Microbiome in Asthma & COPD

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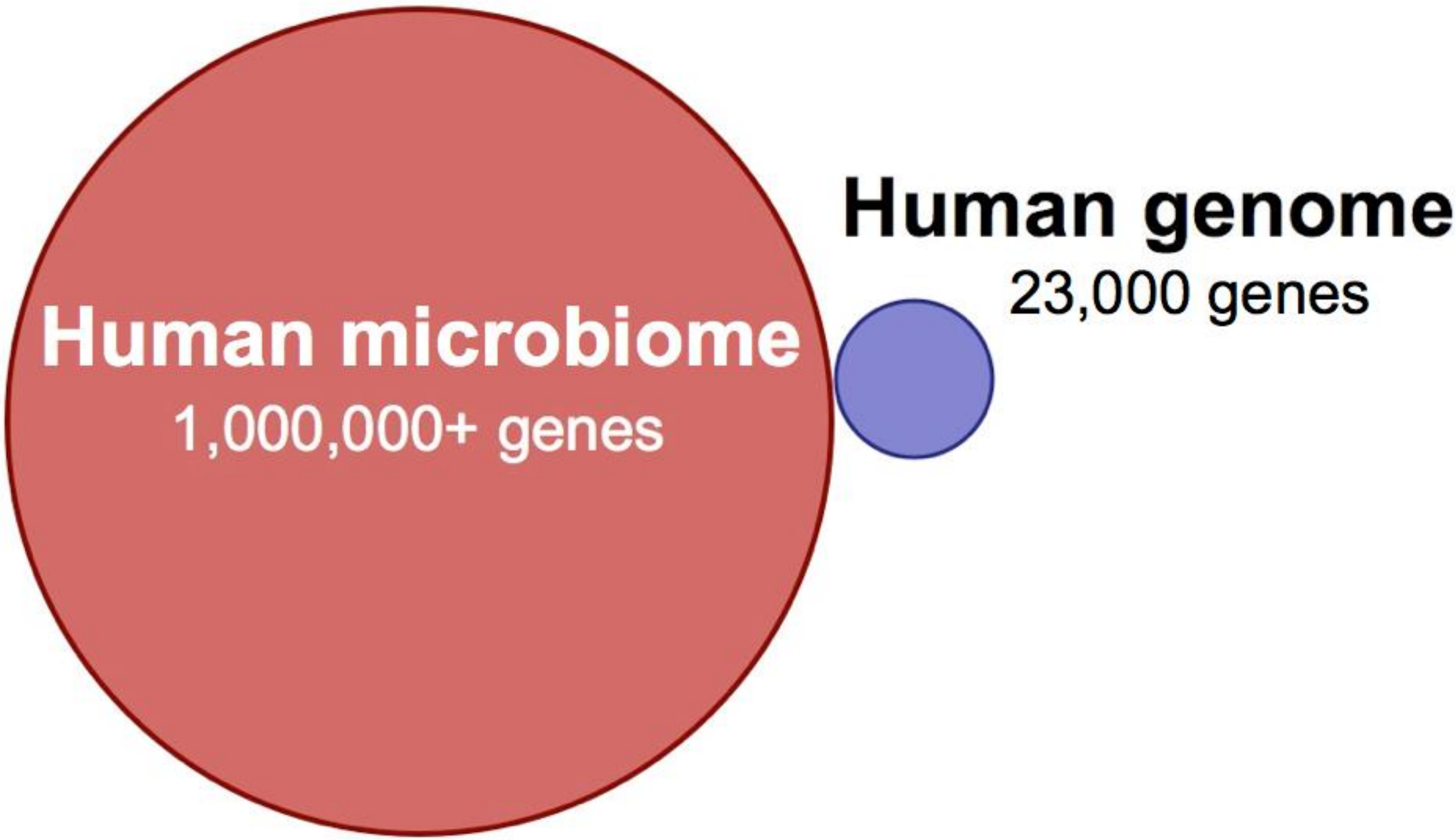
INTRODUCTION

Microbiome

- "The ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space"
- **Microbiota**
  - "collection of organisms"
- **Microbiome**
  - "collection of genes"

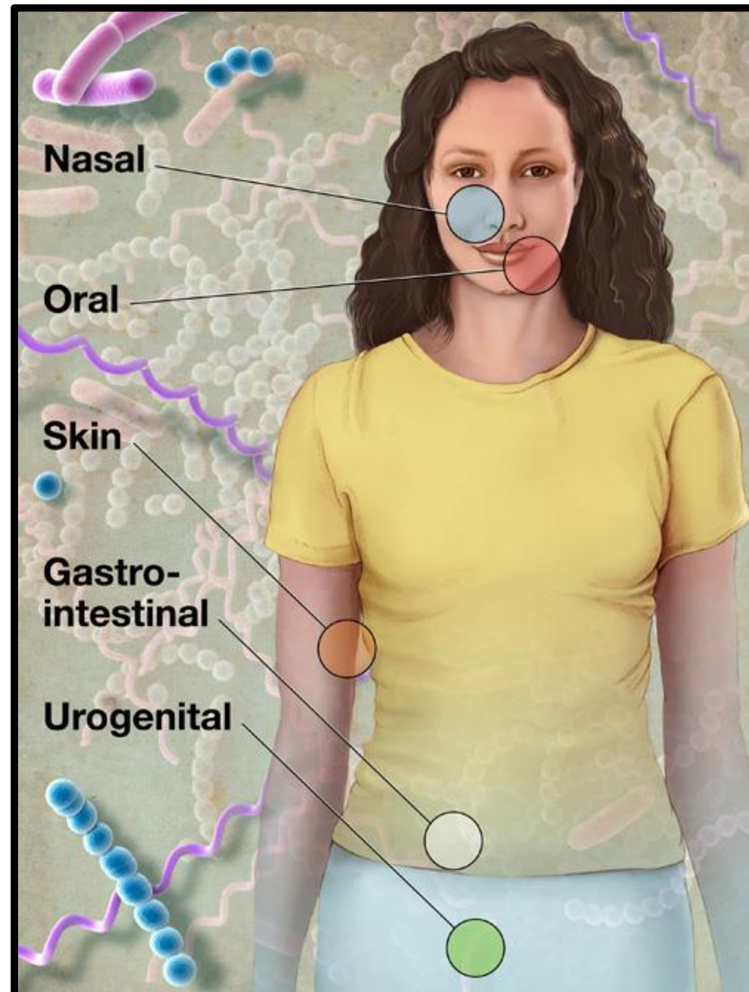

Human microbiome
1,000,000+ genes
Human genome
23,000 genes

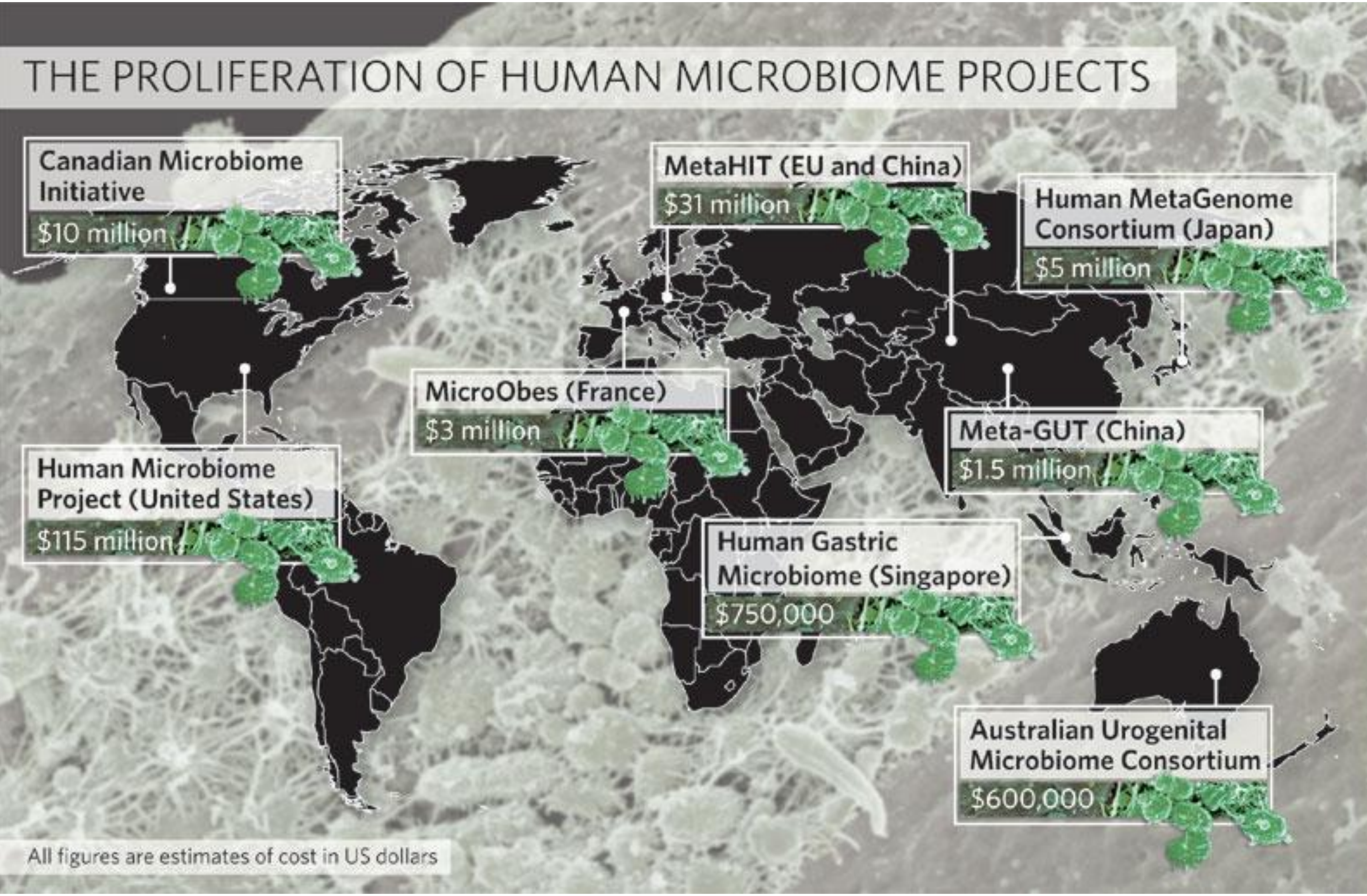
NIH Roadmap for medical research Human Microbiome Project (HMP), 2007

5 years, & 115 million

- Key Goal : Baseline to empower future clinical studies
- Assess microbial diversity of 250 healthy individuals at 5 sites

  - 16s rRNA sequencing





Mullard *Nature* 2008

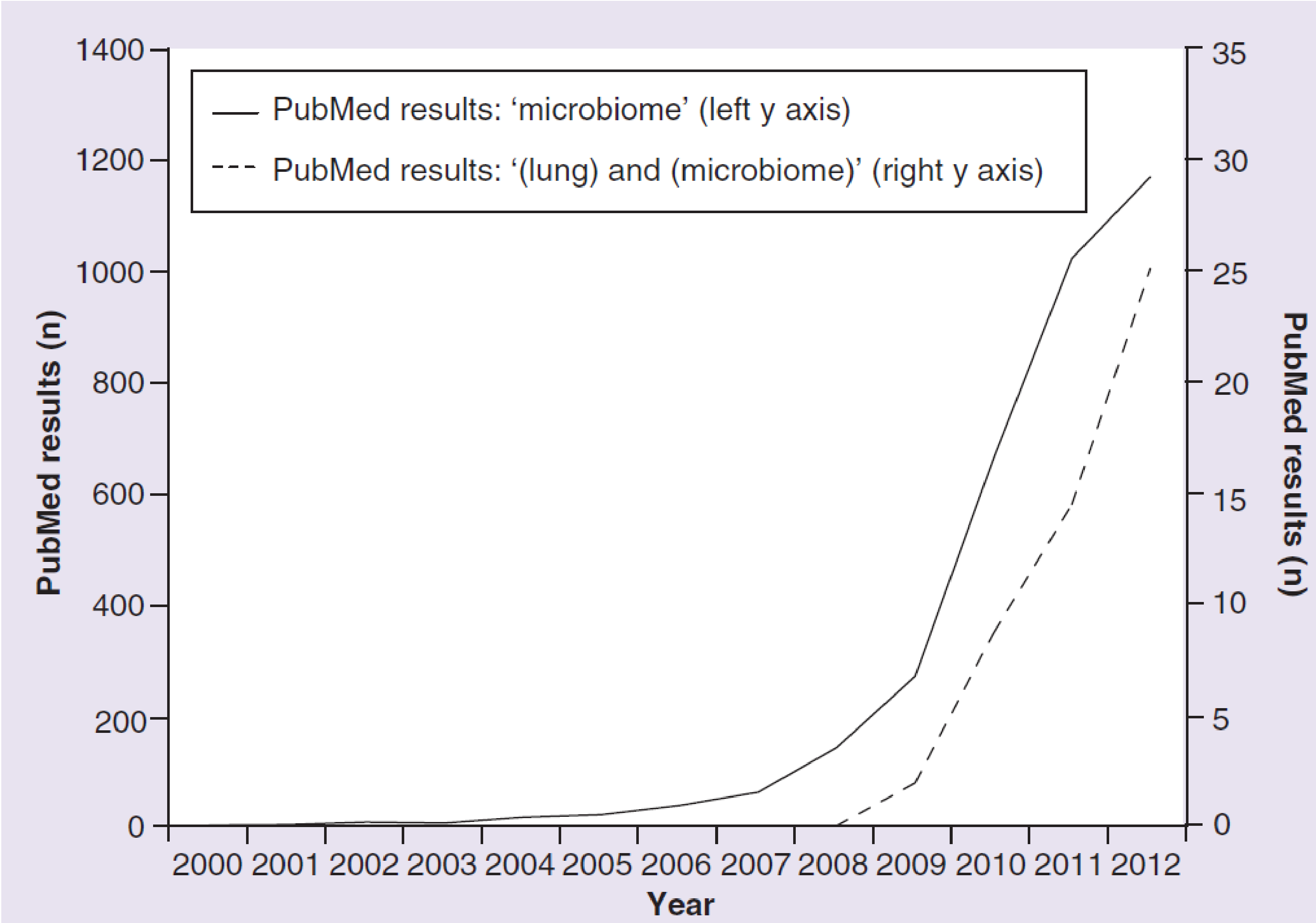


Figure 1. PubMed results by year for 'microbiome' (left y axis) and '(lung) and (microbiome)' (right y axis).

Dickson et al. *Expert Rev Respir Med* 2013;7:245-57

Associations of human conditions with particular microbiota characteristics

Disease	Relevant finding
Psoriasis	Increased ratio of Firmicutes to Actinobacteria
Reflux esophagitis	Esophageal microbiota dominated by gram-negative anaerobes; gastric microbiota with low or absent*Helicobacter pylori*
Obesity	Reduced ratio of Bacteroidetes to Firmicutes
Childhood-onset asthma	Absent gastric *H. pylori* (especially the cytotoxin-associated gene A (*cagA*) genotype)
Inflammatory bowel disease (colitis)	Larger populations of Enterobacteriaceae
Functional bowel diseases	Larger populations of *Veillonella* and *Lactobacillus*
Colorectal carcinoma	Larger populations of *Fusobacterium spp*.
Cardiovascular disease	Gut-microbiota-dependent metabolism of phosphatidylcholine

Cho and Blaser. *Nat Rev Genet* 2012;13:260-270

Genomic vs. Culture Based Methods for Detecting Bacteria

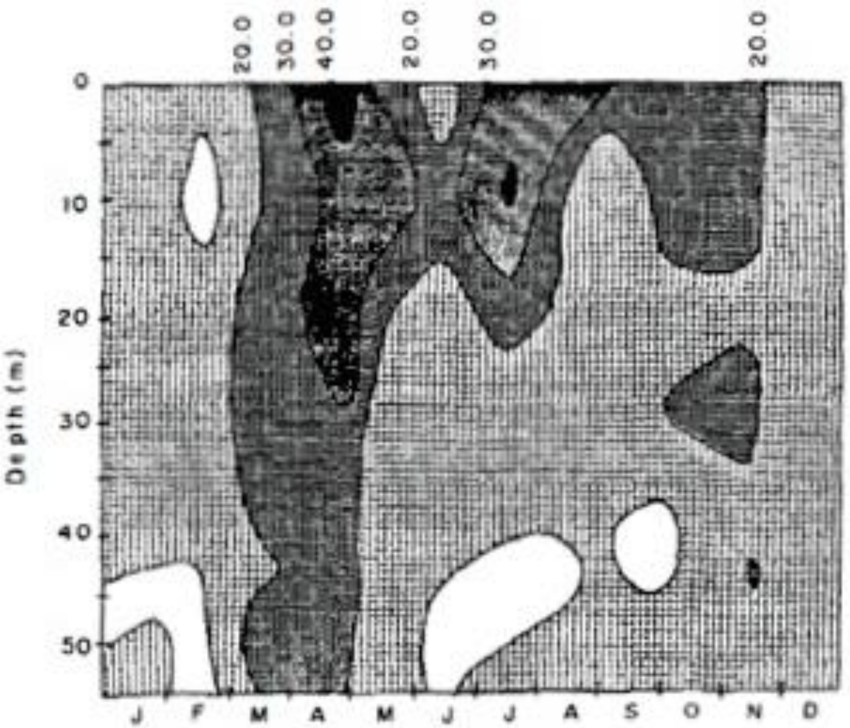


Figure 1. Direct microscopic count of bacteria in a sample of water from Lake Washington using acridine orange, a dye which illuminates bacterial DNA and RNA.

