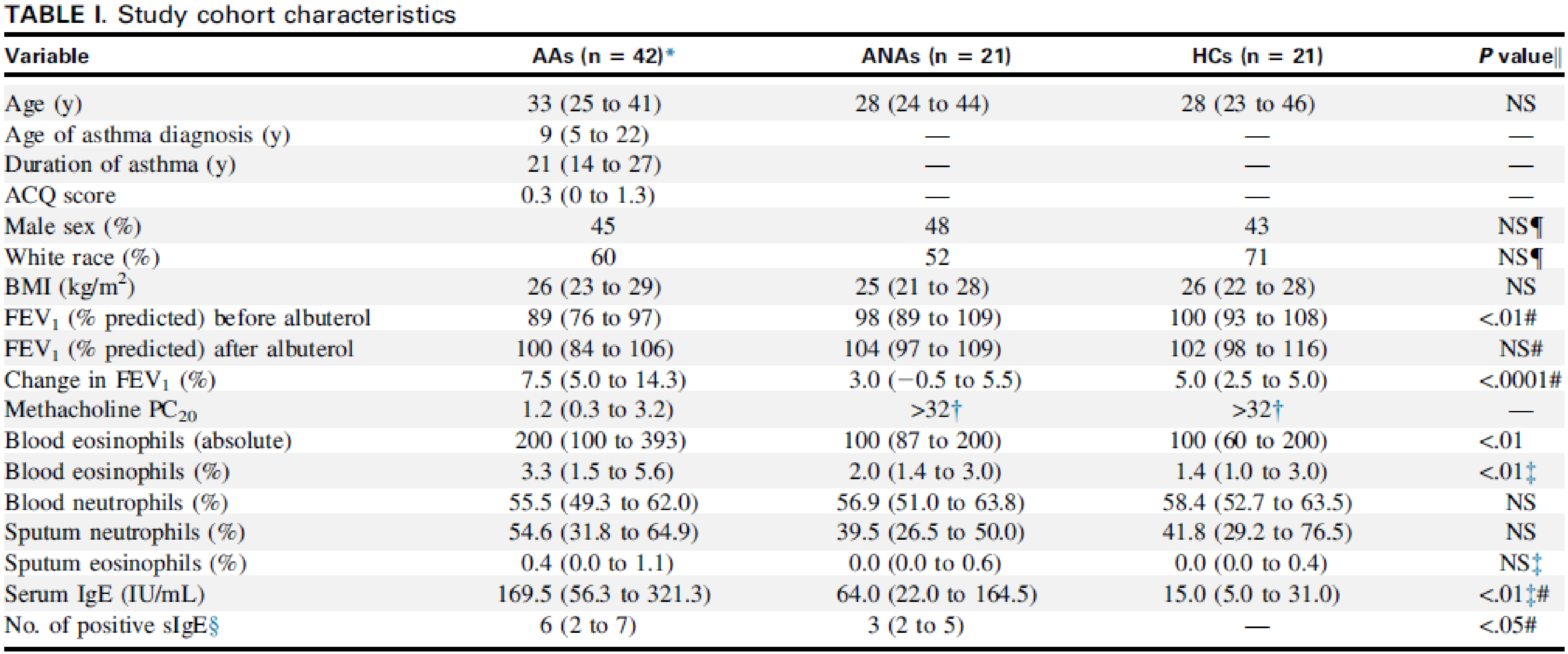

**TABLE I.** Study cohort characteristics

| Variable | AAs (n = 42)* | ANAs (n = 21) | HCs (n = 21) | *P* value‖ |
|---|---|---|---|---|
| Age (y) | 33 (25 to 41) | 28 (24 to 44) | 28 (23 to 46) | NS |
| Age of asthma diagnosis (y) | 9 (5 to 22) | — | — | — |
| Duration of asthma (y) | 21 (14 to 27) | — | — | — |
| ACQ score | 0.3 (0 to 1.3) | — | — | — |
| Male sex (%) | 45 | 48 | 43 | NS¶ |
| White race (%) | 60 | 52 | 71 | NS¶ |
| BMI (kg/m$^2$) | 26 (23 to 29) | 25 (21 to 28) | 26 (22 to 28) | NS |
| FEV$_1$ (% predicted) before albuterol | 89 (76 to 97) | 98 (89 to 109) | 100 (93 to 108) | <.01# |
| FEV$_1$ (% predicted) after albuterol | 100 (84 to 106) | 104 (97 to 109) | 102 (98 to 116) | NS# |
| Change in FEV$_1$ (%) | 7.5 (5.0 to 14.3) | 3.0 (−0.5 to 5.5) | 5.0 (2.5 to 5.0) | <.0001# |
| Methacholine PC$_{20}$ | 1.2 (0.3 to 3.2) | >32† | >32† | — |
| Blood eosinophils (absolute) | 200 (100 to 393) | 100 (87 to 200) | 100 (60 to 200) | <.01 |
| Blood eosinophils (%) | 3.3 (1.5 to 5.6) | 2.0 (1.4 to 3.0) | 1.4 (1.0 to 3.0) | <.01‡ |
| Blood neutrophils (%) | 55.5 (49.3 to 62.0) | 56.9 (51.0 to 63.8) | 58.4 (52.7 to 63.5) | NS |
| Sputum neutrophils (%) | 54.6 (31.8 to 64.9) | 39.5 (26.5 to 50.0) | 41.8 (29.2 to 76.5) | NS |
| Sputum eosinophils (%) | 0.4 (0.0 to 1.1) | 0.0 (0.0 to 0.6) | 0.0 (0.0 to 0.4) | NS‡ |
| Serum IgE (IU/mL) | 169.5 (56.3 to 321.3) | 64.0 (22.0 to 164.5) | 15.0 (5.0 to 31.0) | <.01‡# |
| No. of positive sIgE§ | 6 (2 to 7) | 3 (2 to 5) | — | <.05# |

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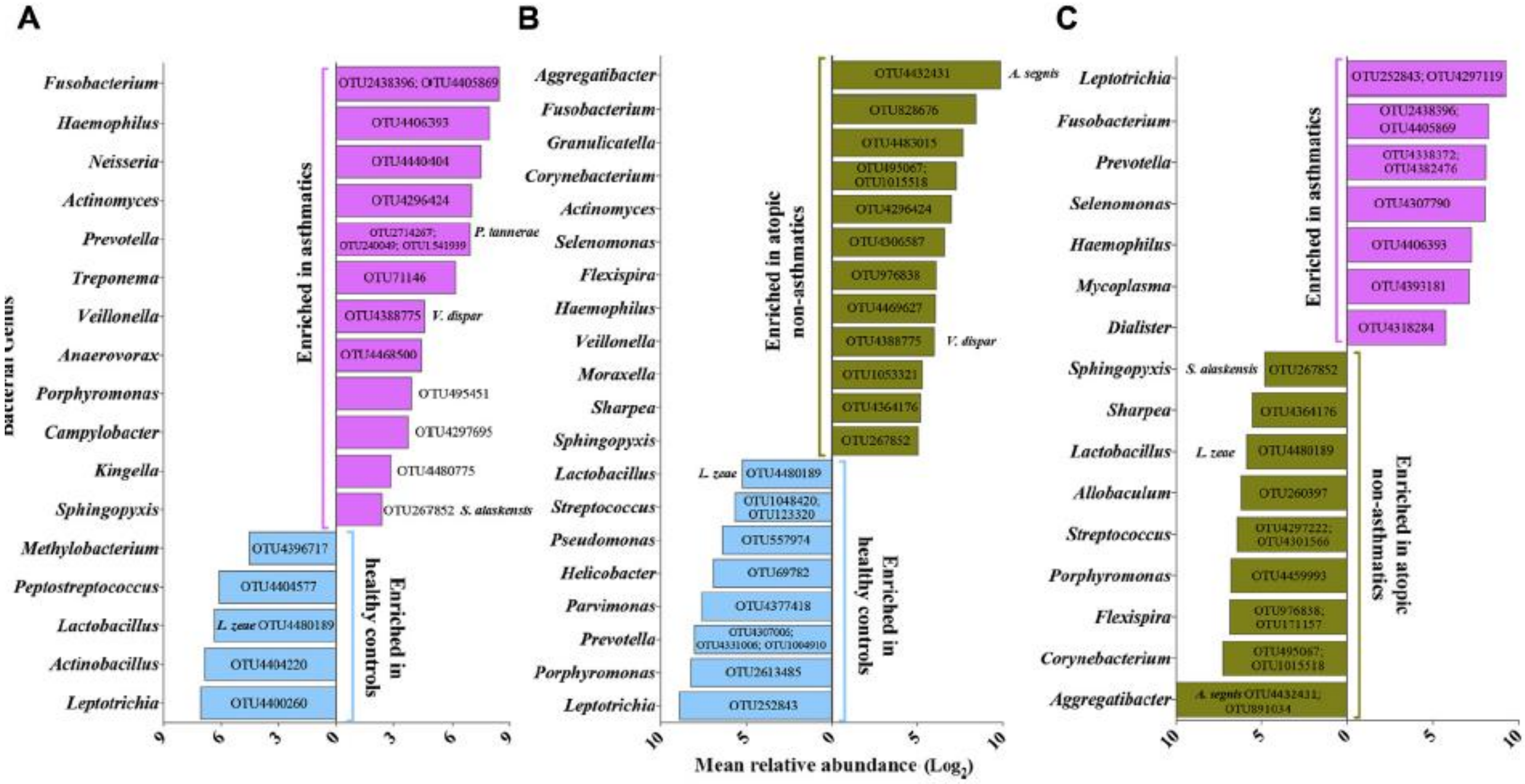
# Relative Abundance (>X2)