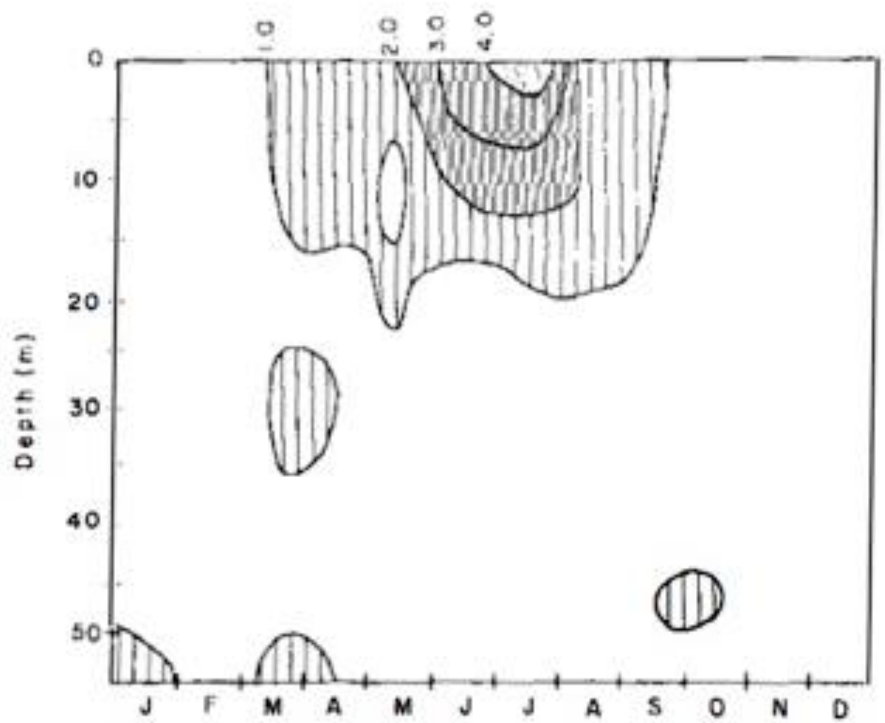


Figure 2. Count of bacteria in a sample of water from Lake Washington using a culture-based method: CPM medium.

Source: Staley JT, Konopka A. (1985). Annu Rev Microbiol, 39: 321-46.

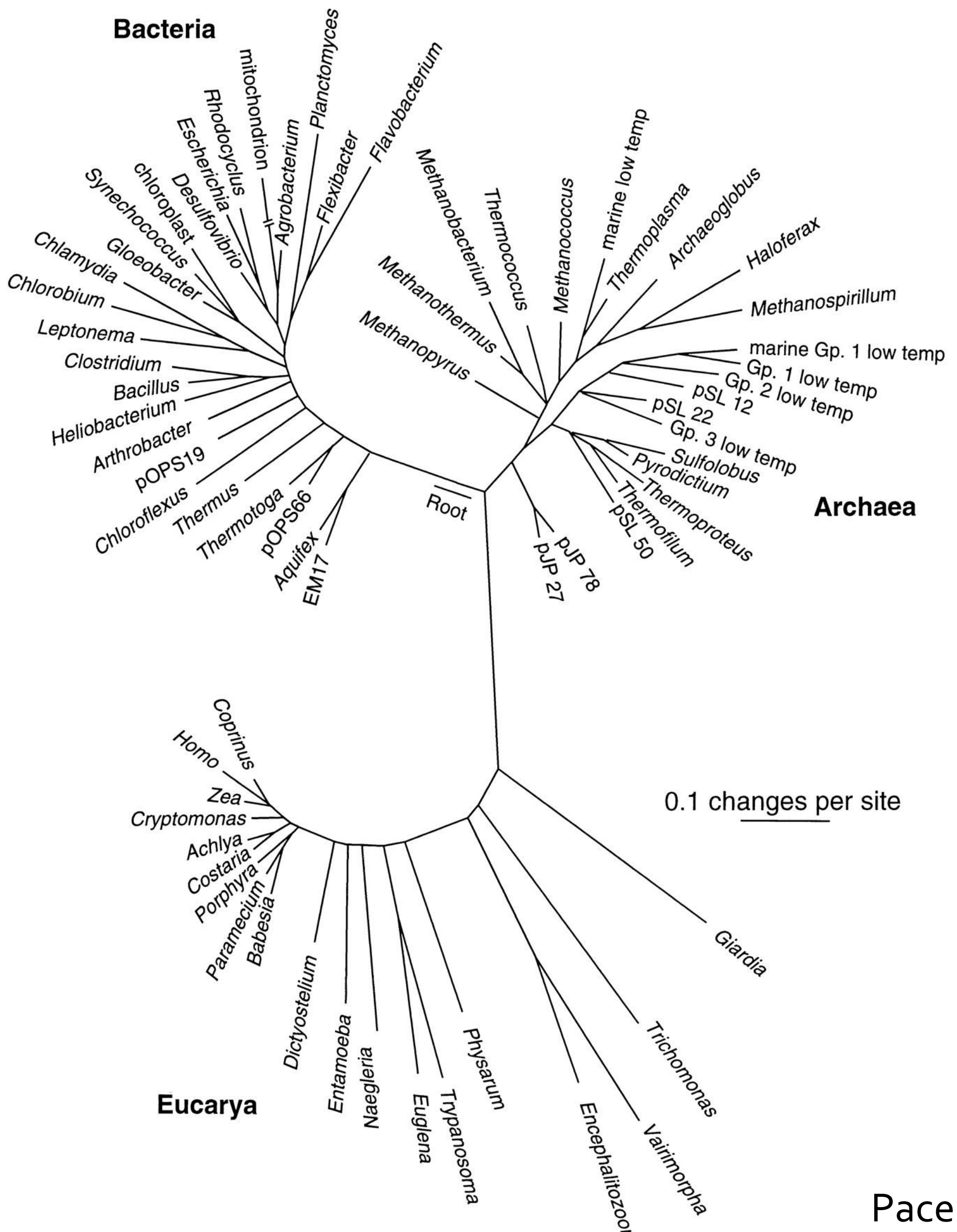
Phylogenetic structure of the prokaryotic domain: The primary kingdoms

Association coefficients (S_{AB}) between representative members of the three primary kingdoms

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *Saccharomyces cerevisiae*, 18S	—	0.29	0.33	0.05	0.06	0.08	0.09	0.11	0.08	0.11	0.11	0.08	0.08
2. *Lemna minor*, 18S	0.29	—	0.36	0.10	0.05	0.06	0.10	0.09	0.11	0.10	0.10	0.13	0.07
3. L cell, 18S	0.33	0.36	—	0.06	0.06	0.07	0.07	0.09	0.06	0.10	0.10	0.09	0.07
4. *Escherichia coli*	0.05	0.10	0.06	—	0.24	0.25	0.28	0.26	0.21	0.11	0.12	0.07	0.12
5. *Chlorobium vibrioforme*	0.06	0.05	0.06	0.24	—	0.22	0.22	0.20	0.19	0.06	0.07	0.06	0.09
6. *Bacillus firmus*	0.08	0.06	0.07	0.25	0.22	—	0.34	0.26	0.20	0.11	0.13	0.06	0.12
7. *Corynebacterium diphtheriae*	0.09	0.10	0.07	0.28	0.22	0.34	—	0.23	0.21	0.12	0.12	0.09	0.10
8. *Aphanocapsa* 6714	0.11	0.09	0.09	0.26	0.20	0.26	0.23	—	0.31	0.11	0.11	0.10	0.10
9. Chloroplast (*Lemna*)	0.08	0.11	0.06	0.21	0.19	0.20	0.21	0.31	—	0.14	0.12	0.10	0.12
10. *Methanobacterium thermoautotrophicum*	0.11	0.10	0.10	0.11	0.06	0.11	0.12	0.11	0.14	—	0.51	0.25	0.30
11. *M. ruminantium* strain M-1	0.11	0.10	0.10	0.12	0.07	0.13	0.12	0.11	0.12	0.51	—	0.25	0.24
12. *Methanobacterium* sp., Cariaco isolate JR-1	0.08	0.13	0.09	0.07	0.06	0.06	0.09	0.10	0.10	0.25	0.25	—	0.32
13. *Methanosarcina barkeri*	0.08	0.07	0.07	0.12	0.09	0.12	0.10	0.10	0.12	0.30	0.24	0.32	—

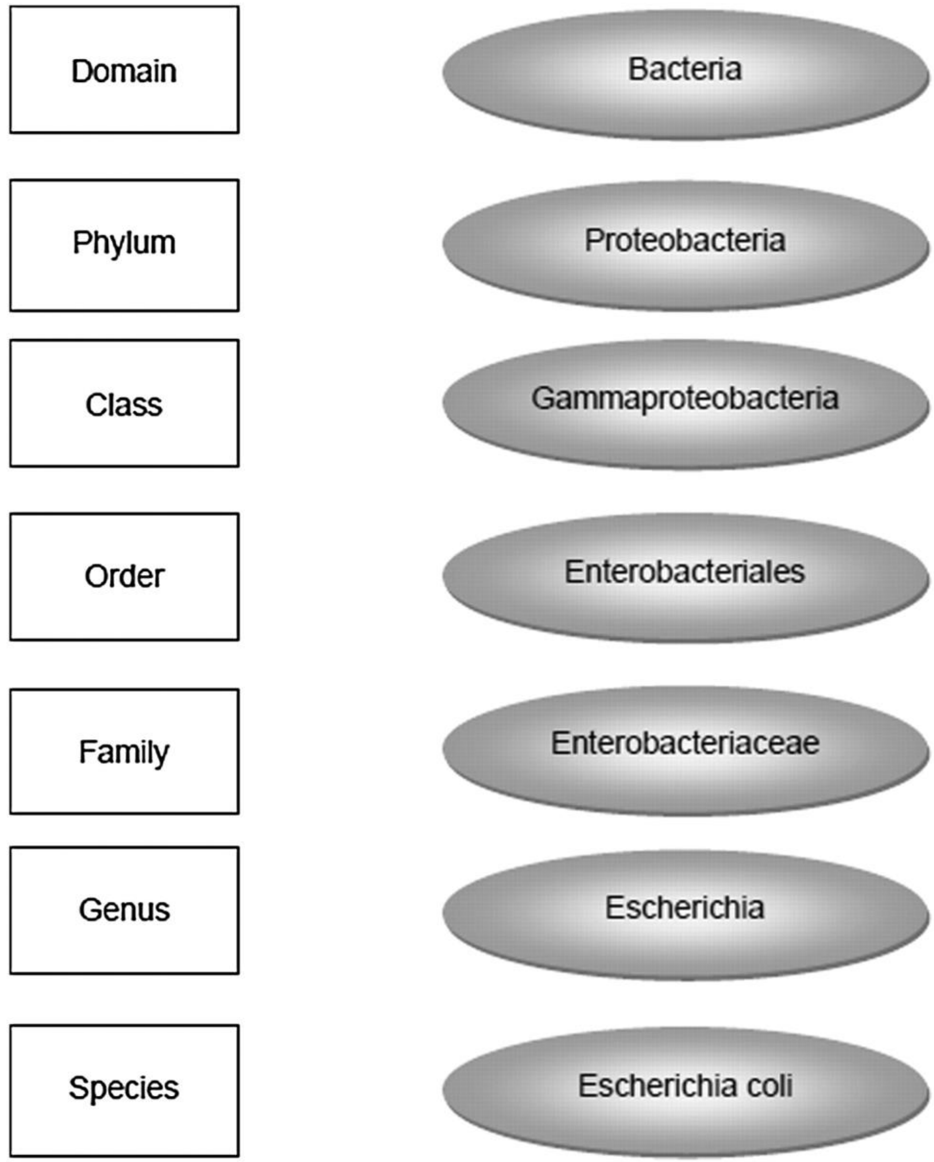
Woese and Fox. *Proc Natl Acad Sci USA* 1977;74:5088–90

Universal phylogenetic tree based on rRNA sequences



Pace. *Science* 1997;276:734-40

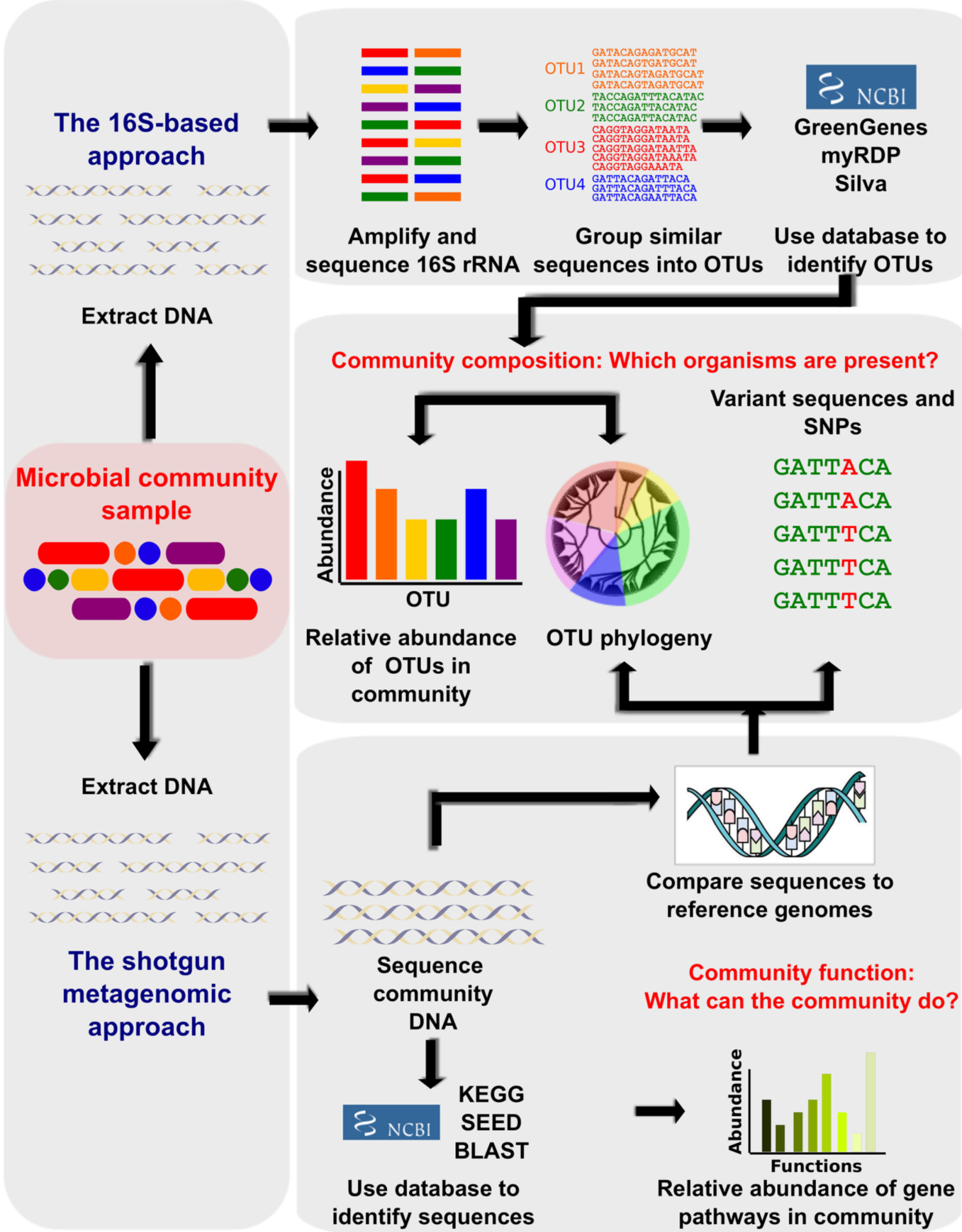
Bacterial classification system using *Escherichia coli*