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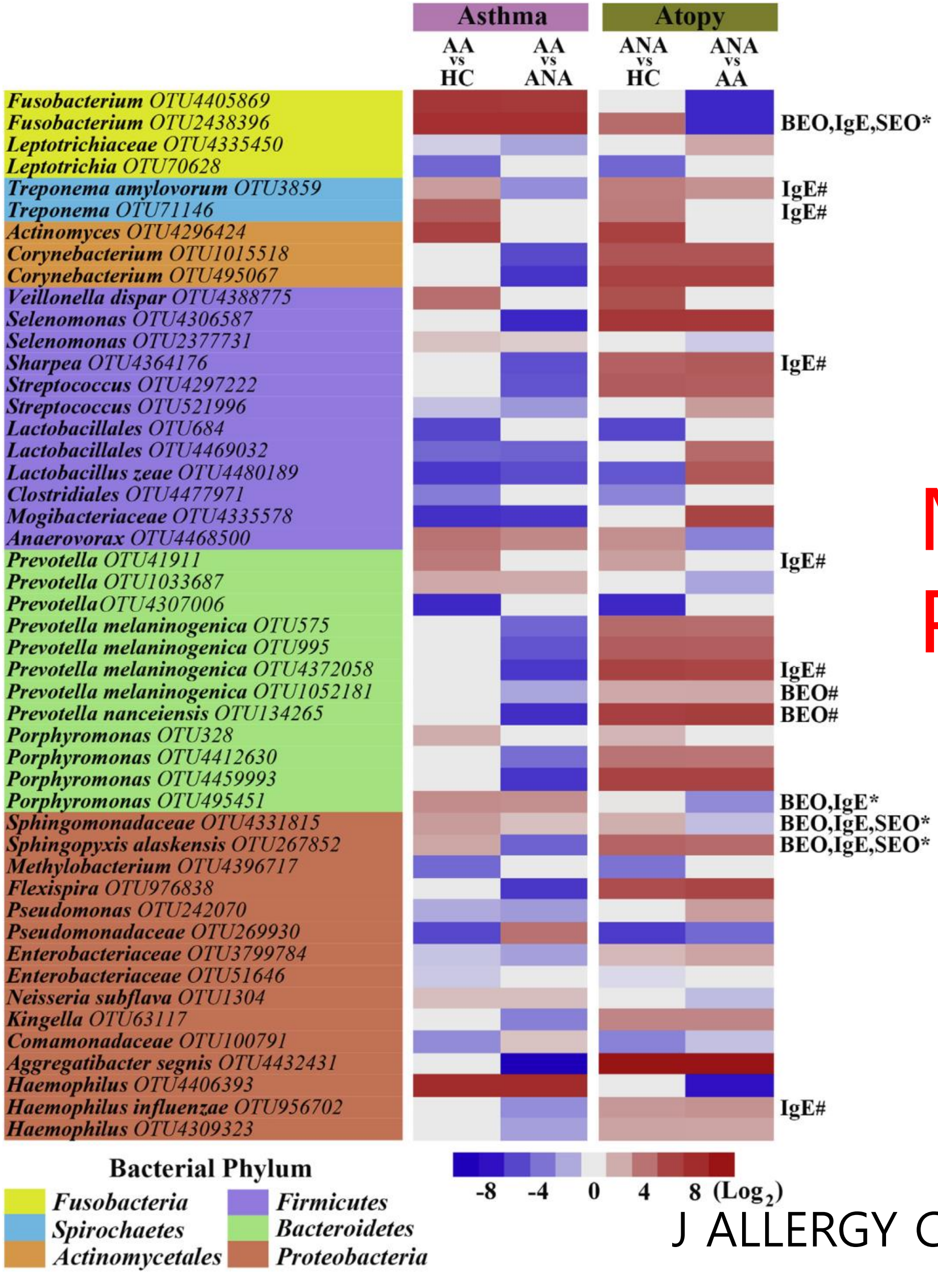
# Taxa positively correlated with blood eosinophil counts (BEO), serum IgE levels (IgE), or sputum eosinophil counts (SEO) in AAs (*) or ANAs (#)



Metagenomic Profiling

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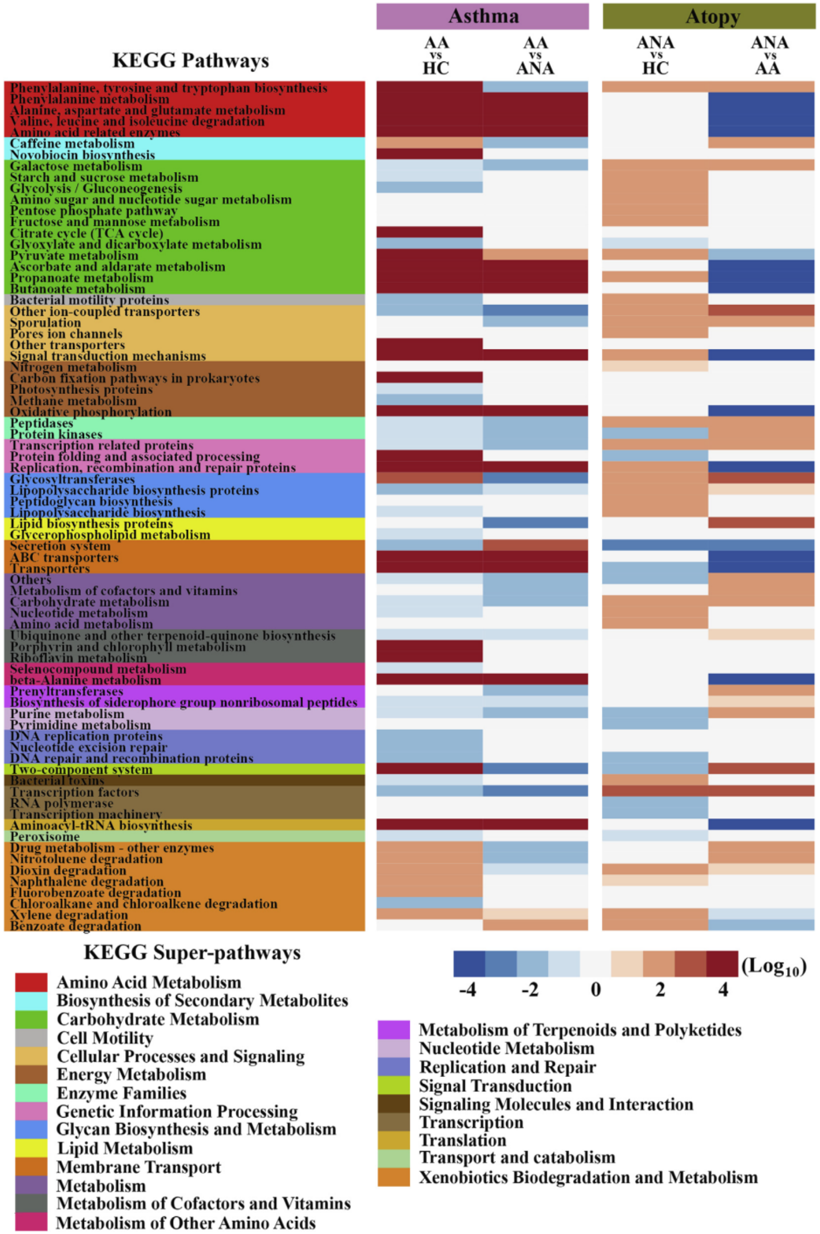
# Mean difference in predicted Kyoto Encyclopedia of Genes and Genomes orthologs (KOs) between groups



Functional Metagenomic Data

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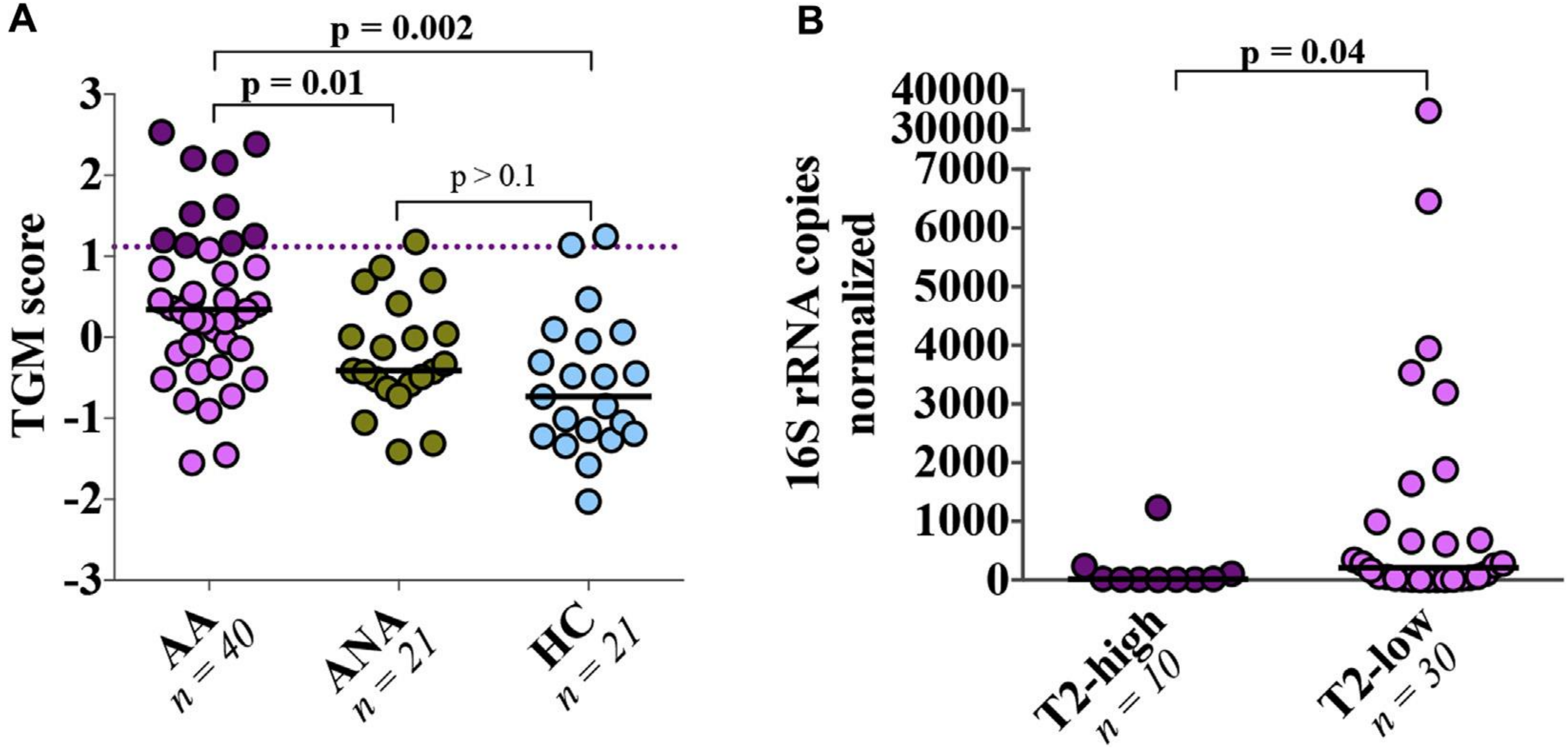
# Epithelial Genes & Bacterial Burden in T2-high Asthma