Han et al. *Thorax* 2012;67:456-463

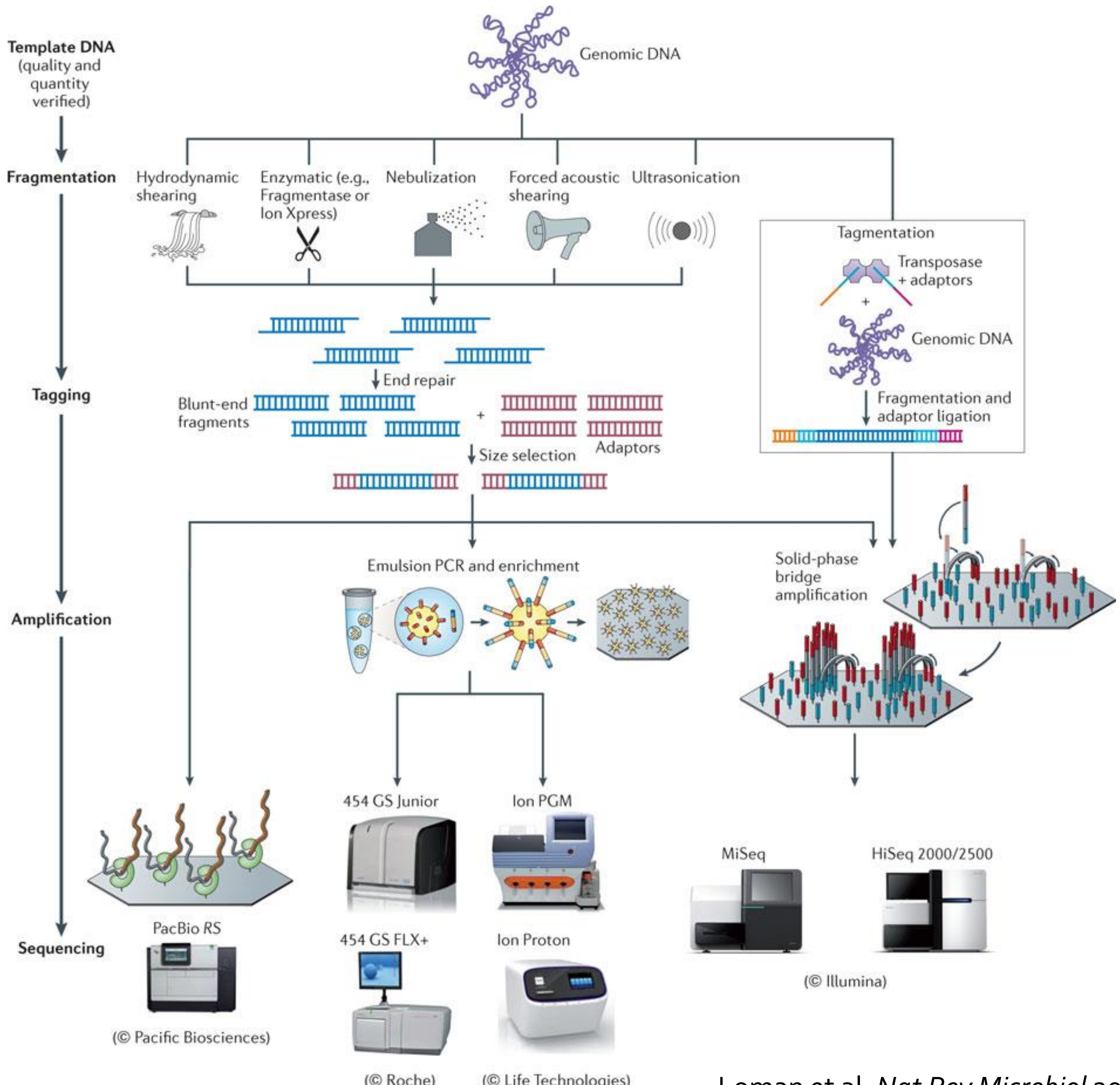
Techniques

- Real-time quantitative PCR (qPCR)
- Fluorescent in situ hybridization (FISH)
- Denaturing gradient gel electrophoresis (DGGE)
- Terminal restriction fragment length polymorphism (T-RFLP)



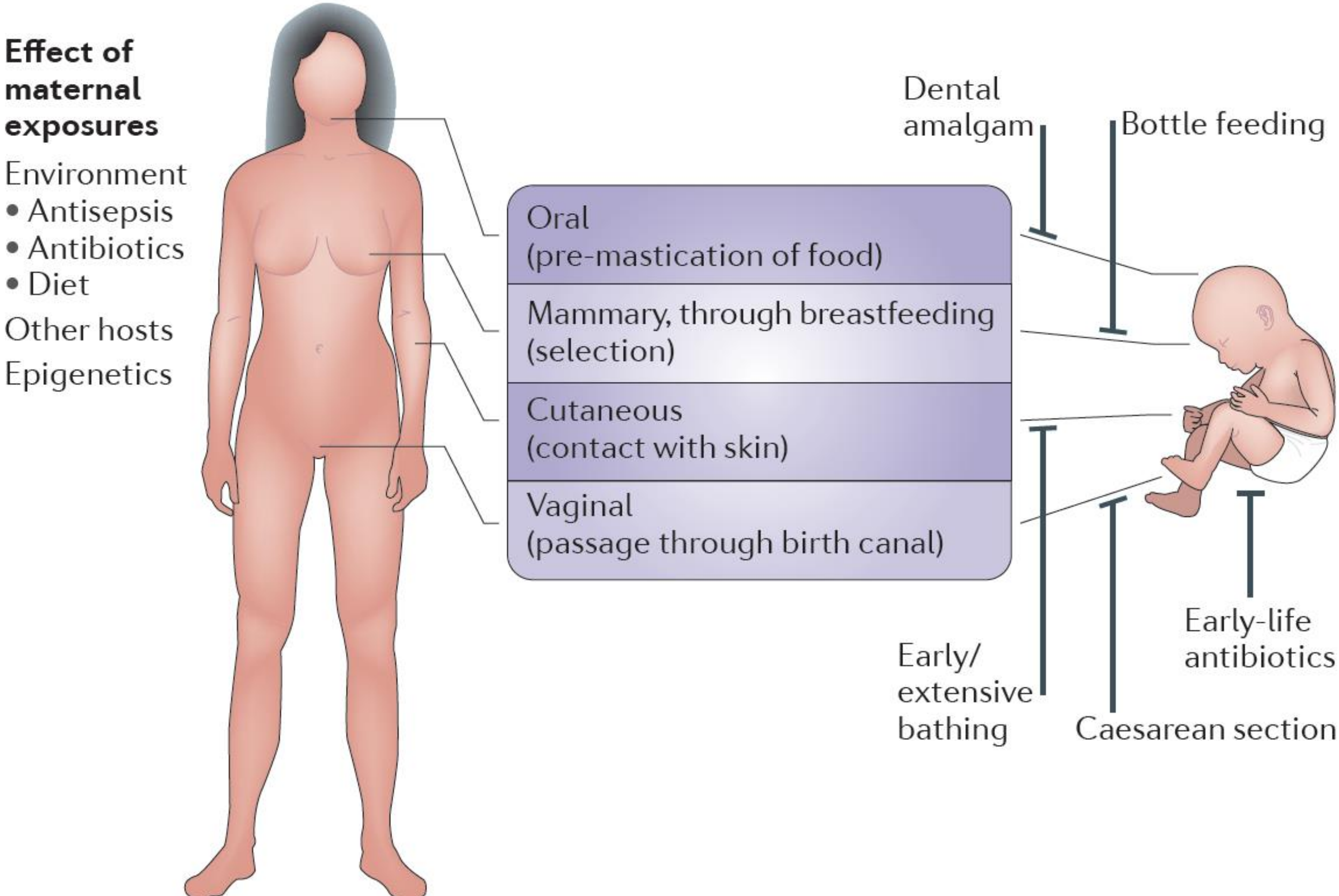
OTU = Operational Taxonomic Unit, a group of very similar 16S sequences

Morgan et al. *PLoS Comput Biol* 2012;8: e1002808



Loman et al. *Nat Rev Microbiol* 2012;10:599-606

MICROBIOME OF THE HEALTHY LUNG



Cho and Blaser. *Nat Rev Genet* 2012;13:260-270

How to sample the airways

- Sputum (spontaneous and/or induced)
- Bronchoalveolar lavage
- Protected specimen brush
- Biopsy
- Sterile tissue sample

Disordered Microbial Communities in Asthmatic Airways

Markus Hilty[1], Conor Burke[2], Helder Pedro[3,4], Paul Cardenas[1], Andy Bush[1], Cara Bossley[1], Jane Davies[1], Aaron Ervine[2], Len Poulter[2], Lior Pachter[4], Miriam F. Moffatt[1], William O. C. Cookson[1*]

Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD

John R. Erb-Downward[1], Deborah L. Thompson[1], Meilan K. Han[1], Christine M. Freeman[1,2], Lisa McCloskey[1,2], Lindsay A. Schmidt[1], Vincent B. Young[1], Galen B. Toews[1,2], Jeffrey L. Curtis[1,2], Baskaran Sundaram[1], Fernando J. Martinez[1¶], Gary B. Huffnagle[1*¶]

Topographical Continuity of Bacterial Populations in the Healthy Human Respiratory Tract

Emily S. Charlson[1,2], Kyle Bittinger[1], Andrew R. Haas[2], Ayannah S. Fitzgerald[2], Ian Frank[3], Anjana Yadav[2], Frederic D. Bushman[1*], and Ronald G. Collman[1,2*]

The Lung Tissue Microbiome in Chronic Obstructive Pulmonary Disease

Marc A. Sze[1], Pedro A. Dimitriu[2], Shizu Hayashi[1], W. Mark Elliott[1], John E. McDonough[1], John V. Gosselink[1], Joel Cooper[3], Don D. Sin[1], William W. Mohn[2], and James C. Hogg[1]

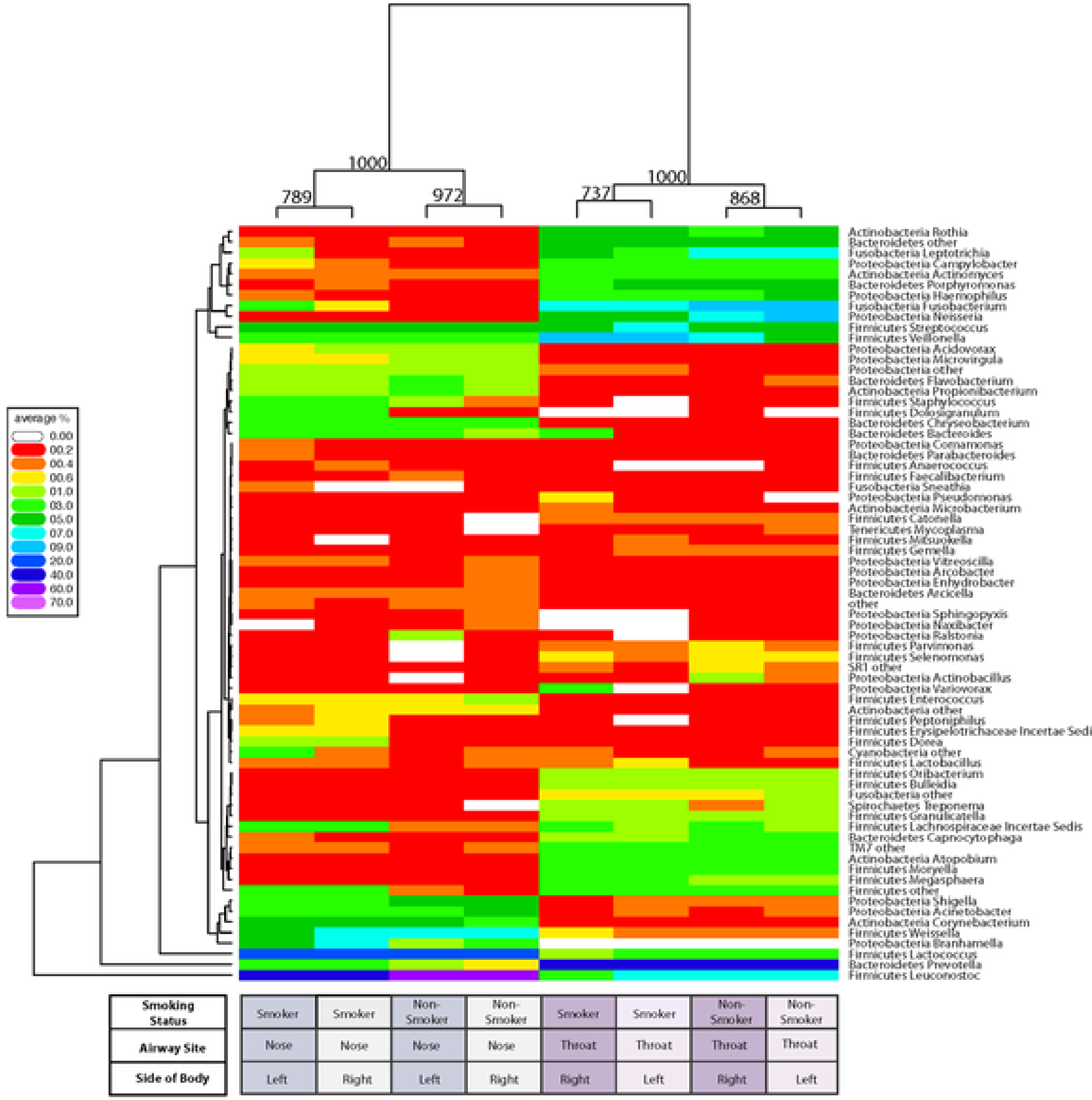
[1]The James Hogg Research Centre, Providence Heart-Lung Institute at St. Paul's Hospital, Departments of Medicine and Pathology and Laboratory Medicine, and [2]Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada; and [3]Department of Cardiovascular and Thoracic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania

Am J Respir Crit Care Med 2012;185:1073-1080

Taxonomy of phyla and genera in human lung microbiome

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

Smoking & Microbiota



Charlson et al. *PLoS ONE* 2010;5: e15216

Unanswered Questions

- How are the bacterial communities of the lung established after delivery?
- Are the bacterial communities of the lung stable or dynamic over time?
- Is there geographic variability in the constitution of lung microbial communities?

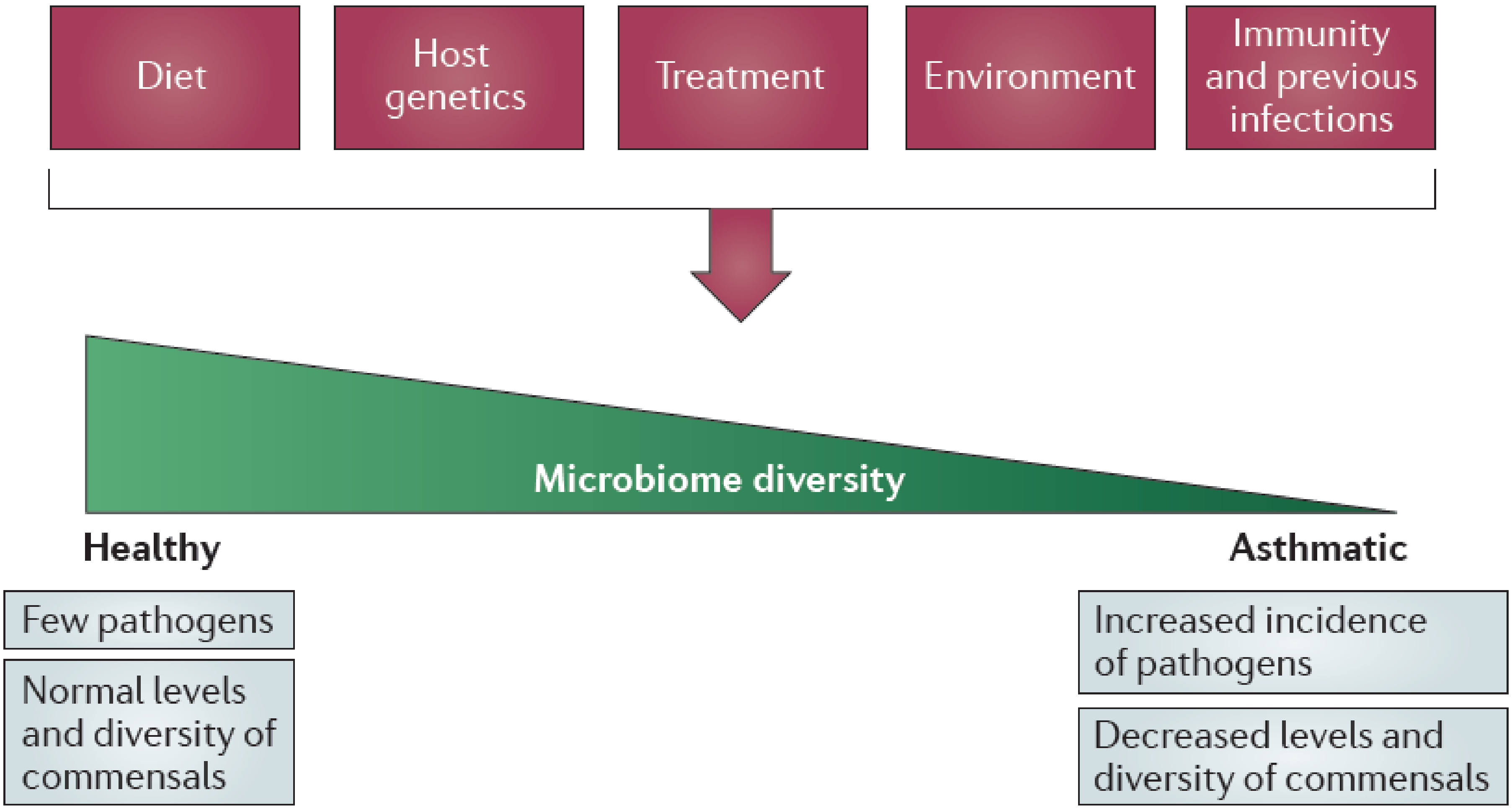
Unanswered Questions

- How do microbiota of the lung influence and are they influenced by microbes in other compartments of the body?

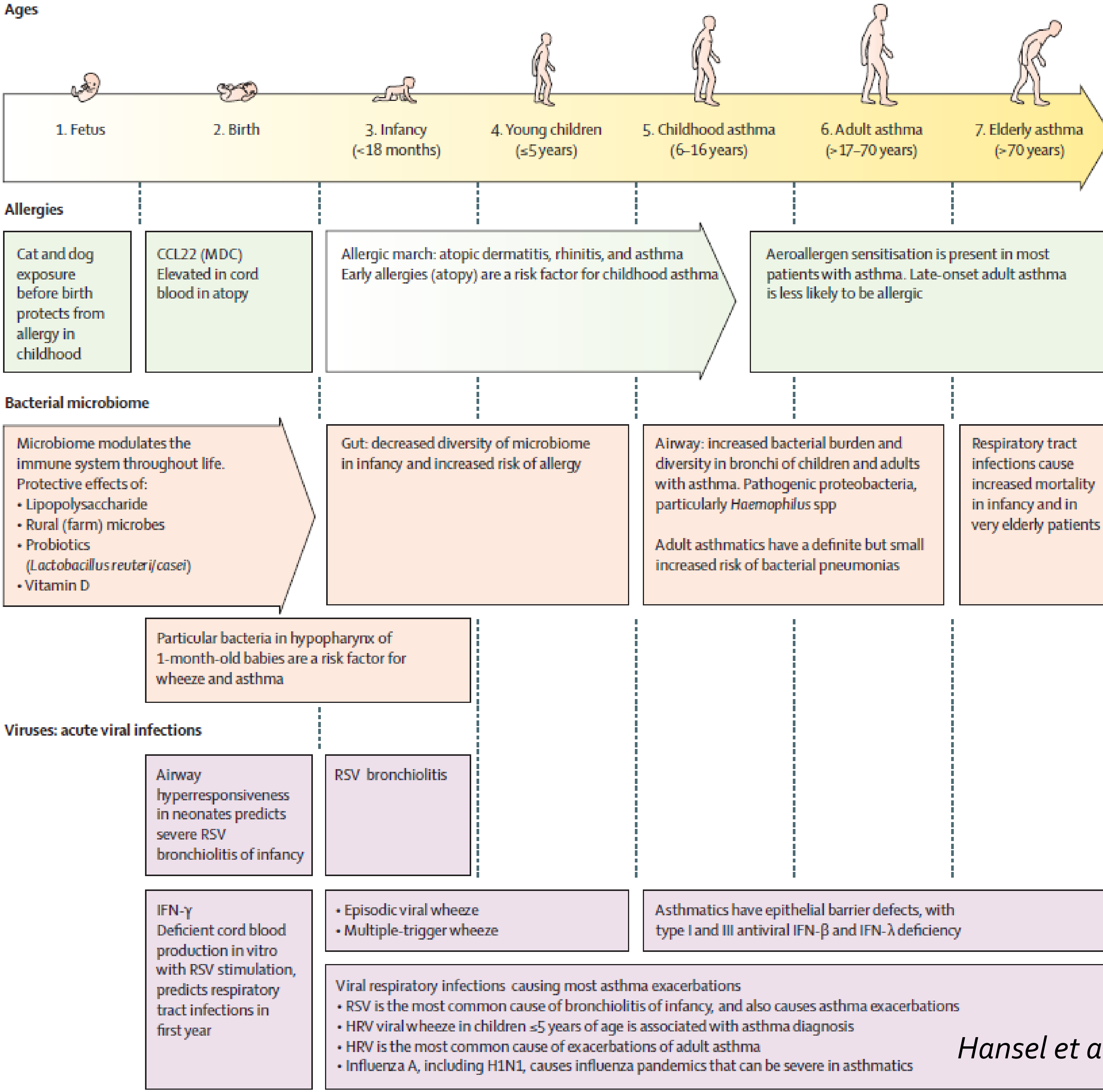
- How are lung microbial communities influenced by clinical factors and exposures such as gender, living environment and vaccination history?

MICROBIOME

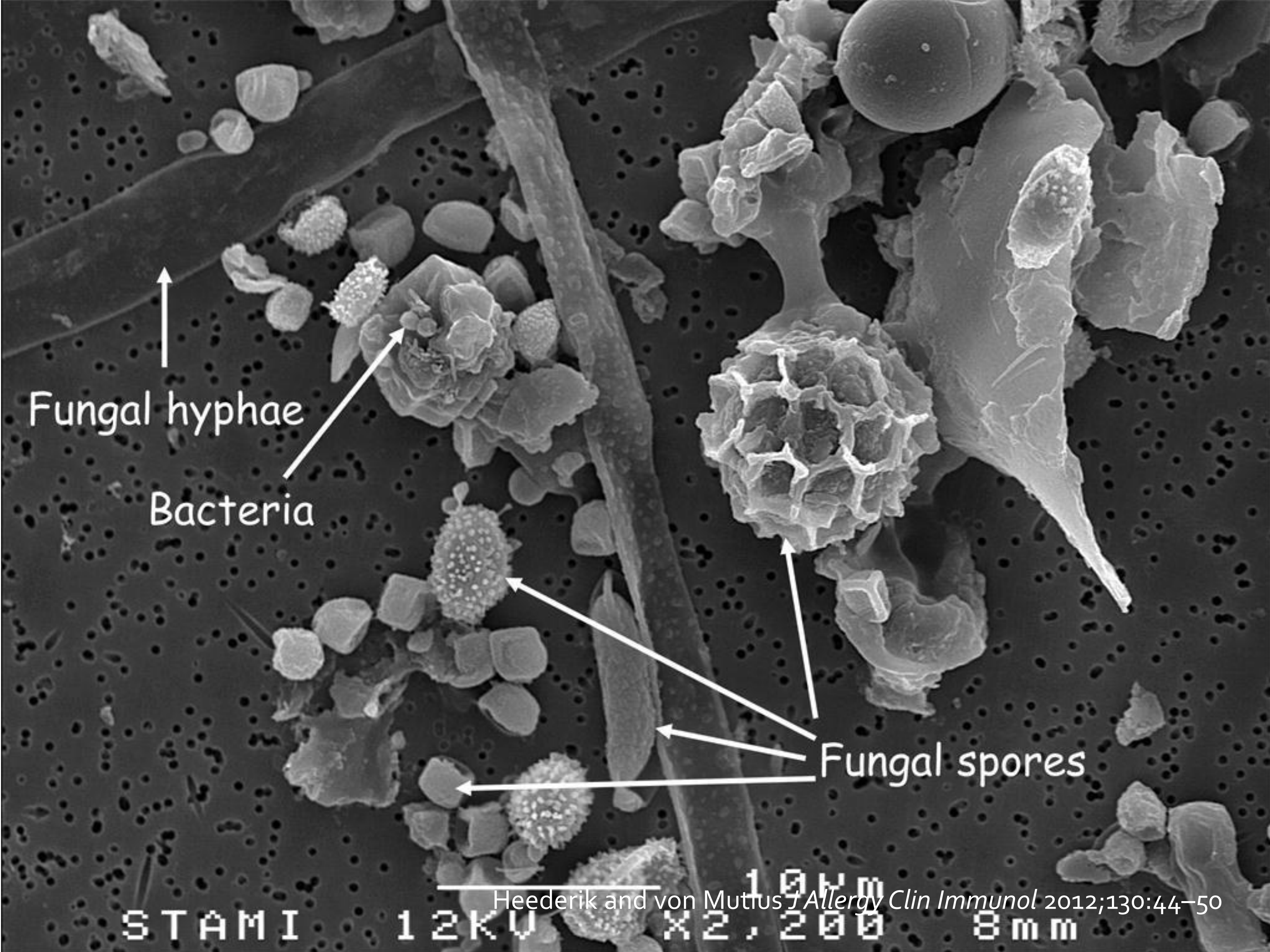
IN ASTHMA



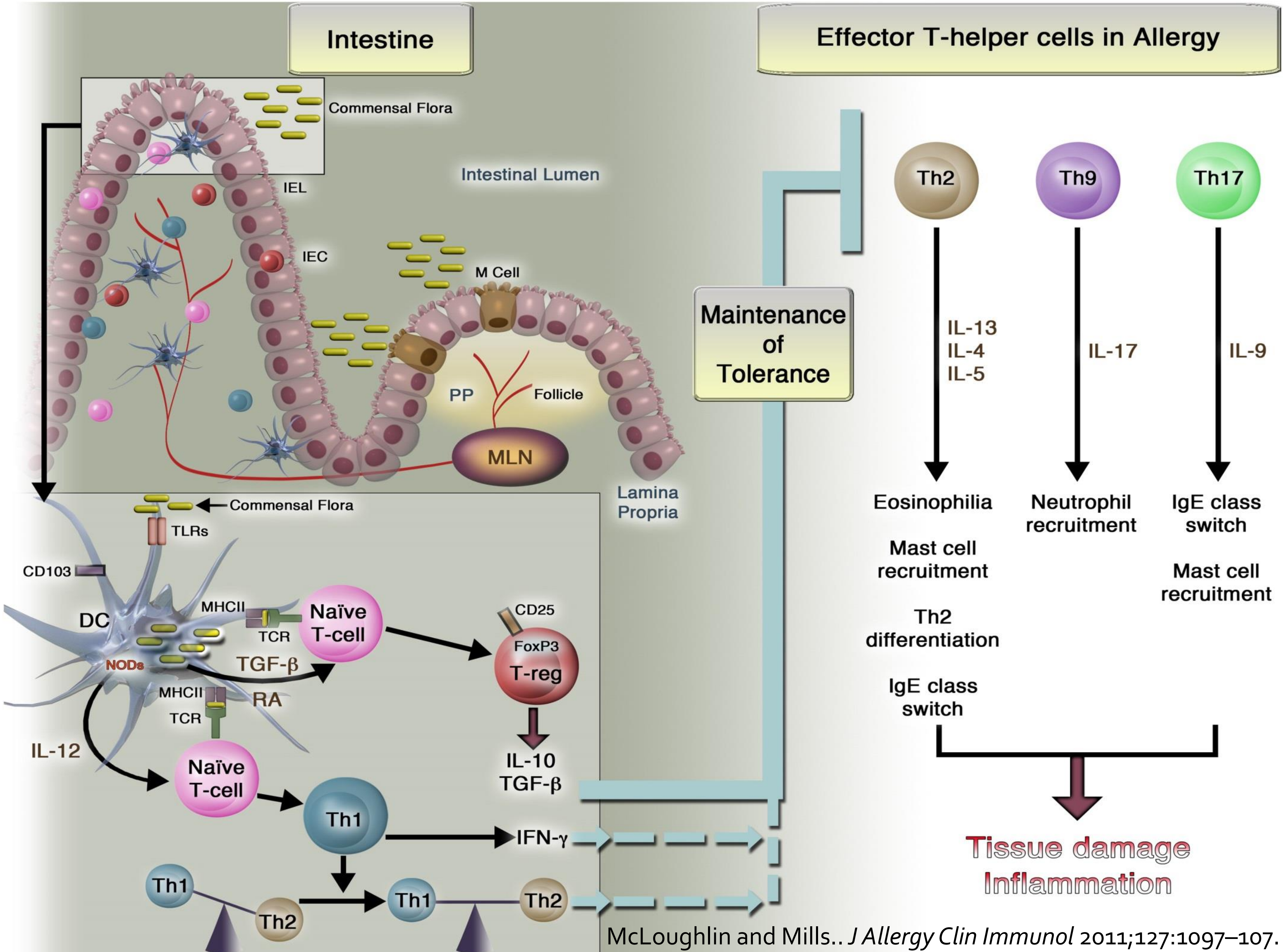
Edwards et al. *Nat Rev Microbiol* 2012;10:459-471



Hansel et al. Lancet 2013

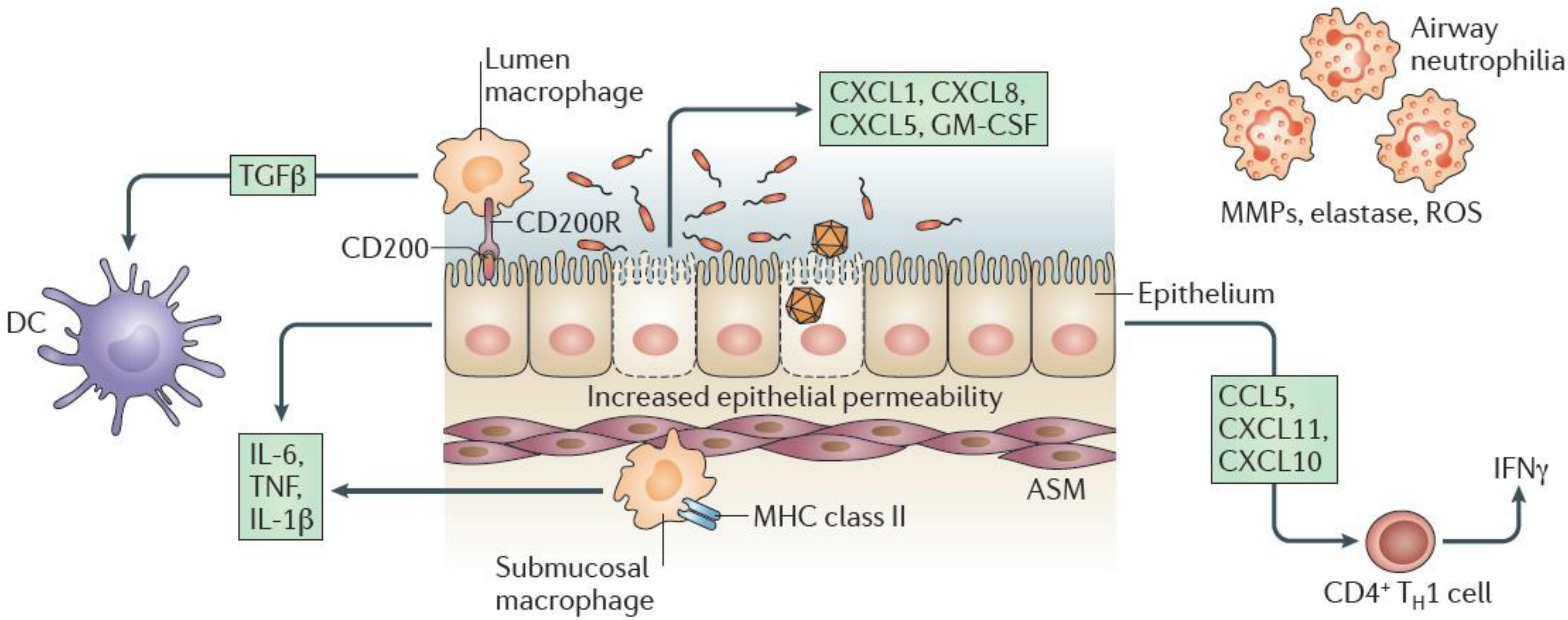


Heederik and von Mutius *J Allergy Clin Immunol* 2012;130:44–50



McLoughlin and Mills.. *J Allergy Clin Immunol* 2011;127:1097–107.