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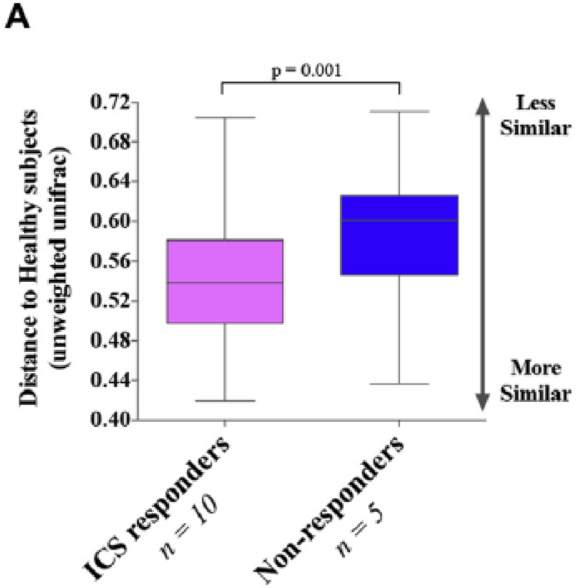
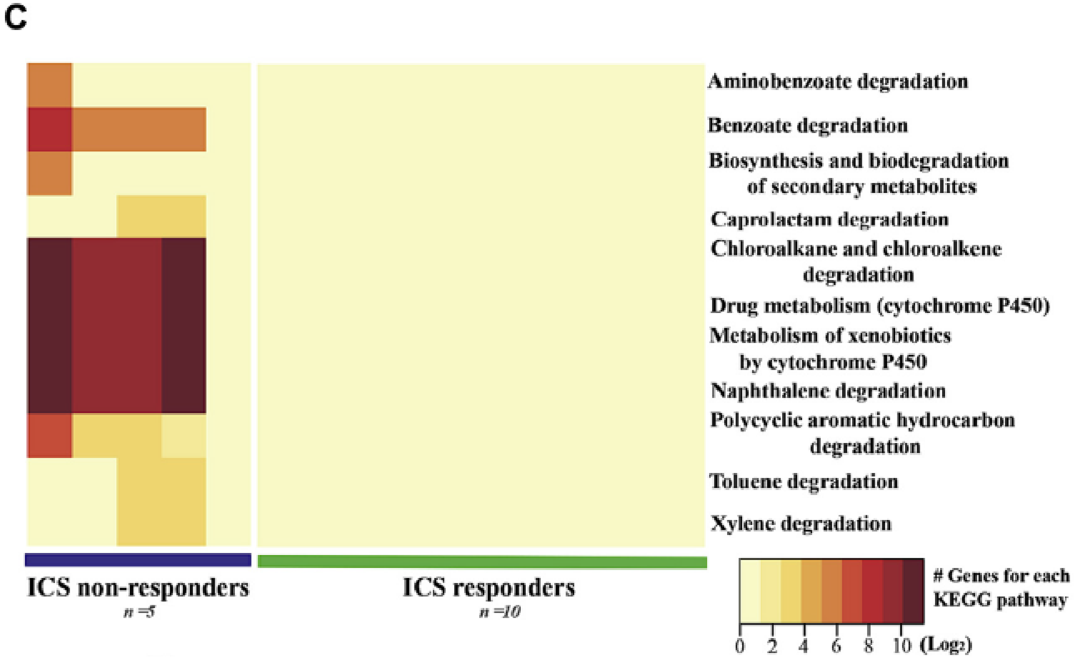
# Responsiveness to ICS





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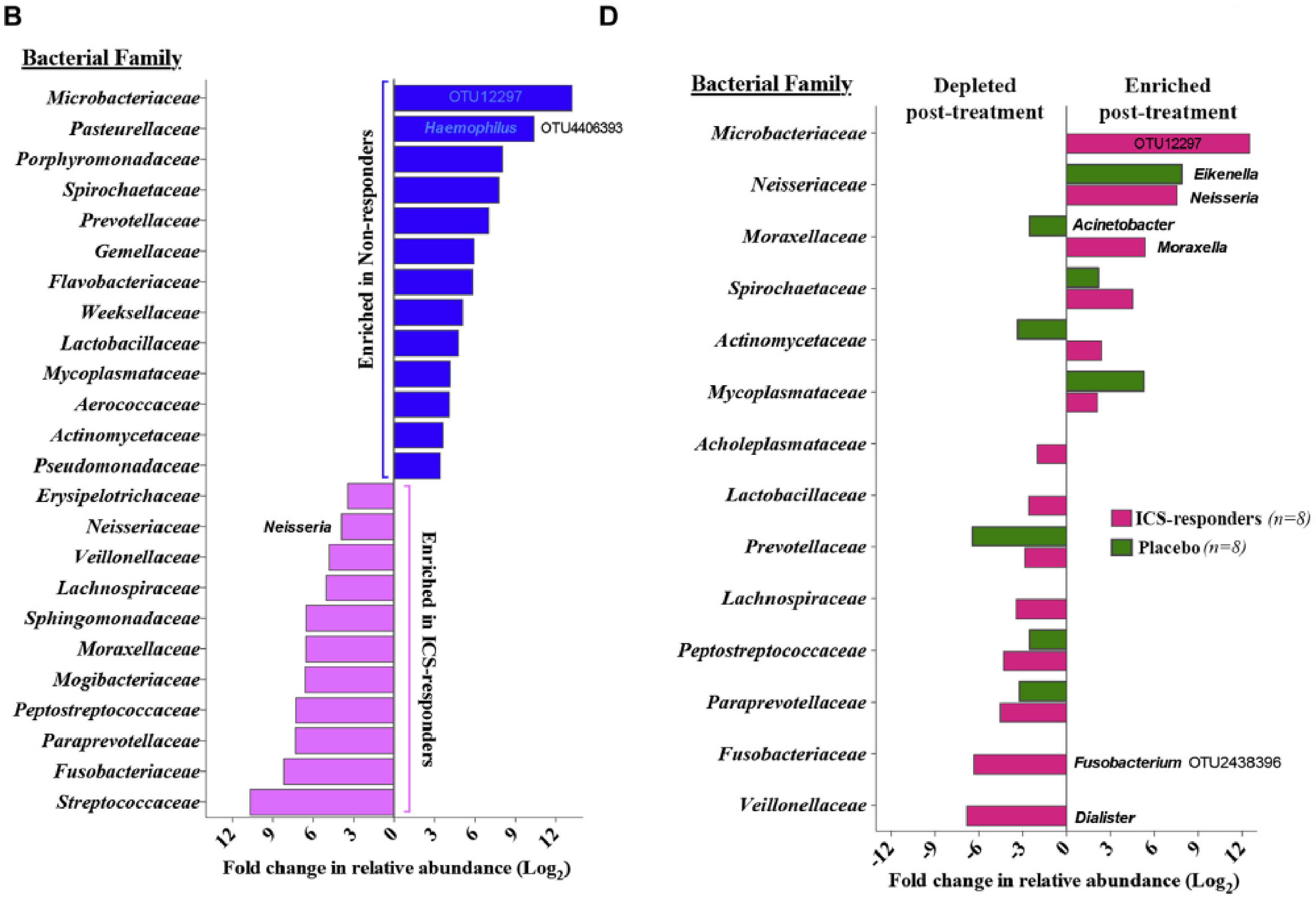
# ICS Treatment



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# Suggestion

- Different bronchial bacterial microbiome in three mild atopic asthma (steroid naive), atopy alone, control.

- Asthma:

  high *Haemophilus, Neisseria, Fusobacterium,* and *Porphyromonas* spp., Sphingomonodaceae

  no *Lactobacillus* spp.

- T2-high asthma **phenotype** -low bacterial burden

- **ICS response** is linked to a distinct microbiome & functional profile

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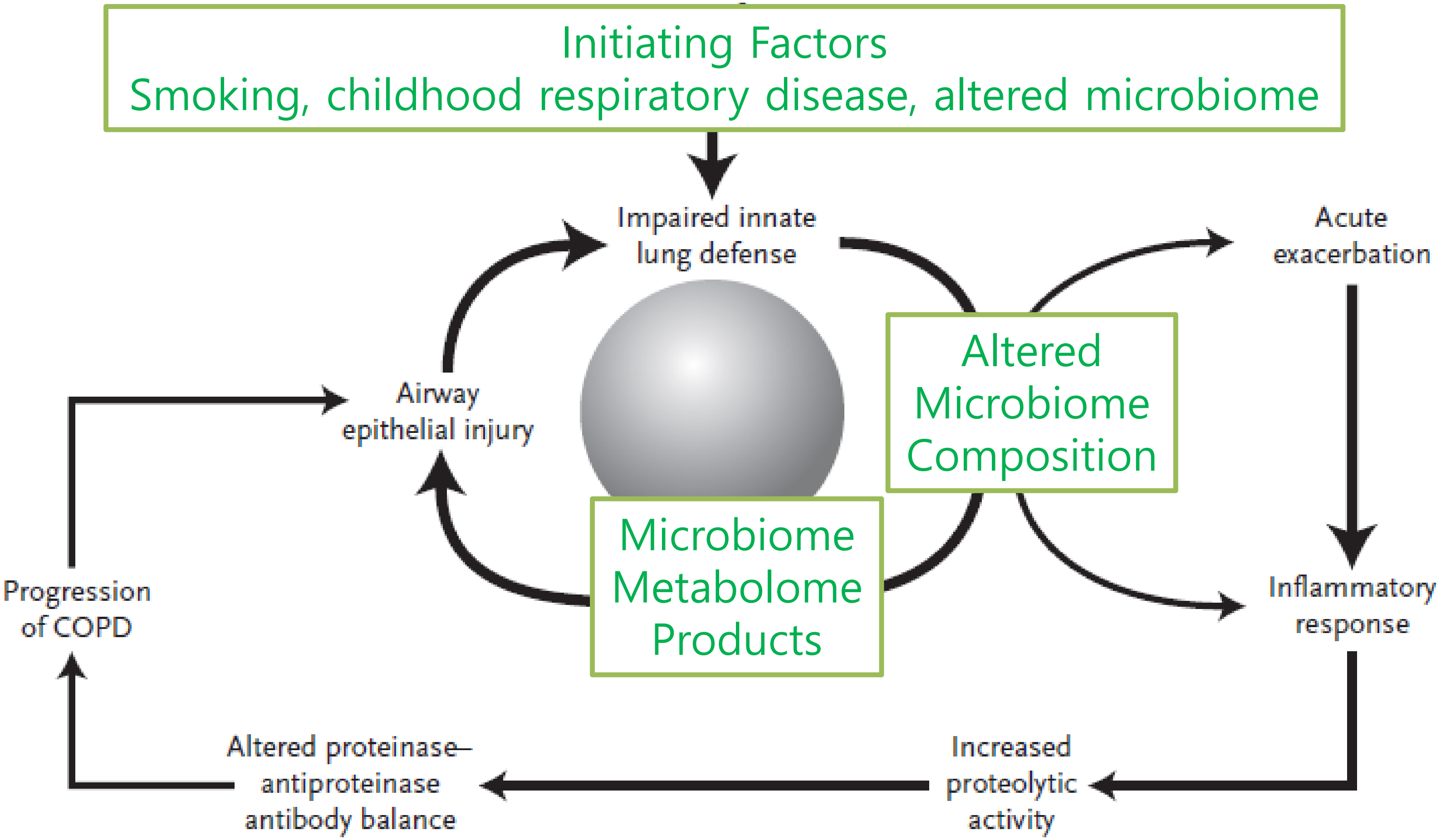
# COPD