Respiratory Microbiota & Asthma



Edwards et al. *Nat Rev Microbiol* 2012;10:459-471

Disordered Microbial Communities in Asthmatic Airways

Markus Hilty[1], Conor Burke[2], Helder Pedro[3,4], Paul Cardenas[1], Andy Bush[1], Cara Bossley[1], Jane Davies[1], Aaron Ervine[2], Len Poulter[2], Lior Pachter[4], Miriam F. Moffatt[1], William O. C. Cookson[1]*

1 National Heart and Lung Institute, Imperial College London, London, England, **2** Department of Respiratory Medicine, Connolly Hospital, Dublin, Ireland, **3** Instituto Gulbenkian de Ciência, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, **4** Department of Mathematics, University of California, Berkeley, California, United States of America

PLoS ONE 2010;5:e8578

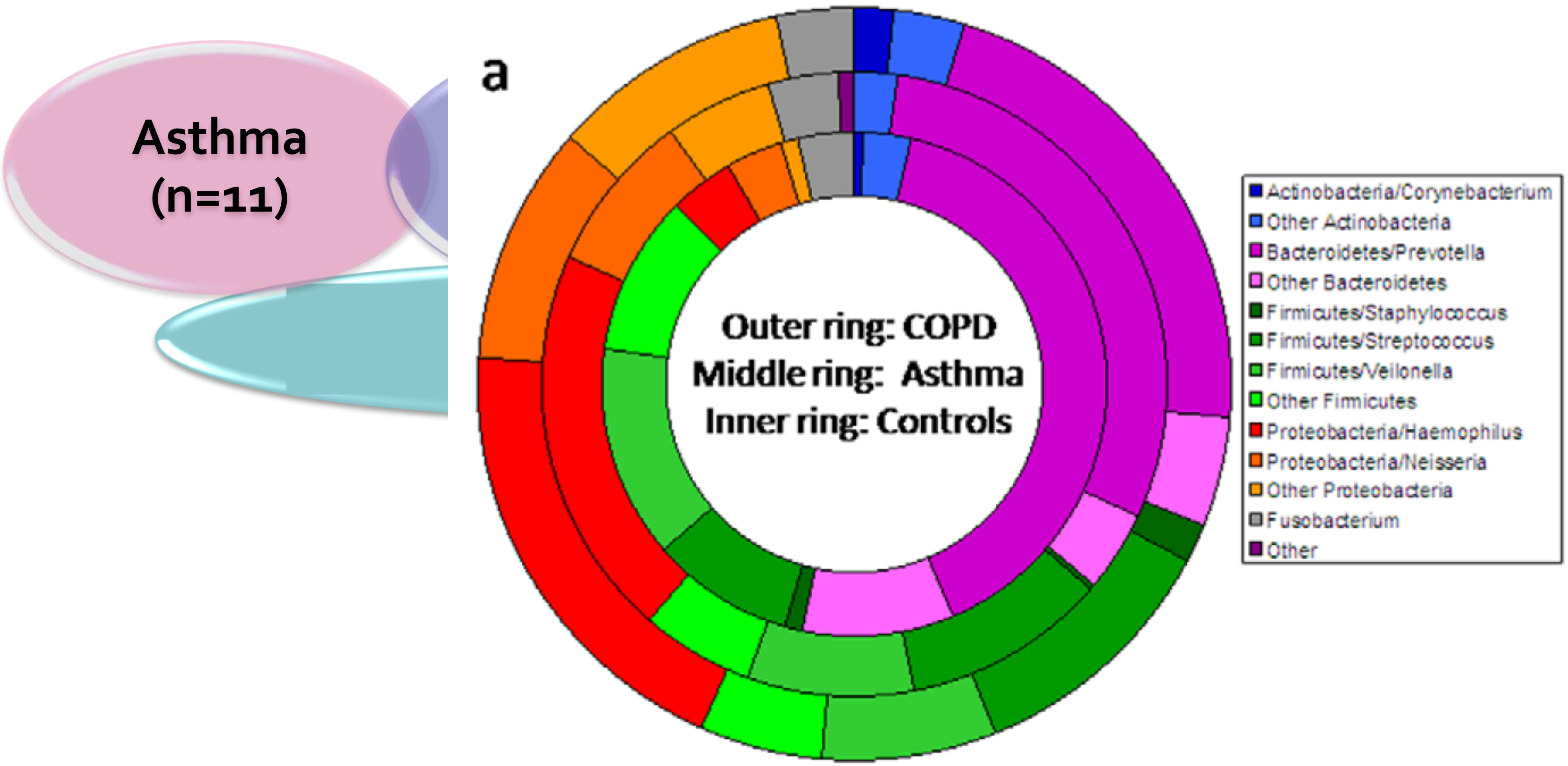


Table 3. Cladistic association analysis: *P*-values for differences in phyla and genera from left upper lobe brushings between adult subject groups.

PHYLA	COPD	Asthma	Control	*P** COPD vs. Controls	*P* Asthma vs. Controls
Proteobacteria	94	181	27	7.70E−15	2.16E−14
Bacterioidetes	62	179	151	3.38E−05	7.17E−03
Firmicutes	60	134	103		
Fusobacteria	8	19	11		
Actinobacteria	11	12	11		
GENERA					
Actinobacteria/Corynebacterium	4	0	2		
Other Actinobacteria	7	12	9		
Bacteroidetes/Prevotella	51	158	121	7.55E−03	
Other Bacteroidetes	11	21	30		
Firmicutes/Staphylococcus	4	2	3		
Firmicutes/Streptococcus	26	56	28		
Firmicutes/Veillonella	18	45	41		
Other Firmicutes	12	31	31		
Proteobacteria/Haemophilus	46	108	13	2.06E−05	1.17E−08
Proteobacteria/Neisseria	24	44	11		
Other Proteobacteria	24	29	3	9.22E−04	
Fusobacterium	8	19	11		

The numbers of sequences are shown for each split level.

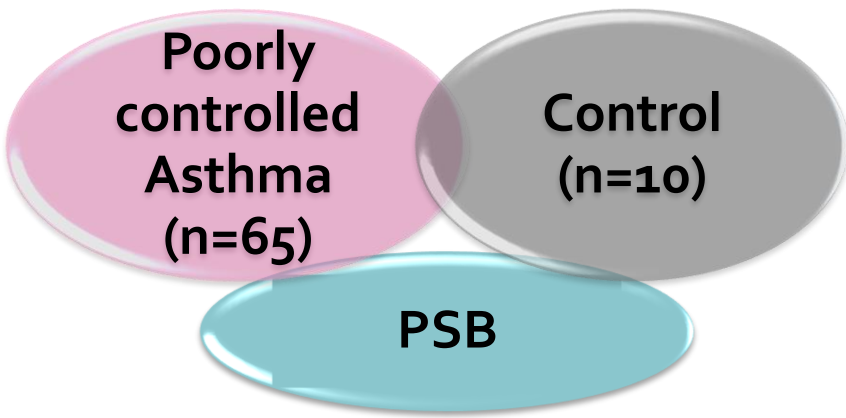
*Only significant P values are shown. The significance levels have been Bonferroni corrected for multiple comparisons.

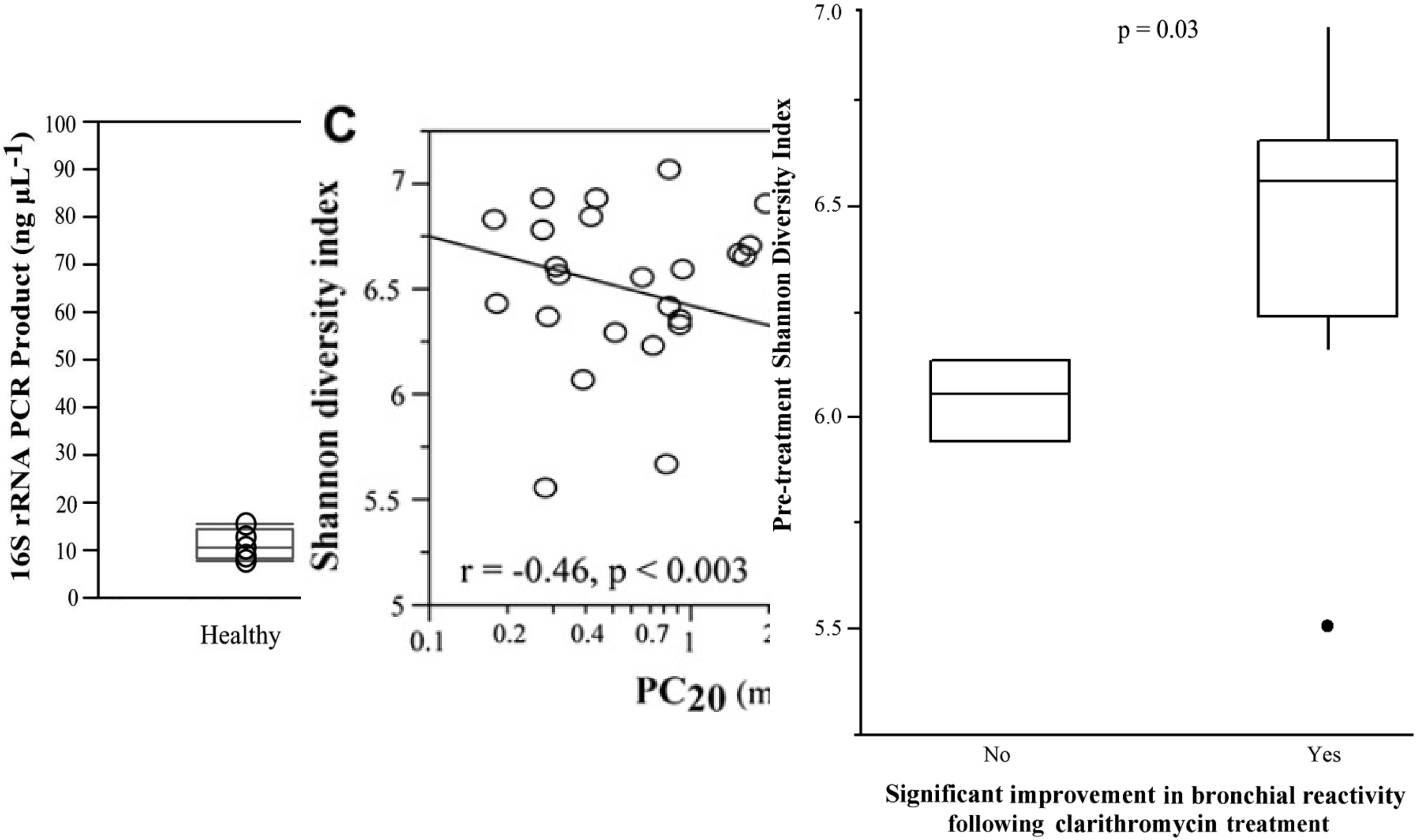
Hilty et al. *PLoS ONE 2010;5:e8578*

Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma

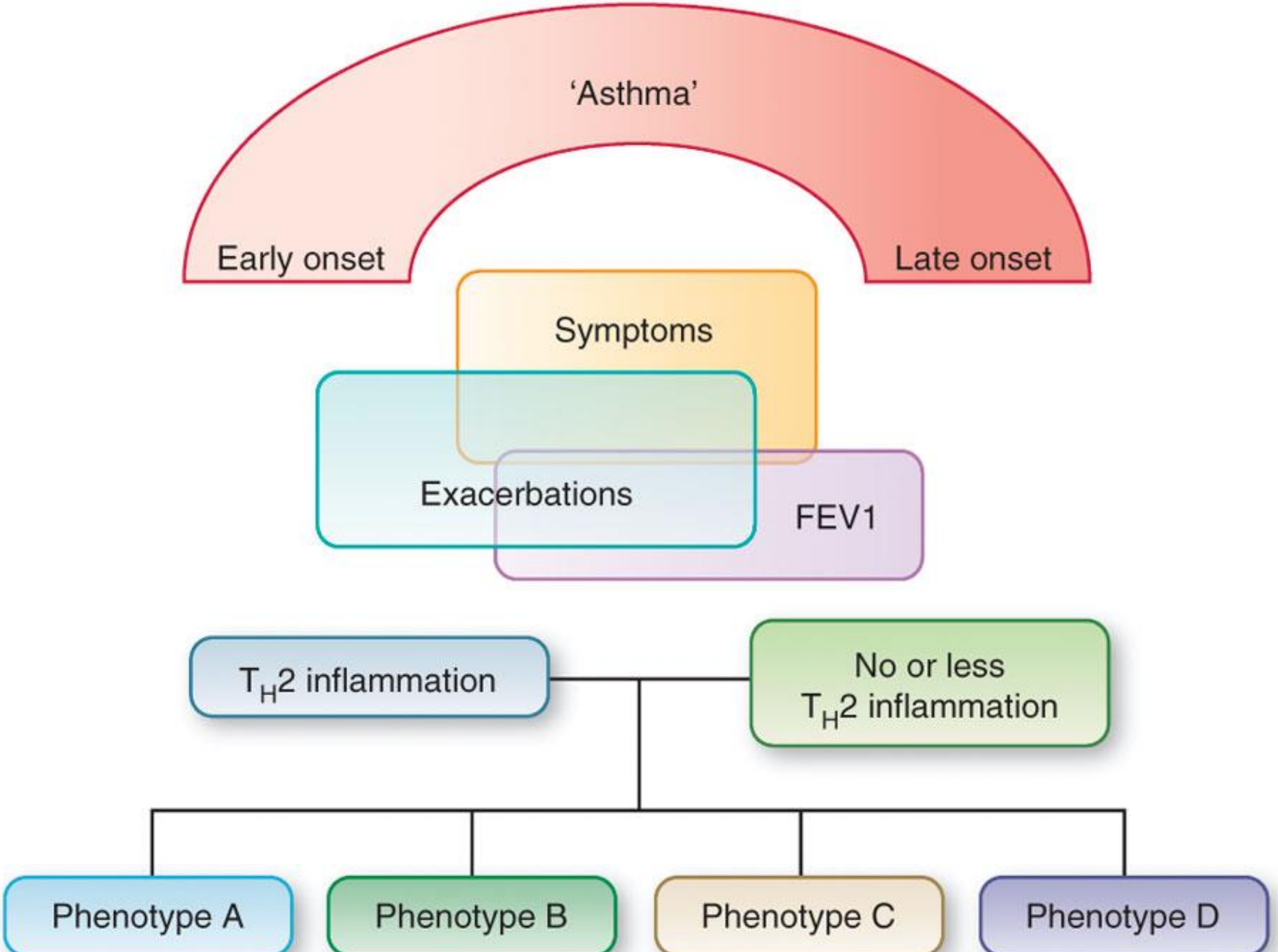
Yvonne J. Huang, MD,[a] Craig E. Nelson, PhD,[b] Eoin L. Brodie, PhD,[c] Todd Z. DeSantis, MS,[c] Marshall S. Baek, BS,[d] Jane Liu, MS,[a] Tanja Woyke, PhD,[e] Martin Allgaier, PhD,[d] Jim Bristow, MD,[e] Jeanine P. Wiener-Kronish, MD,[d] E. Rand Sutherland, MD, MPH,[f] Tonya S. King, PhD,[g] Nikolina Icitovic, MAS,[g] Richard J. Martin, MD,[f] William J. Calhoun, MD,[h] Mario Castro, MD,[i] Loren C. Denlinger, MD, PhD,[j] Emily DiMango, MD,[k] Monica Kraft, MD,[l] Stephen P. Peters, MD, PhD,[m] Stephen I. Wasserman, MD,[n] Michael E. Wechsler, MD,[o] Homer A. Boushey, MD,[a] and Susan V. Lynch, PhD,[p] for the National Heart, Lung, and Blood Institute's Asthma Clinical Research Network* *San Francisco, Santa Barbara, Berkeley, Walnut Creek, and San Diego, Calif, Denver, Colo, Hershey, Pa, Galveston, Tex, St Louis, Mo, Madison, Wis, New York, NY, Durham and Winston-Salem, NC, and Boston, Mass*

J Allergy Clin Immunol 2011;127:372-81



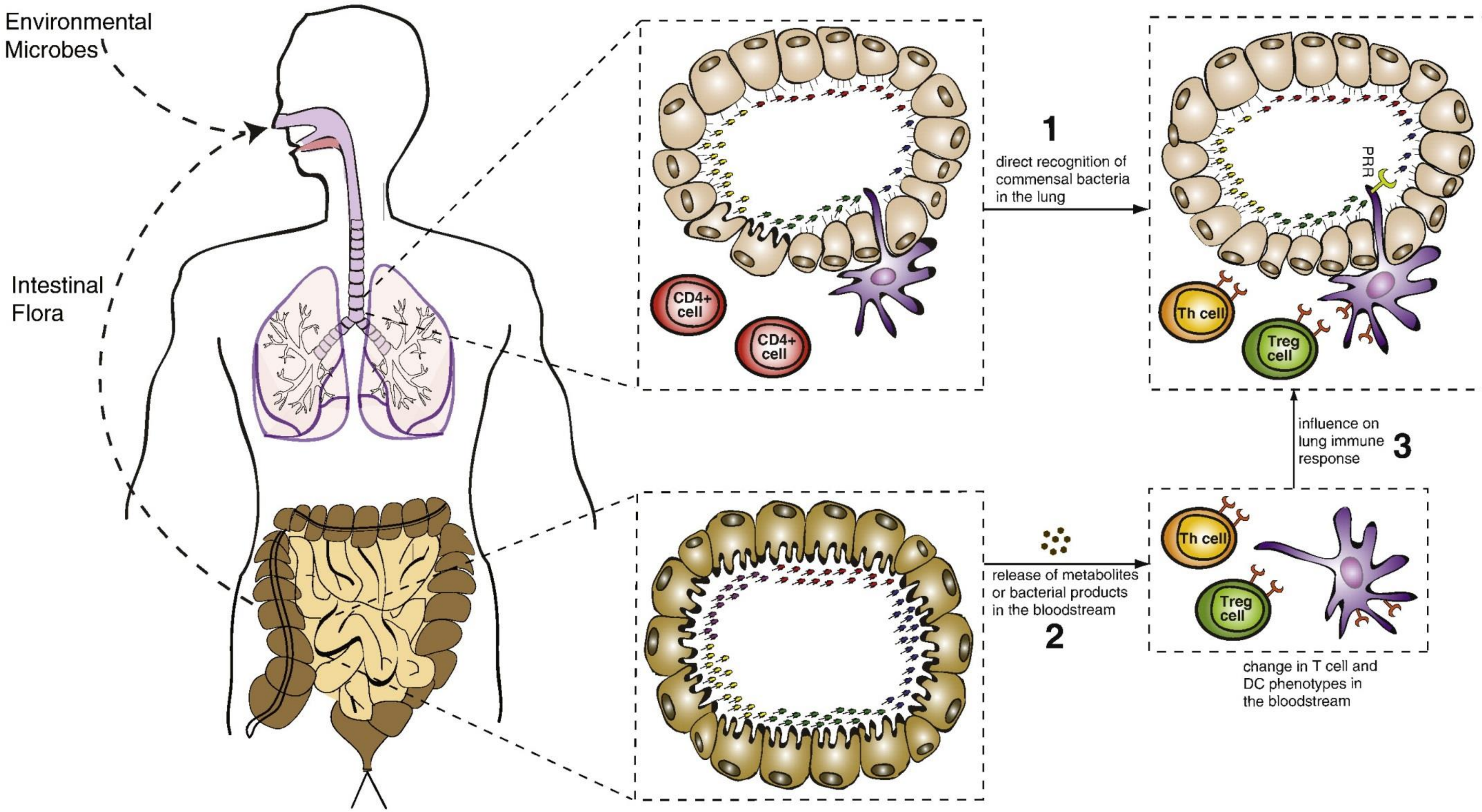


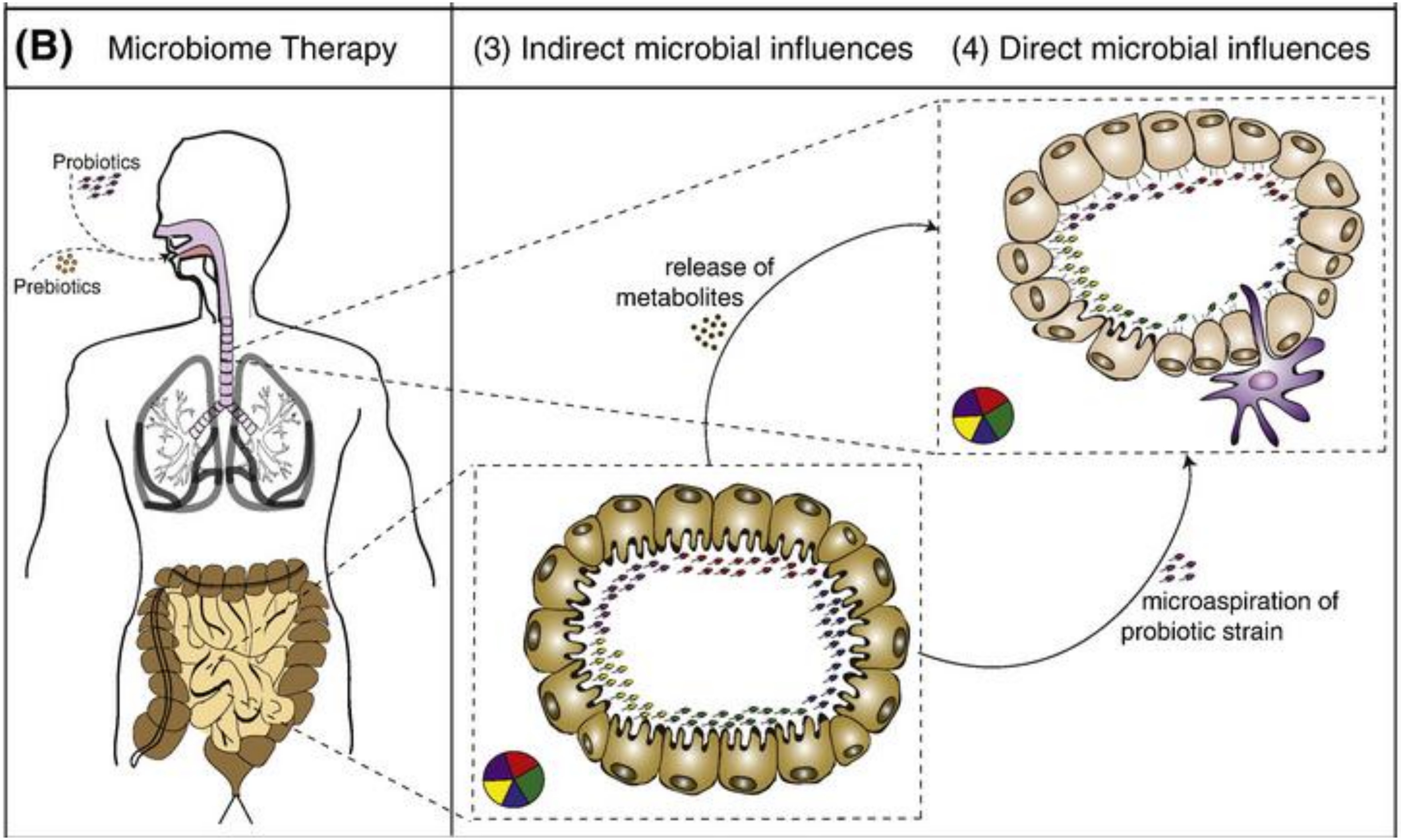
Huang et al. *J Allergy Clin Immunol* 2011;127:372-81



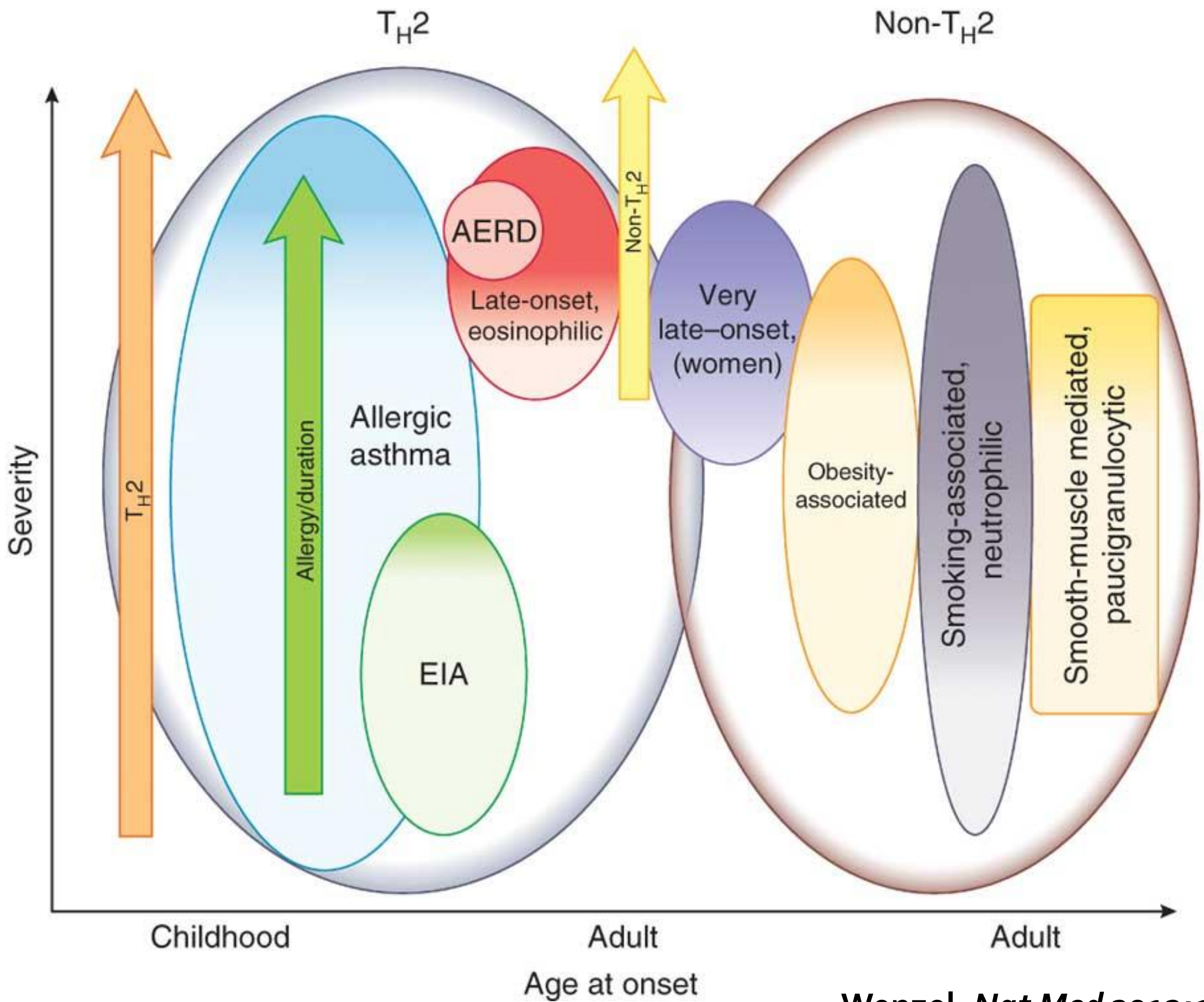
Wenzel. *Nat Med* 2012;18:716–25

1. Early-Life Microbiome Composition & Asthma Development

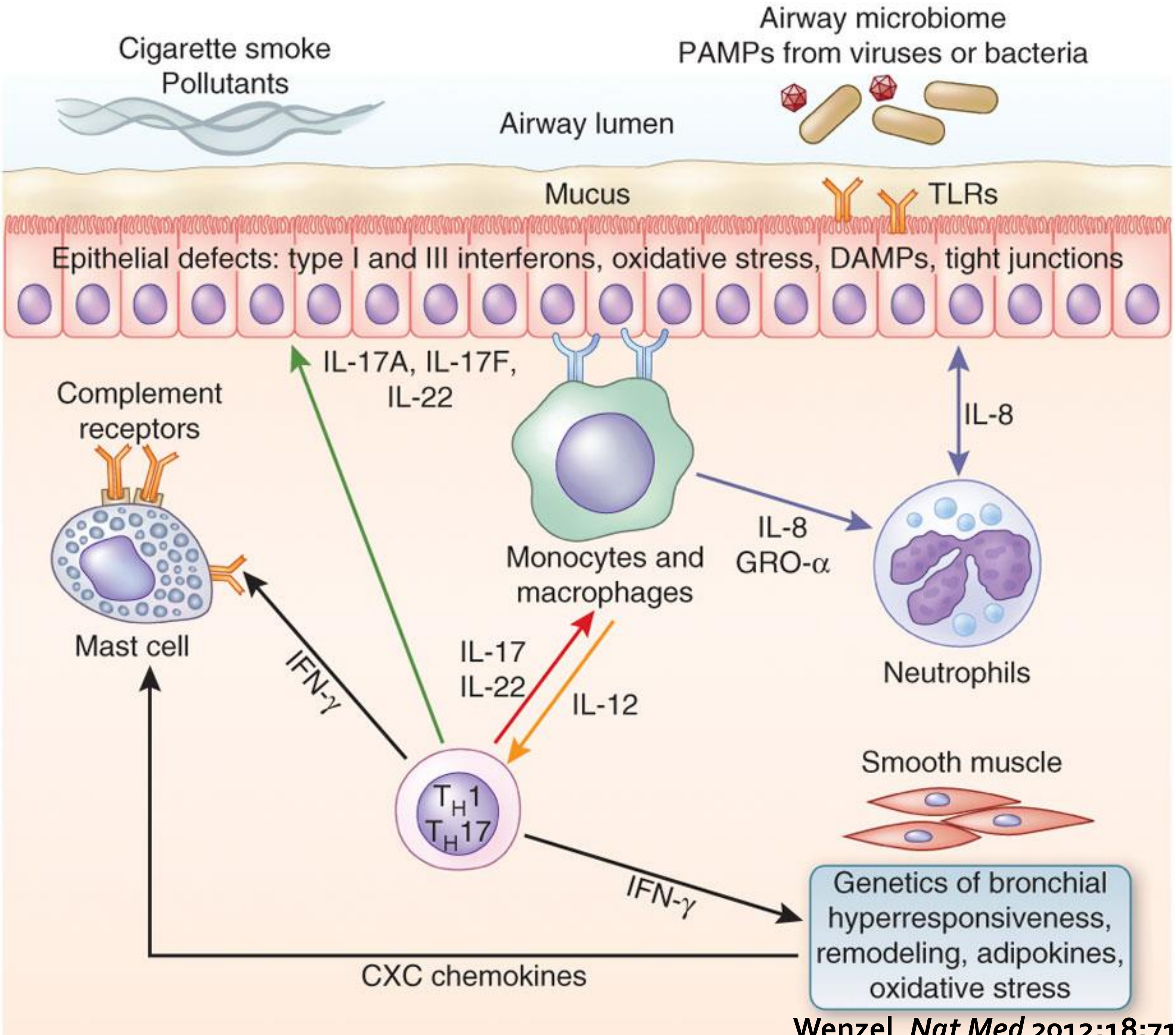



(B) Microbiome Therapy
(3) Indirect microbial influences
(4) Direct microbial influences
Probiotics
Prebiotics
release of metabolites
microaspiration of probiotic strain

2. Neutrophil Variant Forms of Asthma



Wenzel. *Nat Med* 2012;18:716–25



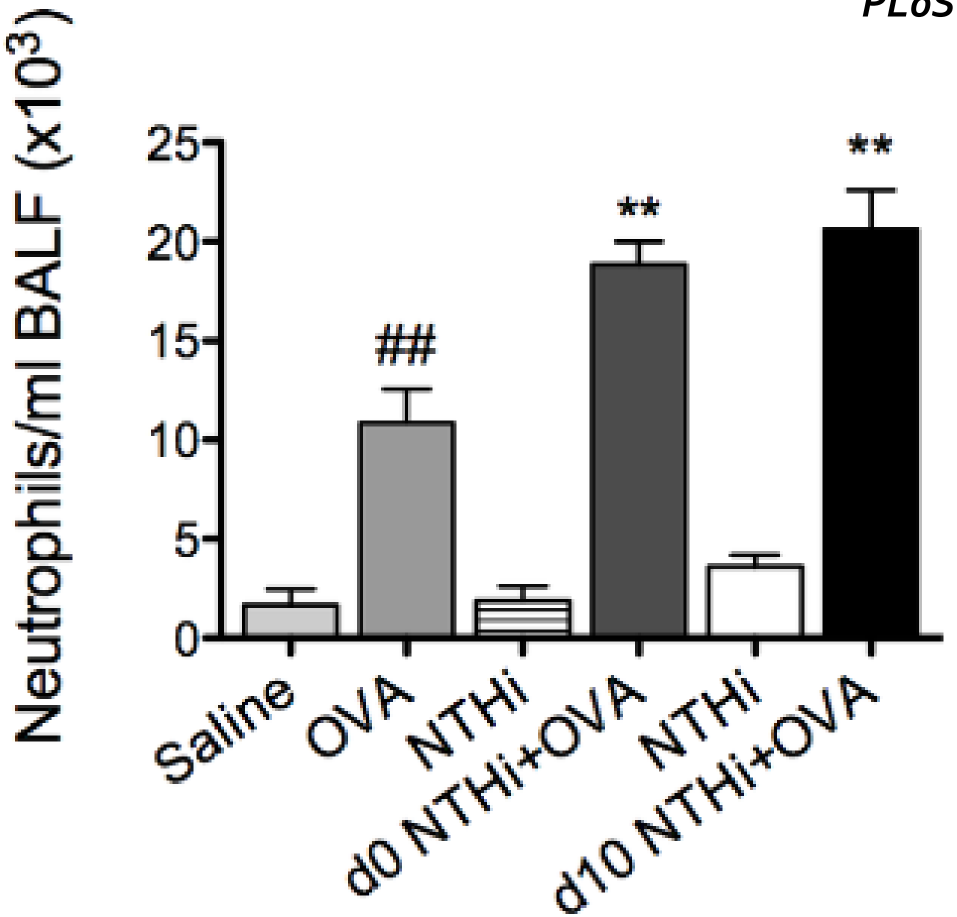
Wenzel. ***Nat Med*** **2012;18:716–25**