# The Vicious-Circle Hypothesis of Infection and Inflammation in COPD.



Initiating Factors
Smoking, childhood respiratory disease, altered microbiome

Impaired innate lung defense

Acute exacerbation

Altered Microbiome Composition

Airway epithelial injury

Microbiome Metabolome Products

Inflammatory response

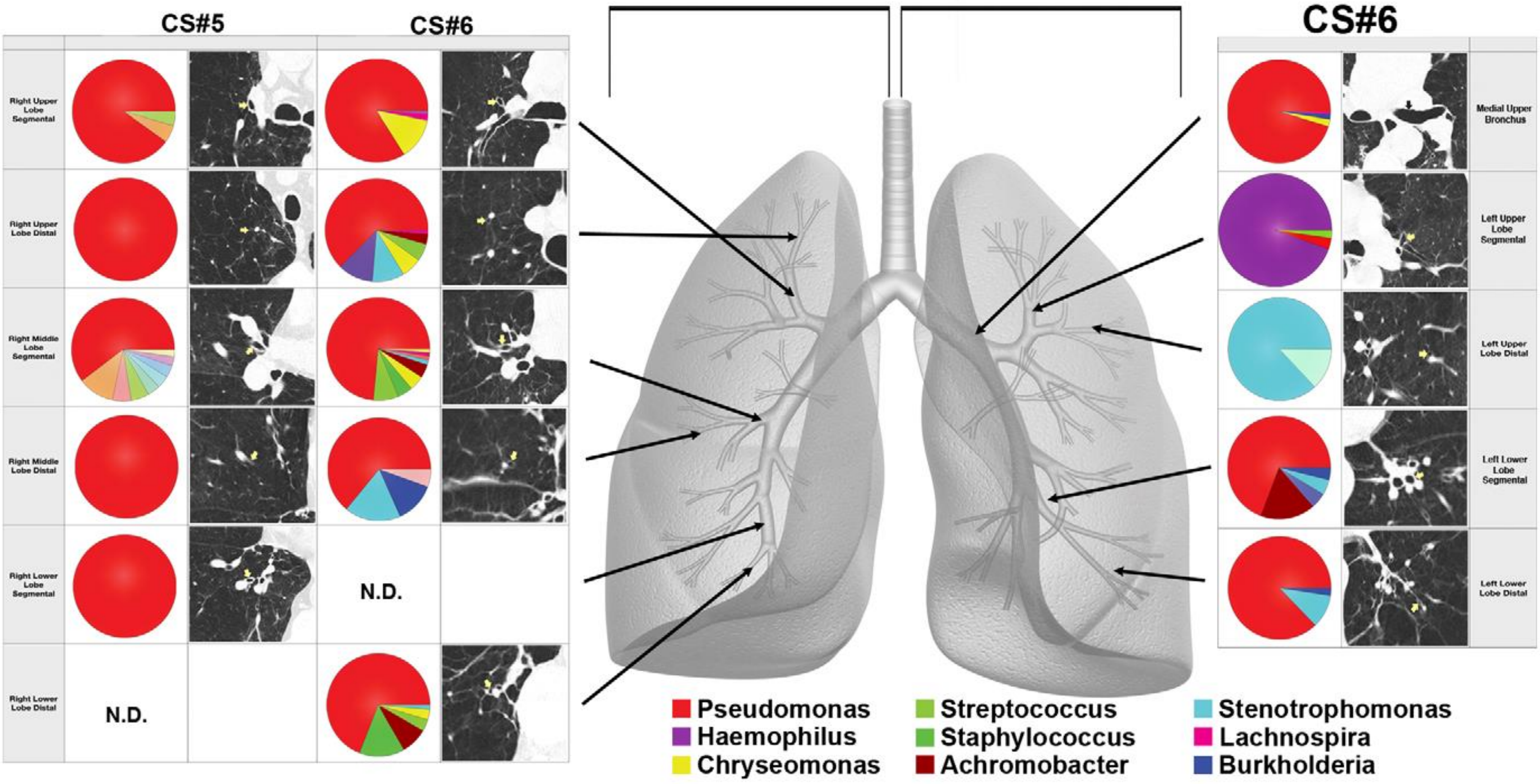
Progression of COPD

Altered proteinase–antiproteinase antibody balance

Increased proteolytic activity

Respirology. 2016;21:590-9.

# Heterogeneity and diversity in bacterial microbiota across different regions of the same lung

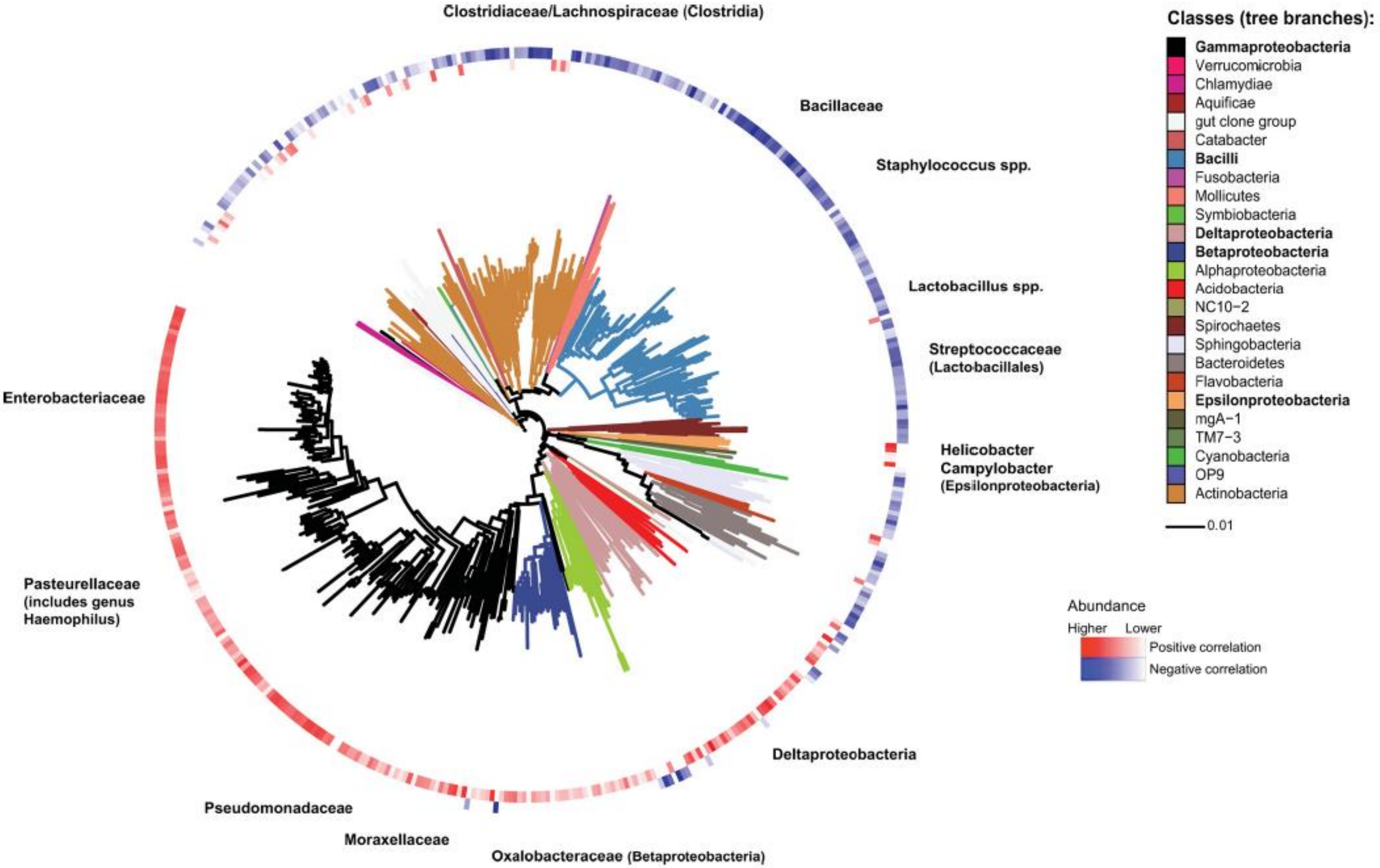


Erb-Downward et al.
PLoS One. 2011; 6(2):e16384

| Author | Year | Type and number of patients | Type of sample | Significant findings |
|---|---|---|---|---|
| | | | | advanced disease. |
| Millares, et al.[54] | 2015 | 8 severe COPD subjects | Sputum collected before and during exacerbation | The bronchial microbiome as a whole is not significantly modified when exacerbation symptoms appear in severe COPD patients, but predicted functional capabilities of microbiota show significant changes in several pathways. |
| Sze et al.[23] | 2015 | 5 subjects with COPD and 4 controls | Surgically excised lung tissue | Decline in microbial diversity associated with emphysematous destruction. Specific OTUs were also associated with neutrophils, eosinophils, and B-cell infiltration. The expression profiles of 859 genes and 235 genes were associated with either enrichment or reductions of Firmicutes and Proteobacteria. |
| Aguirre, et al.[55] | 2015 | 19 subjects with COPD | Expectorated sputum | Using conventional culture, 3 phyla, and 20 bacterial genera were identified, whereas the pyrosequencing approach detected 9 phyla and 43 genera. Enterobacteriaceae, detected frequently in conventional culture, were not significantly detected with pyrosequencing methods. Haemophilus and Moraxella were detected more frequently by 454-pyrosequencing. |
| Su, et al.[56] | 2015 | 6 subjects hospitalized with acute exacerbation of COPD | Expectorated sputum | *Acinetobacter*, *Prevotella*, *Neisseria*, *Rothia*, *Lactobacillus*, *Leptotrichia*, *Streptococcus*, *Veillonella*, and *Actinomyces* were the most commonly identified genera. The fungal population was typically dominated by *Candida*, *Phialosimplex*, *Aspergillus*, *Penicillium*, *Cladosporium*, and *Eutypella*. |
| Wang, et al.[57] | 2016 | 87 subjects with COPD | Sputum samples collected during stable state, exacerbation, 2 and 6 weeks post-therapy | Dynamic lung microbiota where changes appeared to be associated with exacerbation events and indicative of specific exacerbation phenotypes. Antibiotic and steroid treatments appear to have differential effects on the lung microbiome. |