Haemophilus influenzae Infection Drives IL-17-Mediated Neutrophilic Allergic Airways Disease

Ama-Tawiah Essilfie[1], Jodie L. Simpson[1,2], Jay C. Horvat[1], Julie A. Preston[1], Margaret L. Dunkley[1,3], Paul S. Foster[1], Peter G. Gibson[1,2], Philip M. Hansbro[1]*

1 Centre for Asthma and Respiratory Diseases and Hunter Medical Research Institute, The University of Newcastle, Newcastle, New South Wales, Australia, **2** Department of Respiratory and Sleep Medicine, John Hunter Hospital, New Lambton, New South Wales, Australia, **3** Hunter Immunology, Newcastle, Australia

PLoS Pathog **2011;7: e1002244**



3. Treatment-Resistant Asthma

The Effects of Airway Microbiome on Corticosteroid Responsiveness in Asthma

Elena Goleva[1], Leisa P. Jackson[1], J. Kirk Harris[2], Charles E. Robertson[3], E. Rand Sutherland[4], Clifton F. Hall[1], James T. Good, Jr.[4], Erwin W. Gelfand[1,2], Richard J. Martin[4], and Donald Y. M. Leung[1,2]

[1]Department of Pediatrics and [4]Department of Medicine, National Jewish Health, Denver, Colorado; [2]Department of Pediatrics, University of Colorado Denver, Aurora, Colorado; and [3]Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, Colorado

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

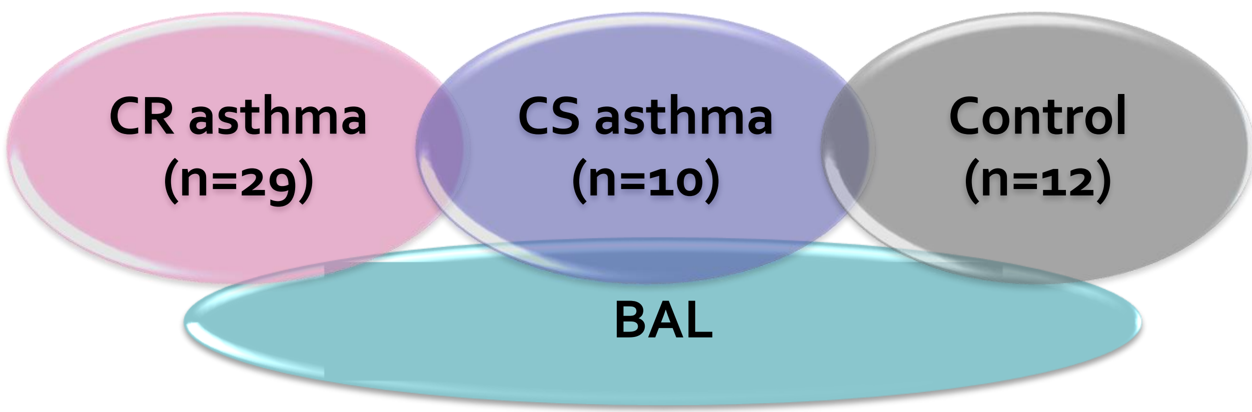


TABLE 1. PATIENT CHARACTERISTICS

	Normal Control Subjects (*n* = 12)	CR Asthma (*n* = 29)	CS Asthma (*n* = 10)
Age, yr (mean ± SD)	31.1 ± 9.2	34.2 ± 11.1	37.9 ± 10.8
Sex, male/female	4/8	14/15	2/8
Race, white/black/other	11/0/1	19/4/6	9/0/1
Body mass index, kg/m^2 (mean ± SD)	24.1 ± 4.7	26.3 ± 6.4	32.2 ± 8.5
IgE, U/ml (mean ± SD)	75 ± 108	253 ± 289	177 ± 211

TABLE 3. UNIQUE MICROORGANISMS EXPANDED IN THE AIRWAYS OF PATIENTS WITH CR 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%)

TABLE 4. UNIQUE MICROORGANISMS EXPANDED IN THE AIRWAYS OF PATIENTS WITH CS 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%)

re; CR = corti-
d nitric oxide;

ICS = inhaled corticosteroids; LABA = long-acting β-agonists.

* $P < 0.0001$ as compared with subjects with CS asthma.

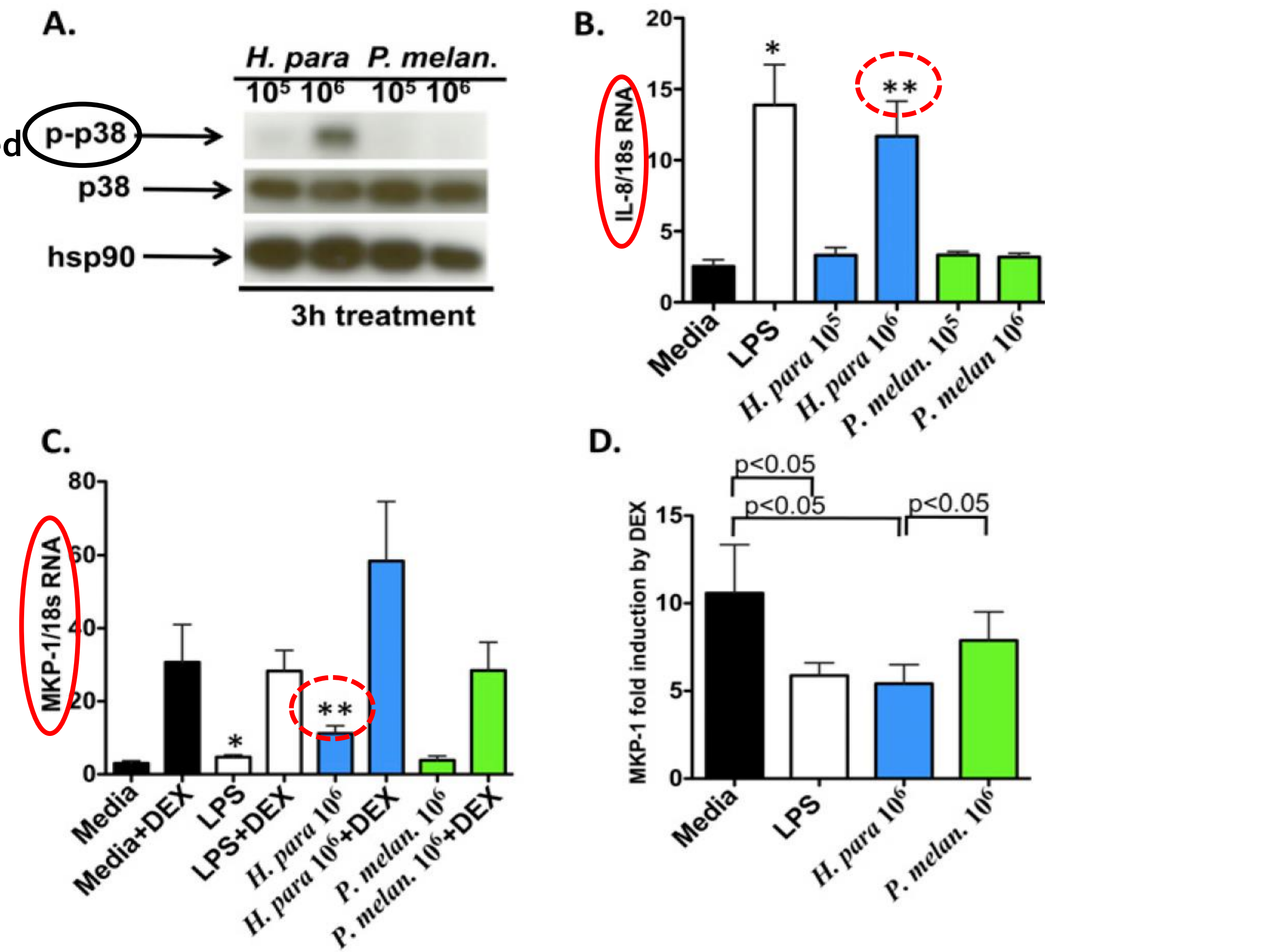
† For the subjects with CR and CS asthma that received ICS/LABA or ICS, the mean ± SD of the ICS dose in budesonide equivalents was 837 ± 713 μg and 1,450 ± 1,034 μg, respectively.

Goleva et al. *Am J Respir Crit Care Med* 2013;188:1193–1201

Effects of Bacteria from the Airways on Cell Activation and Response to Corticosteroids in Airway Macrophages

P38 mitogen-activated protein kinases activation

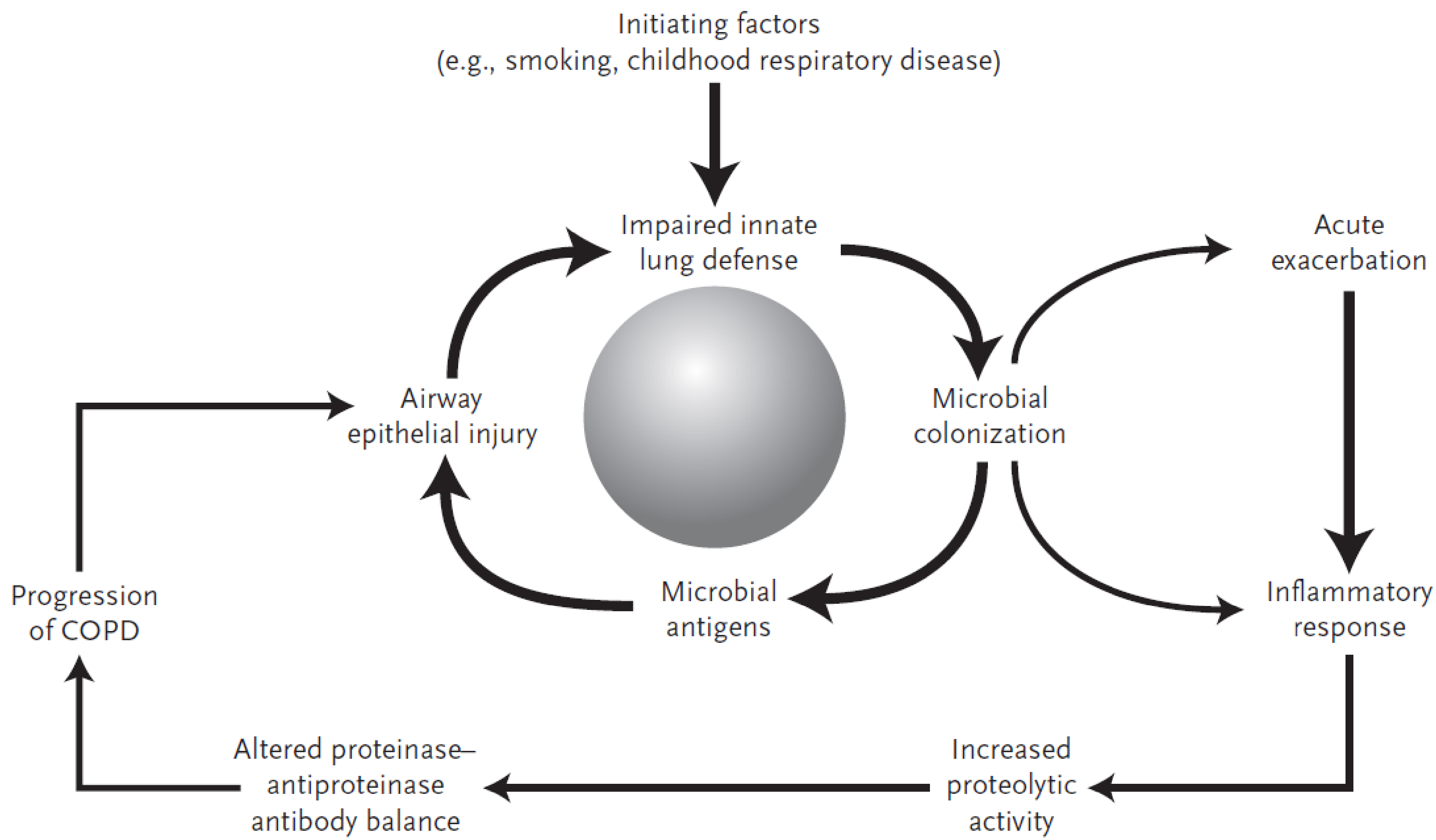
mitogen-activated kinase phosphatase 1 (MKP-1)



Goleva et al. *Am J Respir Crit Care Med* 2013;188:1193–1201

MICROBIOME

IN COPD



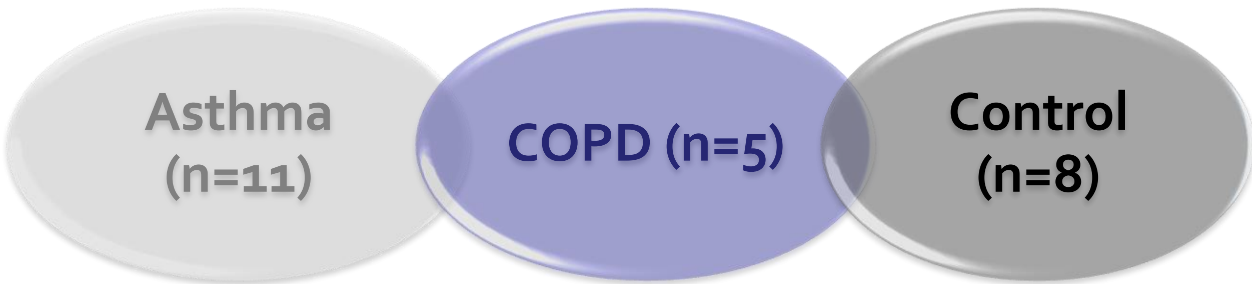
Sethi and Murphy *N Engl J Med* 2008;359:2355-2365

Disordered Microbial Communities in Asthmatic Airways

Markus Hilty[1], Conor Burke[2], Helder Pedro[3,4], Paul Cardenas[1], Andy Bush[1], Cara Bossley[1], Jane Davies[1], Aaron Ervine[2], Len Poulter[2], Lior Pachter[4], Miriam F. Moffatt[1], William O. C. Cookson[1]*

1 National Heart and Lung Institute, Imperial College London, London, England, **2** Department of Respiratory Medicine, Connolly Hospital, Dublin, Ireland, **3** Instituto Gulbenkian de Ciência, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, **4** Department of Mathematics, University of California, Berkeley, California, United States of America

PLoS ONE 2010;5:e8578



- *Distinct from healthy controls*
- ↑ *Proteobacteria* , ↓ *Bacteroides*
- ↑ *Haemophilus* spp.

Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD

John R. Erb-Downward[1], Deborah L. Thompson[1], Meilan K. Han[1], Christine M. Freeman[1,2], Lisa McCloskey[1,2], Lindsay A. Schmidt[1], Vincent B. Young[1], Galen B. Toews[1,2], Jeffrey L. Curtis[1,2], Baskaran Sundaram[1], Fernando J. Martinez[1], Gary B. Huffnagle[1*]

1 University of Michigan, Ann Arbor, Michigan, United States of America, 2 Veterans Affairs Health System, Ann Arbor, Michigan, United States of America

PLoS ONE 2011;6:e16384

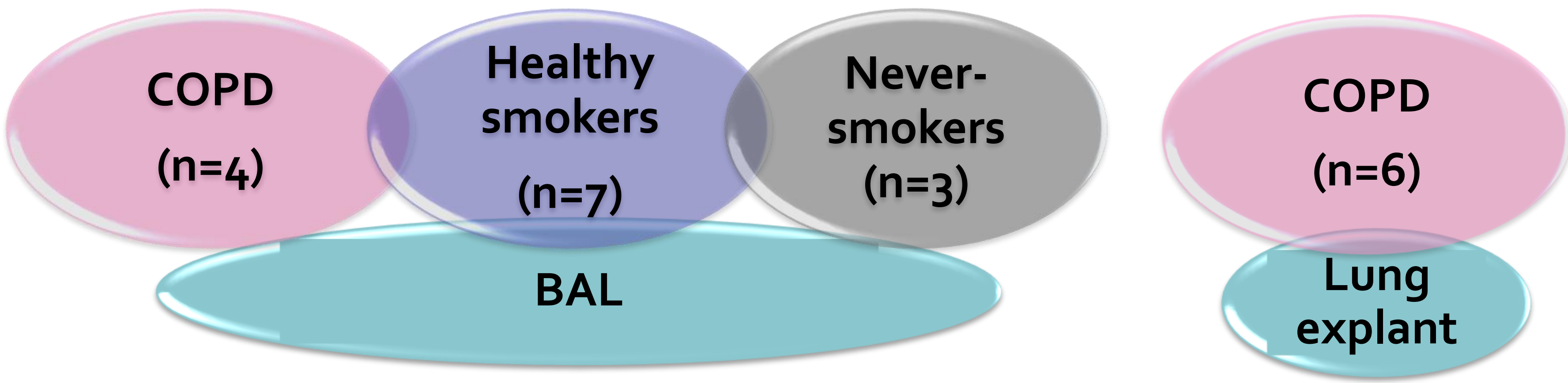


Table 1. Bronchoalveolar Lavage Patient Cohort.