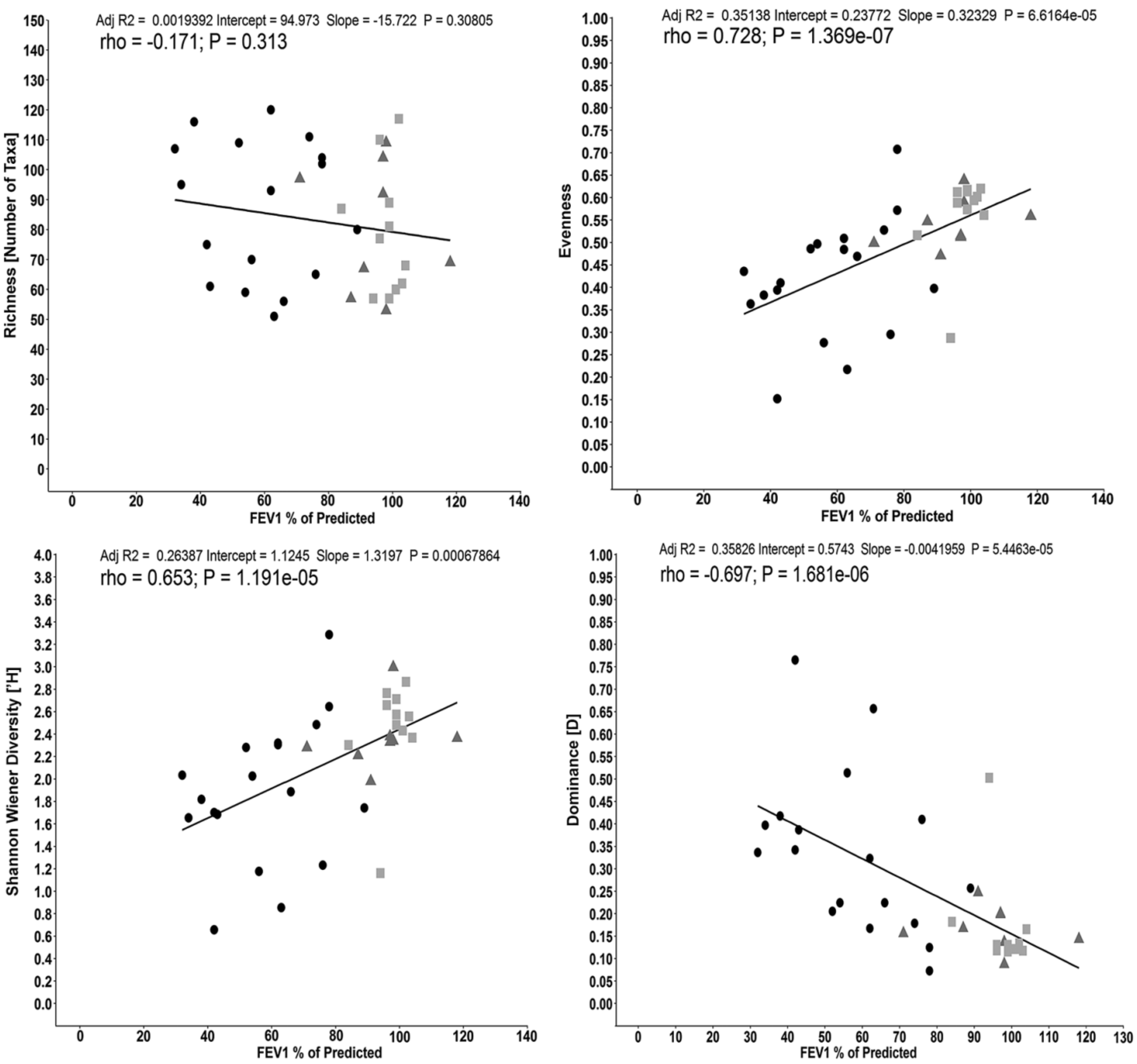
Translational Research 2017;179:71–83

# Correlations between the relative abundance of *H. influenzae* (a *Pasteurellaceae/Gammaproteobacteria*) and that of all other identified taxa



Journal of Clinical Microbiology 2014;52: 2813–23

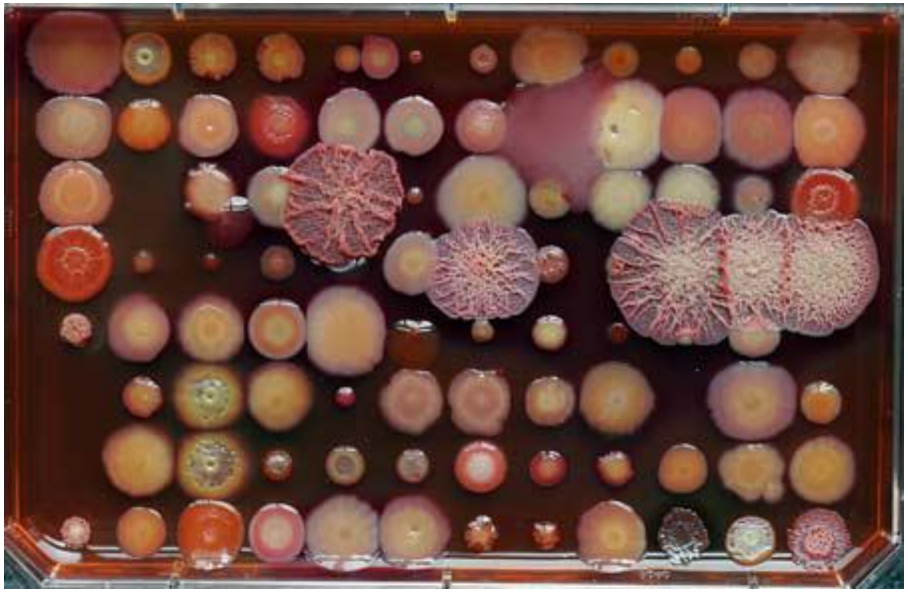
# Microbiome vs. FEV1% in COPD



Thorax 2016;71:795–803

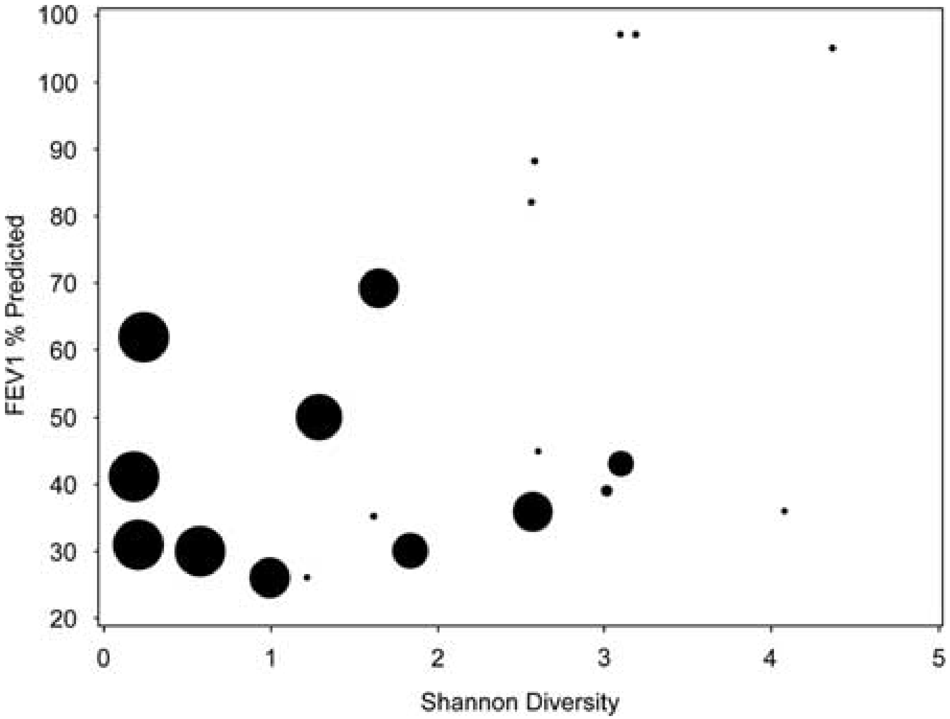
# Pseudomonas aeruginosa in COPD

## Morphotypes

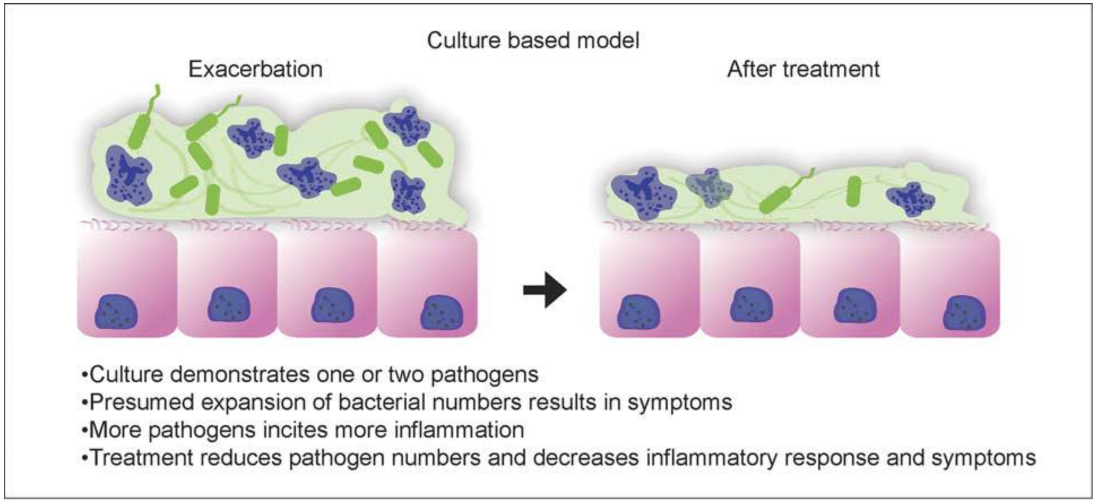


H. Kulasekara and L. Hoffman

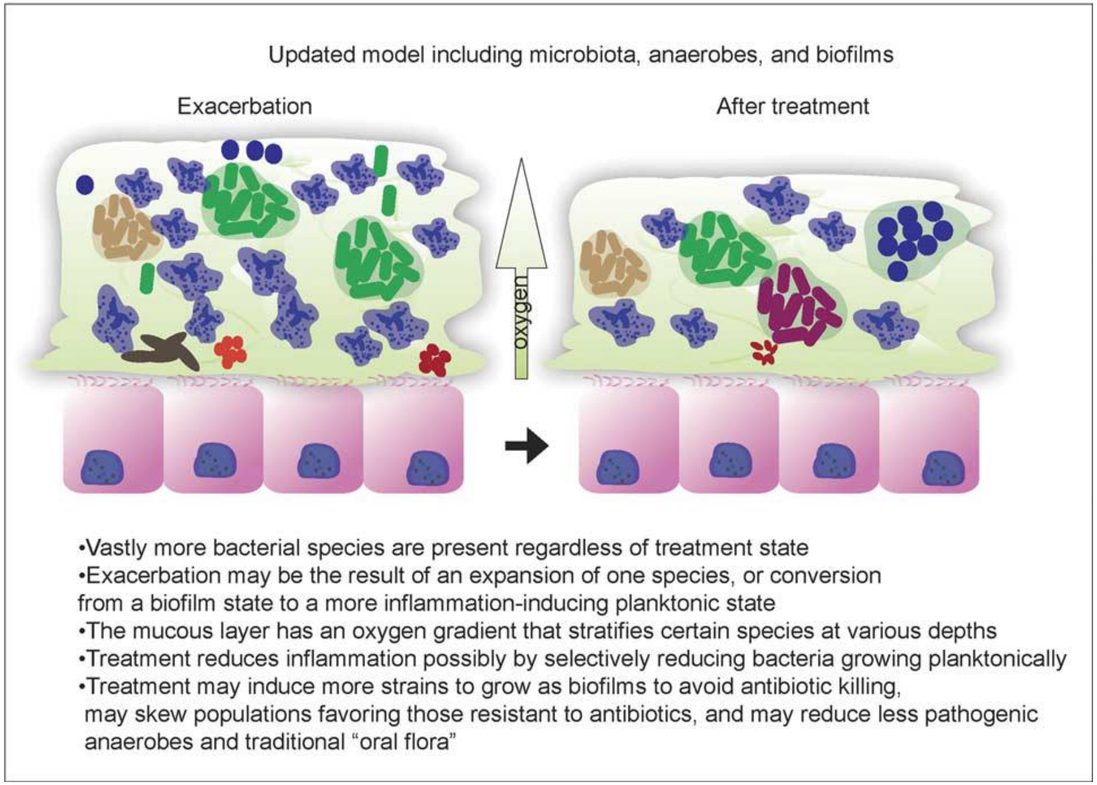
## Diversity of the microbiota VS. lung function



*Clin Pulm Med* 2016;23:57–66



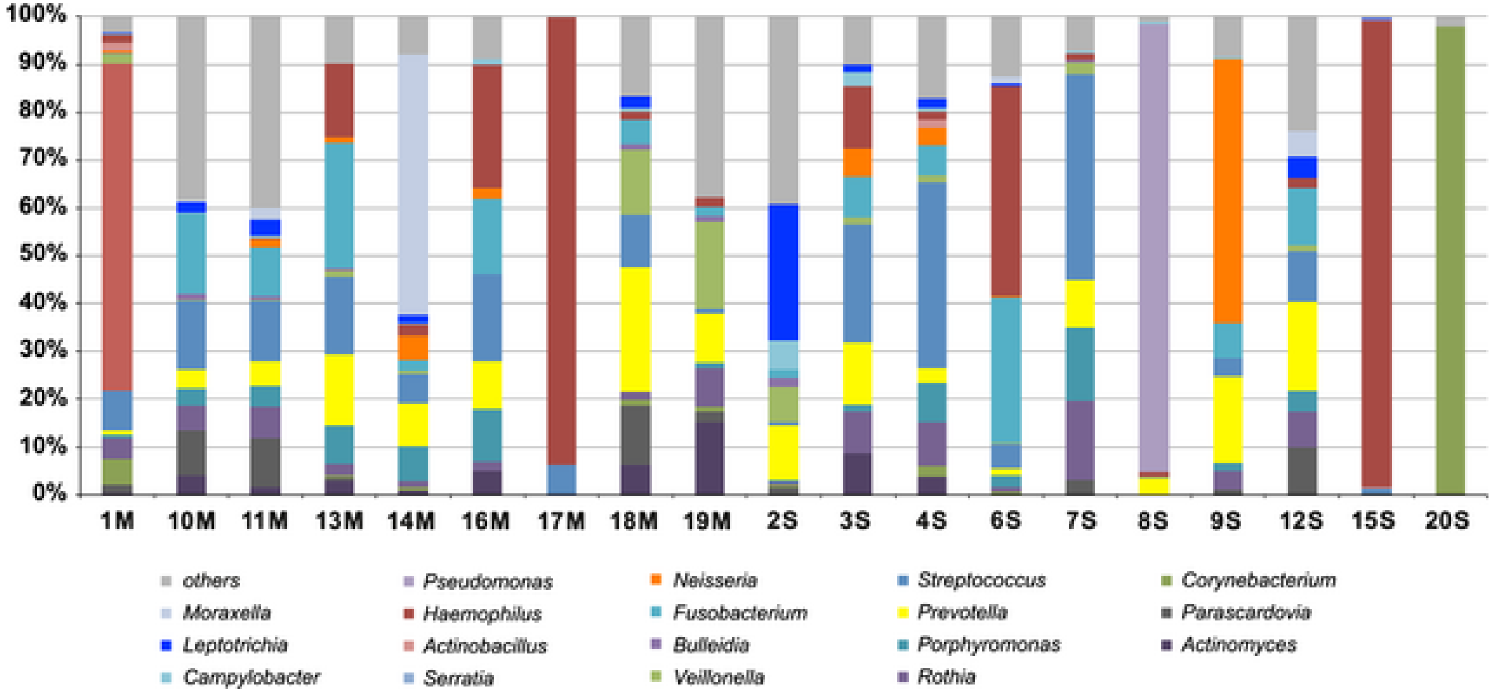
- Culture demonstrates one or two pathogens
- Presumed expansion of bacterial numbers results in symptoms
- More pathogens incites more inflammation
- Treatment reduces pathogen numbers and decreases inflammatory response and symptoms



- Vastly more bacterial species are present regardless of treatment state
- Exacerbation may be the result of an expansion of one species, or conversion from a biofilm state to a more inflammation-inducing planktonic state
- The mucous layer has an oxygen gradient that stratifies certain species at various depths
- Treatment reduces inflammation possibly by selectively reducing bacteria growing planktonically
- Treatment may induce more strains to grow as biofilms to avoid antibiotic killing, may skew populations favoring those resistant to antibiotics, and may reduce less pathogenic anaerobes and traditional "oral flora"

*Clin Pulm Med* 2016;23:57–66

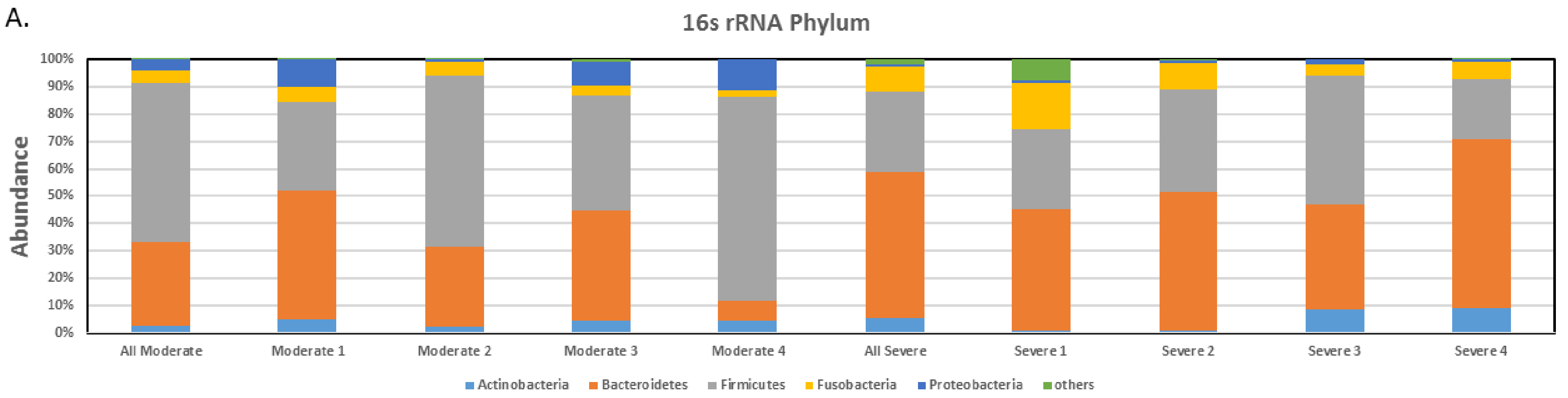
# Relative bacterial composition at the genus level in *moderate and severe (M or S) stable* COPD

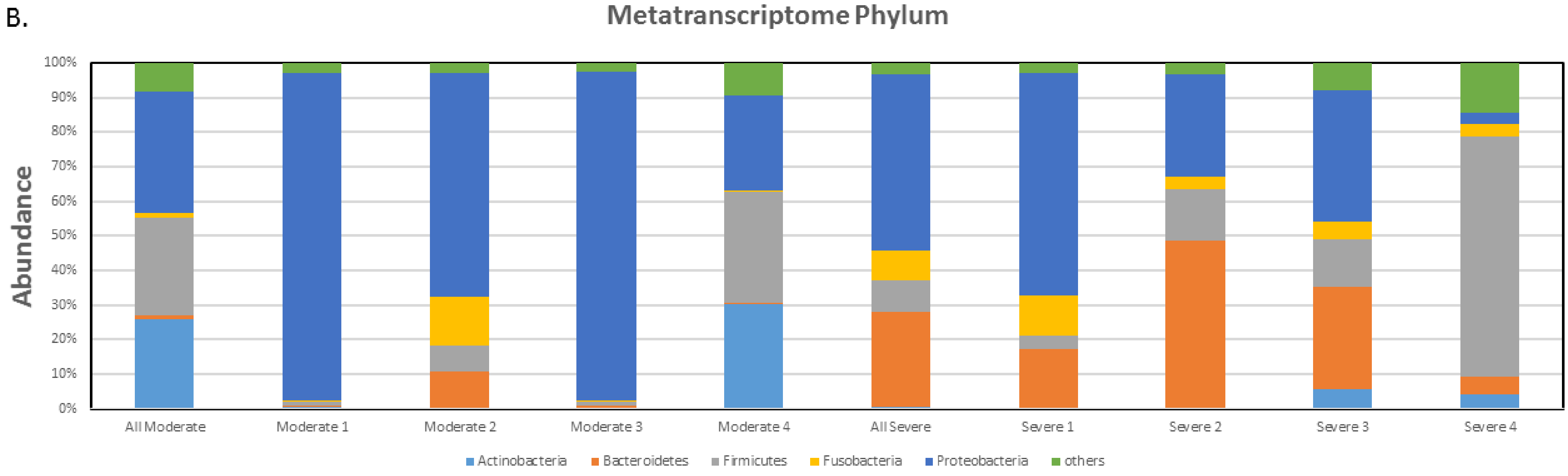


- 19 stable chronic obstructive pulmonary disease patients (mean (SD) FEV1: 47 (18%)
- Haemophilus and Moraxella were detected more frequently with the new genetic procedures

Aquirre E, et al. APMIS. 2015 May;123(5):427-32

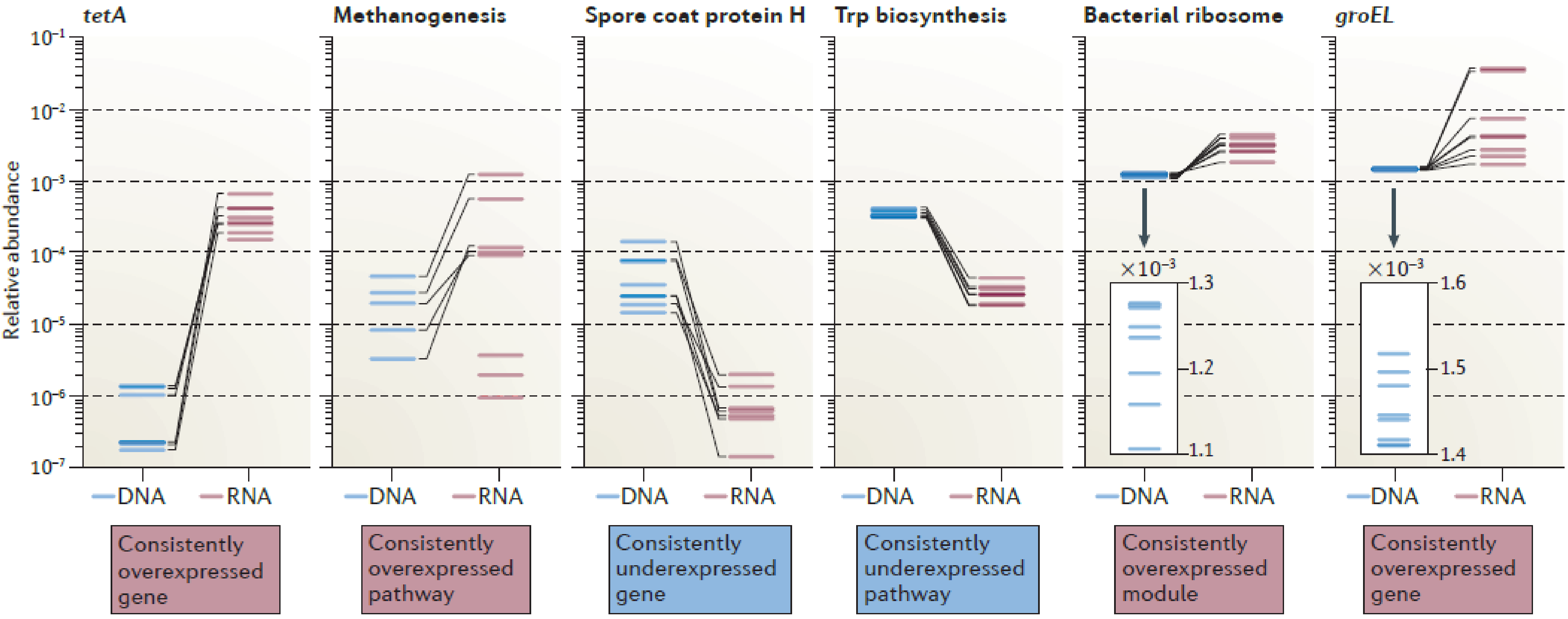
# Metagenome and Metatranscriptome
of Moderate and Severe COPD Sputum





PLOS ONE. 2016 Jul

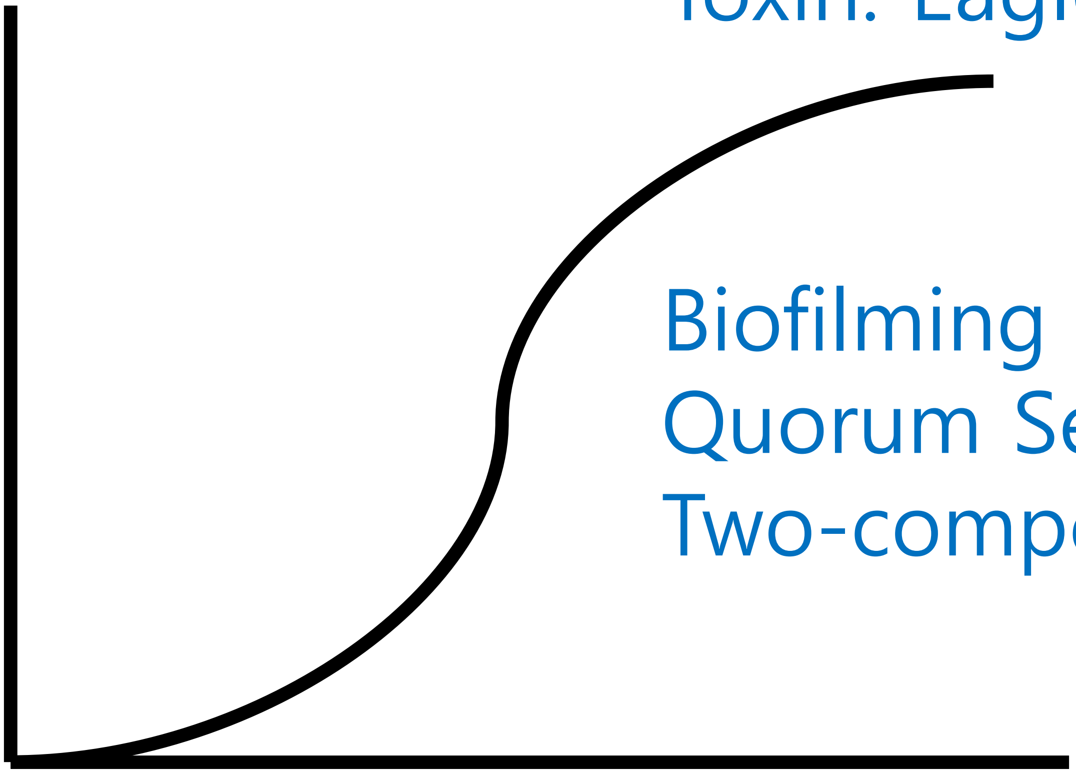
# Metatranscriptome and Metagenome



Nature 2015;13:360-

# Sociomicrobiology

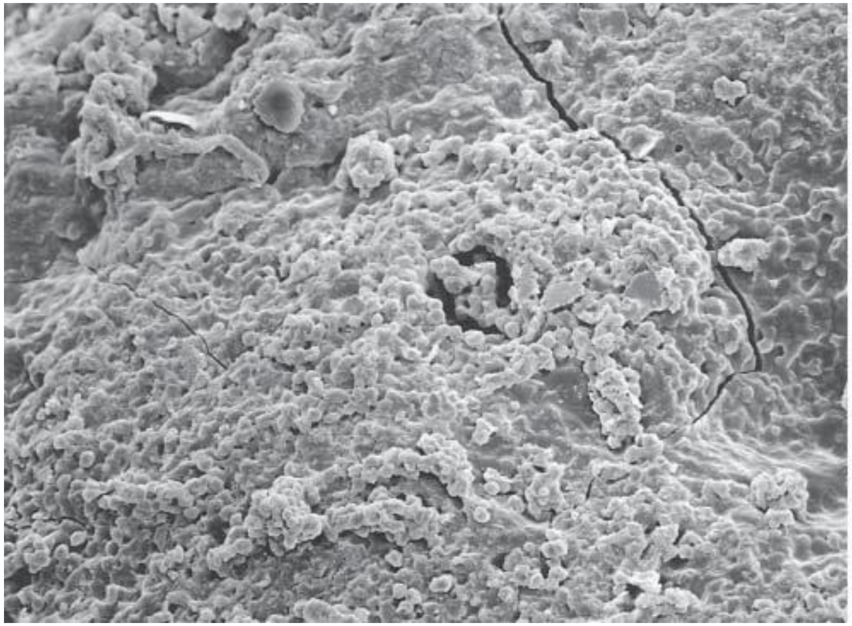
# Bacterial growth and behavior



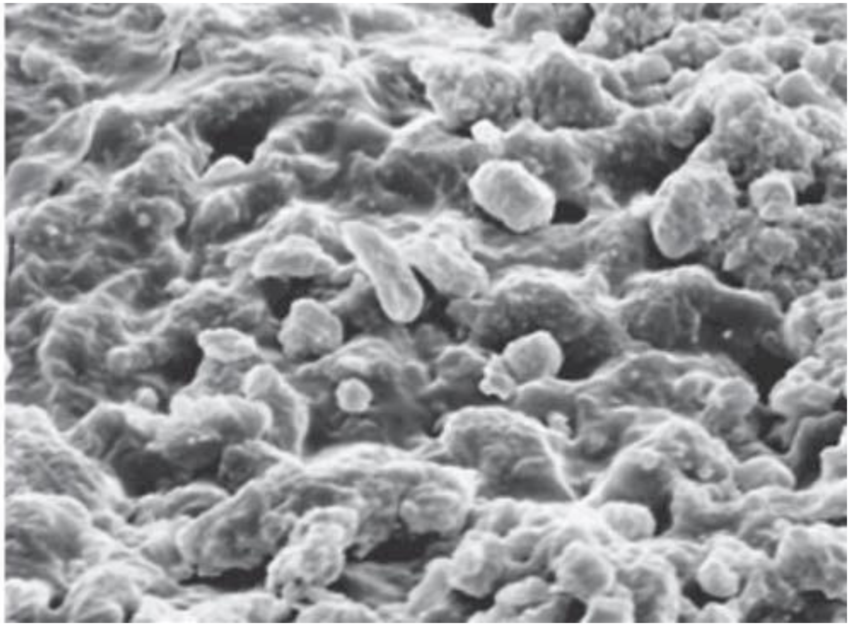
Toxin: Eagle’s effect

Biofilming
Quorum Sensing
Two-component system

# Biofilm Architecture (SEM)



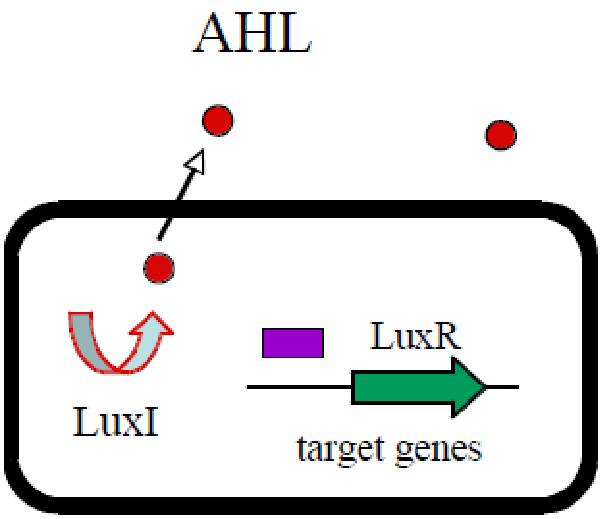
X 500



X 2,000

*Arch Otolaryngol Head Neck Surg. 2007;133:110-114*

# LuxI/LuxR–type quorum sensing in Gram(-) bacteria



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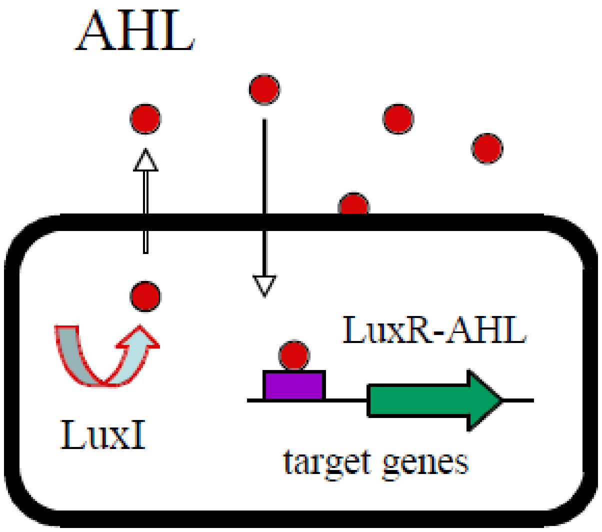
at low cell density



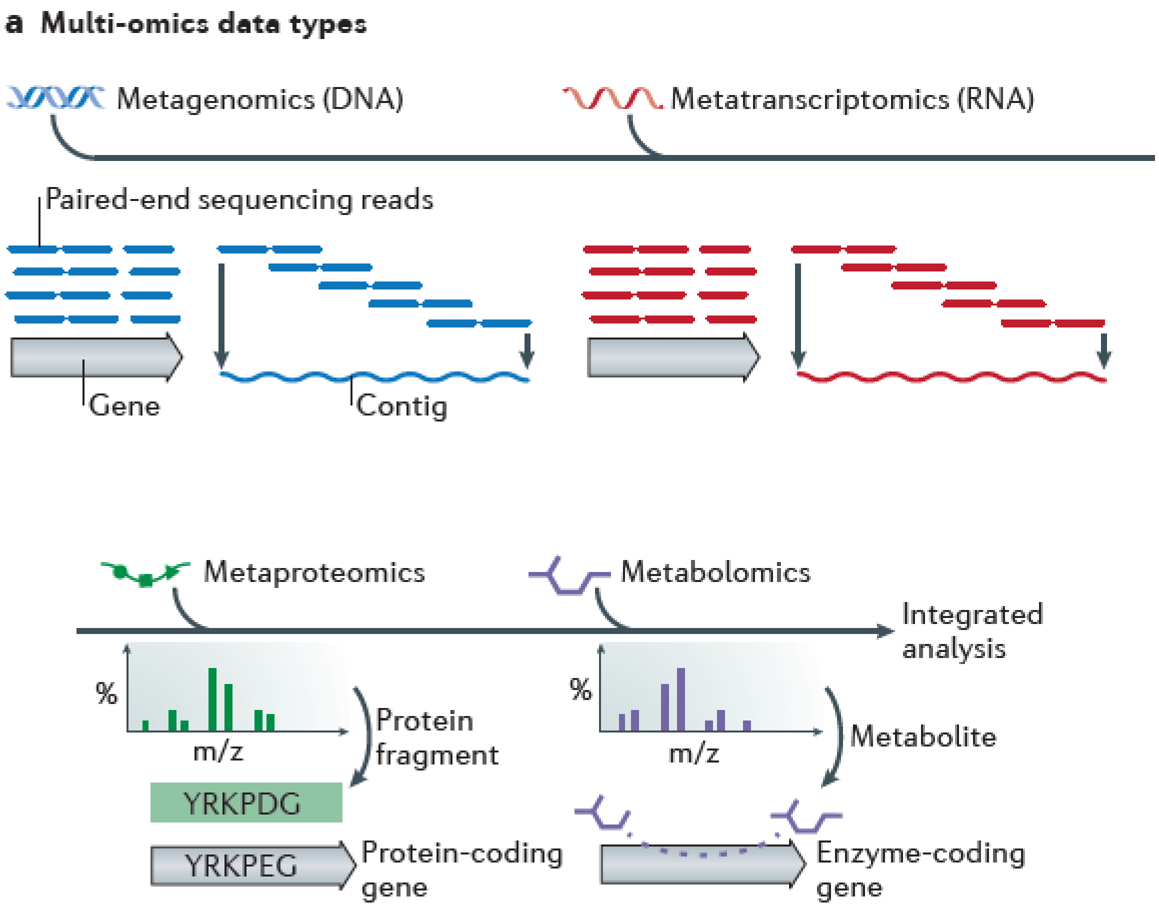
Transcription is activated
at high cell density

Sensors (Basel). 2012; 12(3): 2519–2538.

# Integromics

# Integrating multi-omic data for deeper biological insights

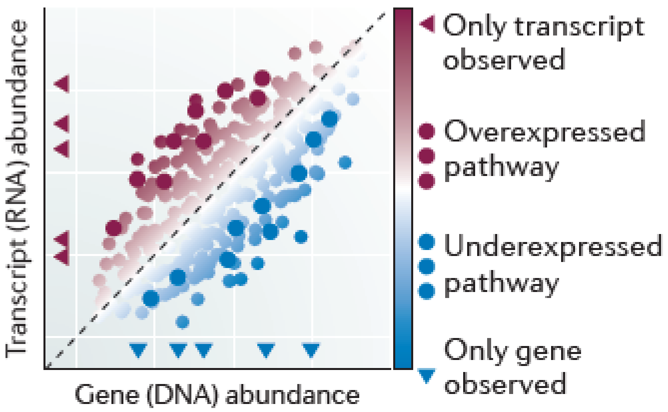
## Multi-omics data types



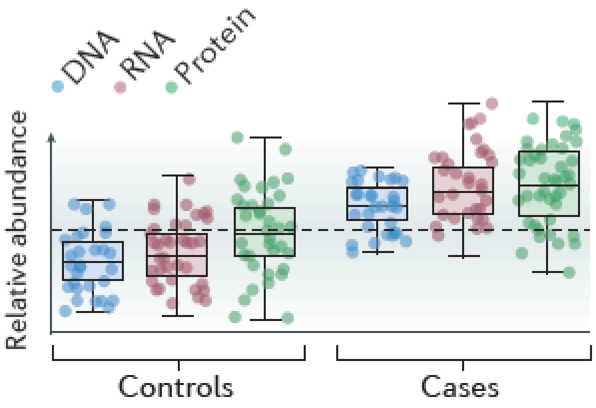
Nature 2015;13:360-

# Integrating multi-omic data for deeper biological insights

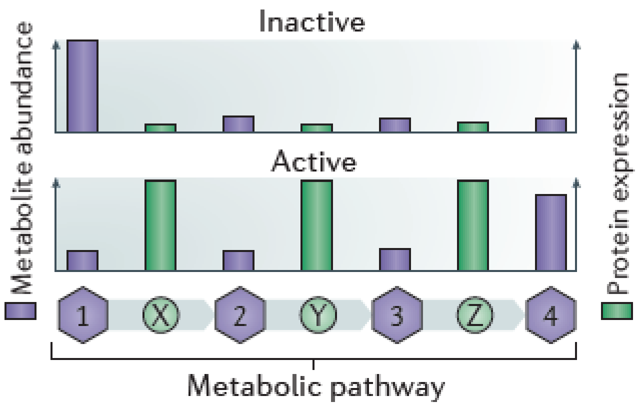
**b Normalization**



**c Strengthening hypotheses**

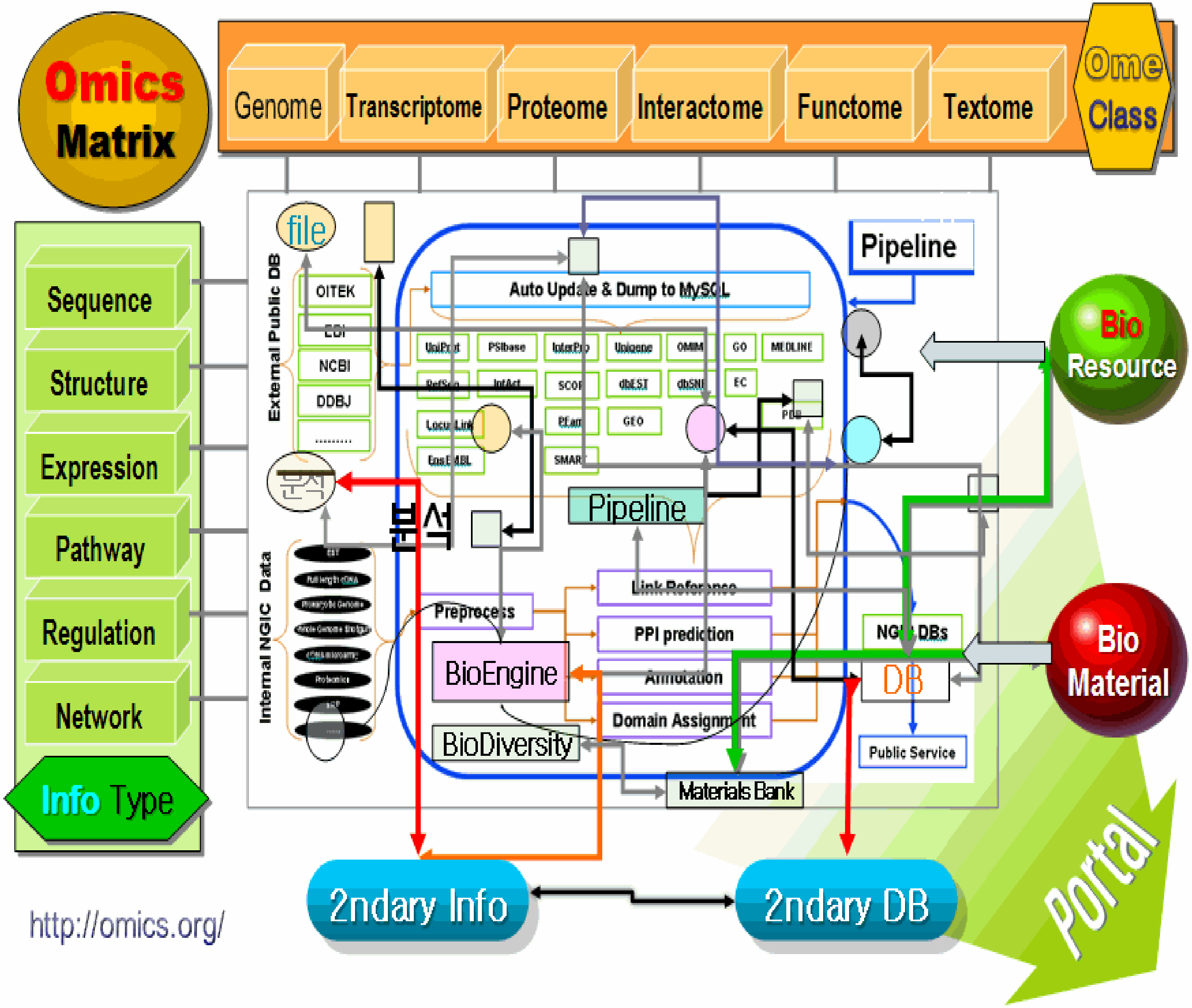


**d Descriptive modelling**



Nature 2015;13:360-

# The Omics Matrix and Integromics



http://omics.org/

# Just Beginning in Airways!

1. Microbiome; metaprofiling -->
2. functional metagenomics, metatranscriptomics, metaproteomics, metametabolomics, multiomics, integromics
3. Unique microbimes and interactomics in asthma, COPD, other respiraotry diseases.
4. AsthmaNet, COMETIPF, etc

1. **Clinical Design!**

2. **Clinical Oriented Analysis Software!**

**Clinical Microbiome**

**We Must Know!**

**We Will Know!**

**Respiratory Microbiome.**

**Thank you for your kind attention!**