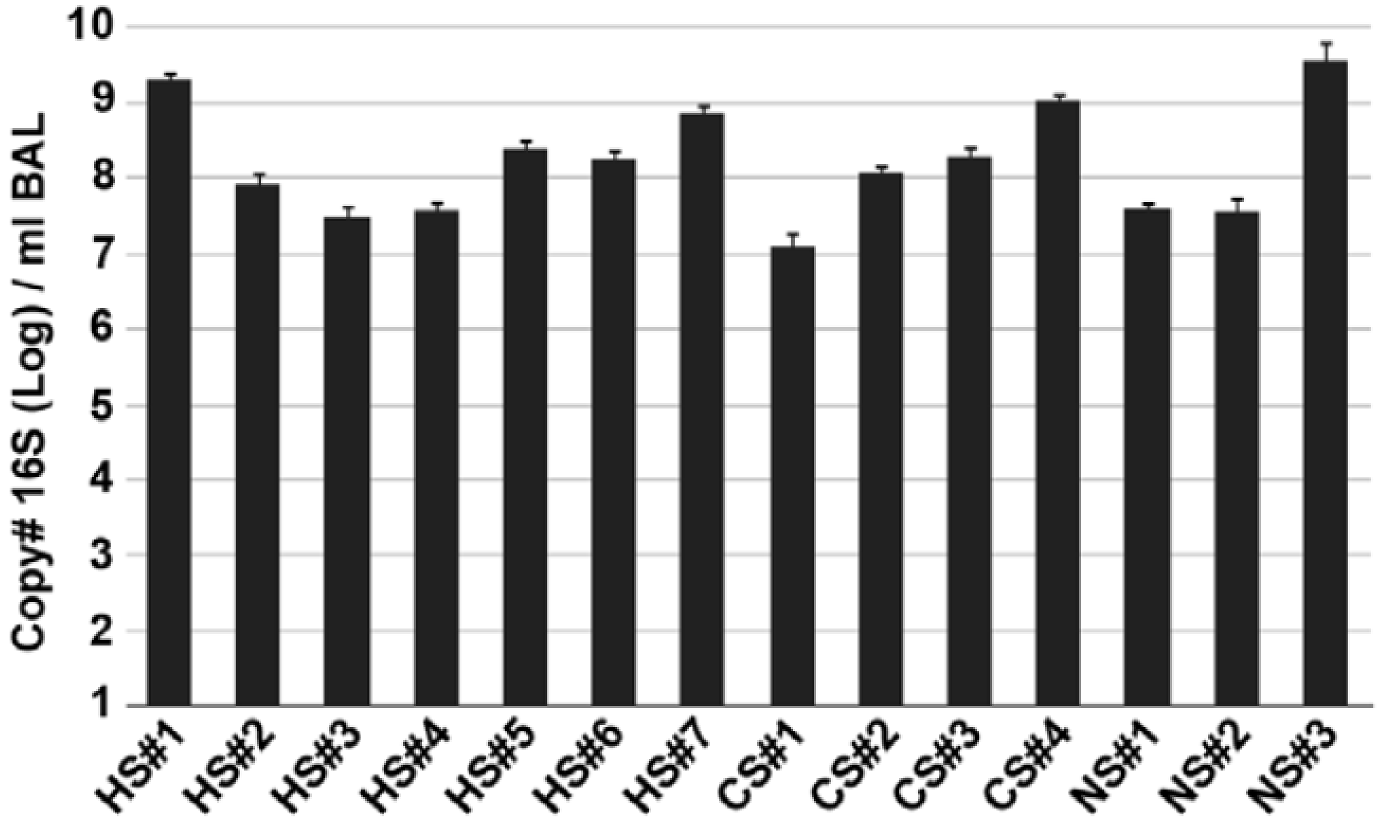
Group	Subject #	Age	Ethnicity	Gender	Smoking history	FEV_1 (%pred)	FEV_1/FVC	Medications	Approach	Current Smoker
HS	1	53	C[1]	F	20	98	0.77	N	Oral	Yes
	2	45	C[1]	F	16	103	0.80	N	Nasal	Yes
	3	45	C[1]	M	20	114	0.93	N	Nasal	Yes
	4	49	AI/NA[2]	F	40	102	0.76	N	Nasal	Yes
	5	50	AA[3]	M	15	99	0.77	N	Nasal	Yes
	6	47	C[1]	F	39	96	0.76	N	Nasal	Yes
	7	66	C[1]	M	32	110	0.80	N	Nasal	No
CS	1	54	C[1]	M	120	79	0.63	ICS[4]/LAB[5]	Nasal	No
	2	62	C[1]	M	68	78	0.68	N	Nasal	Yes
	3	40	AA[3]	M	25	79	0.67	N	Oral	Yes
	4	60	C[1]	M	41	25	0.41	ICS[4]/LAB[5]	Oral	No
NS	1	48	C[1]	F	0	105	0.86	N	Nasal	No
	2	78	C[1]	F	0	83	0.77	N	Nasal	No

Table 2. Explant Cohort (CS).

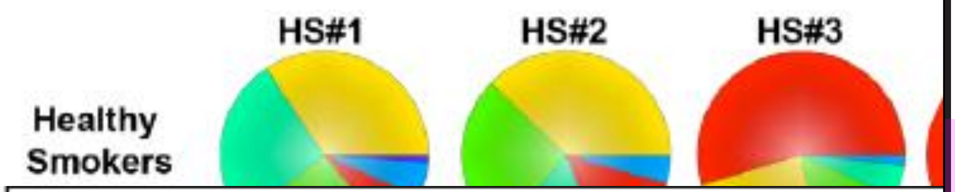
Subject #	Age	Ethnicity	Gender	Smoking history	FEV_1 (%pred)	FEV_1/FVC	Medcations
5 (SLT[6])	66	C[1]	M	No (>6 Months)	18	0.22	ICS[4]/LAB[5]
6 (BLT[7])	57	C[1]	M	No (>6 Months)	13	0.17	ICS[4]/LAB[5]
7 (BLT)	62	C[1]	M	No (>6 Months)	15	19	ICS
8 (SLT)	59	C[1]	M	No (>6 Months)	9	16	None
9 (SLT)	59	C[1]	M	No (>6 Months)	25	44	ICS/LAB
10 (SLT)	64	C[1]	M	No (>6 Months)	16	33	ICS/LAB

[6] = Single Lung Transplant;
[7] = Bilateral Lung Transplant.

16S qPCR of BAL Samples



Erb-Downward et al. *PLoS ONE* 2011;6:e16384



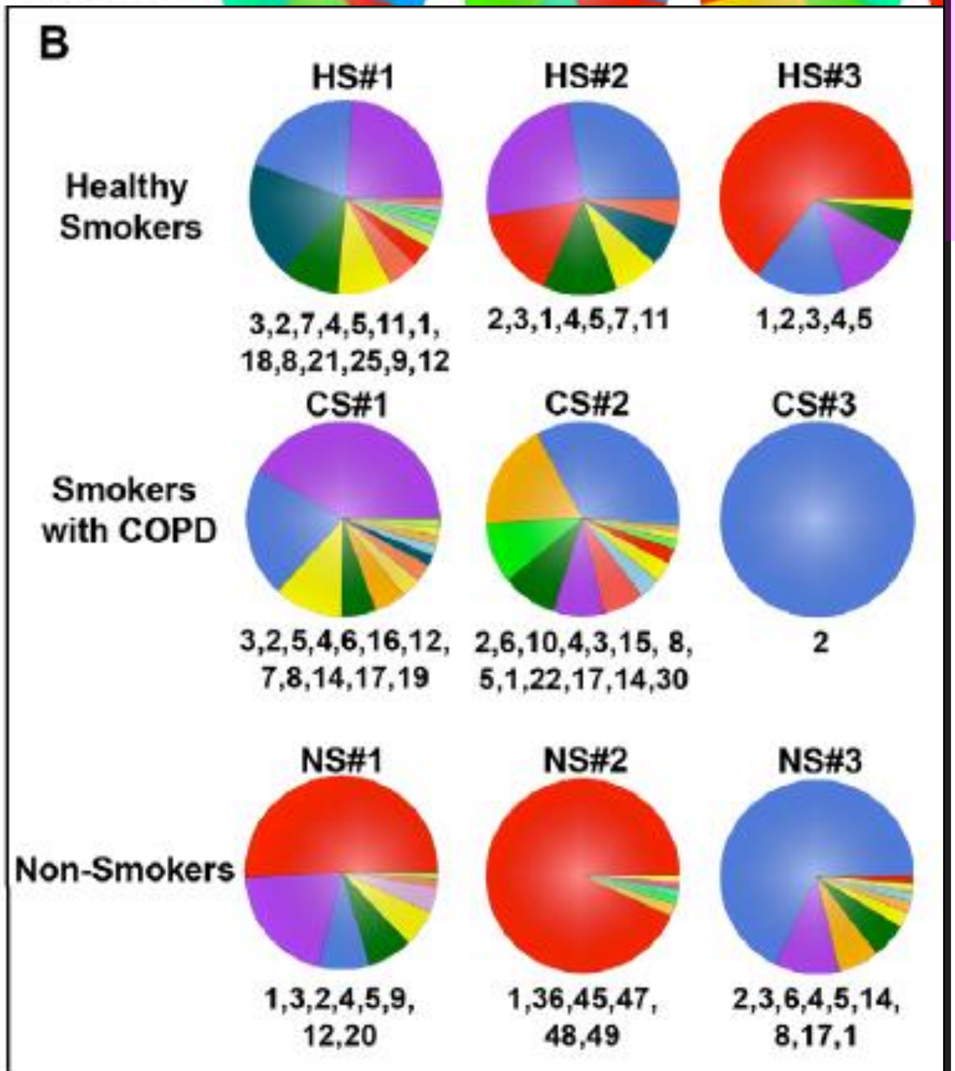
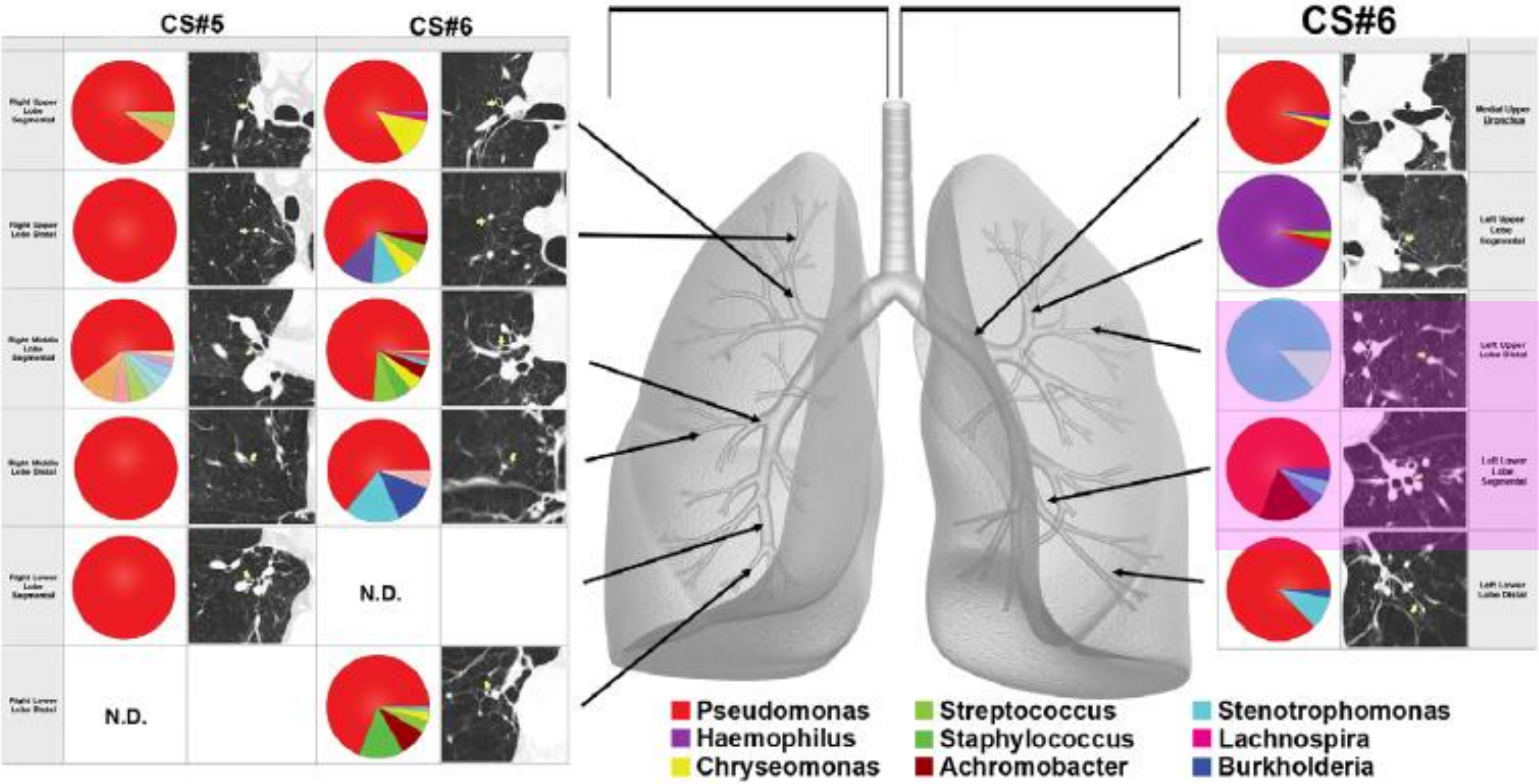


Table 3. BAL Abundance Table.

Rank	Name	Total # Sequences	# Subjects Occurred/Total
1	Pseudomonas	78319	12/14
2	Streptococcus	23253	12/14
3	Prevotella	19916	10/14
4	Fusobacterium	8784	11/14
5	Veillonella	5937	9/14
6	Porphyromonas	4366	8/14
7	Leptotrichia	3801	5/14
8	Haemophilus	2765	8/14
9	Oribacterium	1577	6/14
10	Actinobacillus	1539	4/14
11	Actinomyces	1188	6/14
12	Megasphaera	1017	4/14
13	Sneathia	879	2/14
14	Gemella	828	7/14
15	Tropheryma	783	1/14
16	Neisseria	748	4/14
17	Granulicatella	731	5/14
18	Campylobacter	535	2/14
19	Atopobium	511	3/14
20	Bulleidia	480	4/14
21	Lachnospira	474	3/14
22	Parvimonas	379	3/14
23	Flavimonas	352	3/14

Bacterial Communities Present in Individual Lung Airways



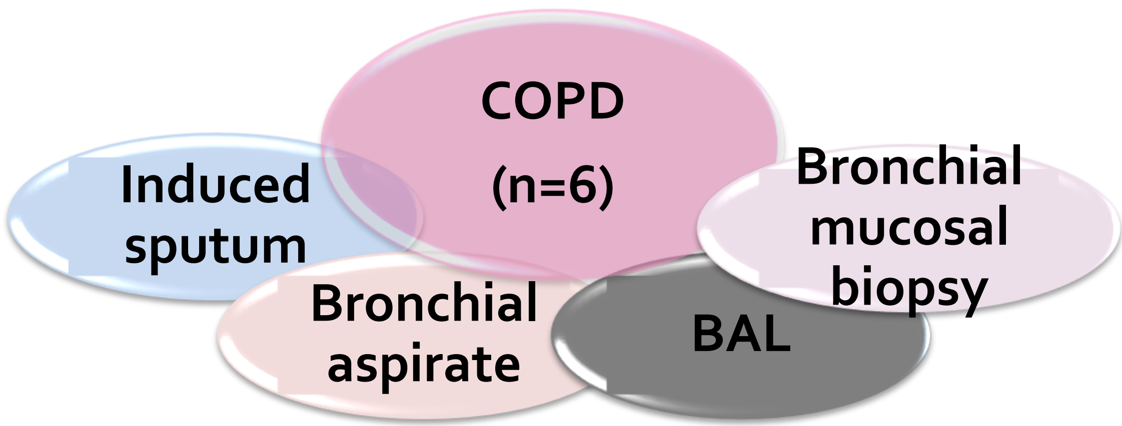
Erb-Downward et al. *PLoS ONE* 2011;6:e16384

Microbiome Diversity in the Bronchial Tracts of Patients with Chronic Obstructive Pulmonary Disease

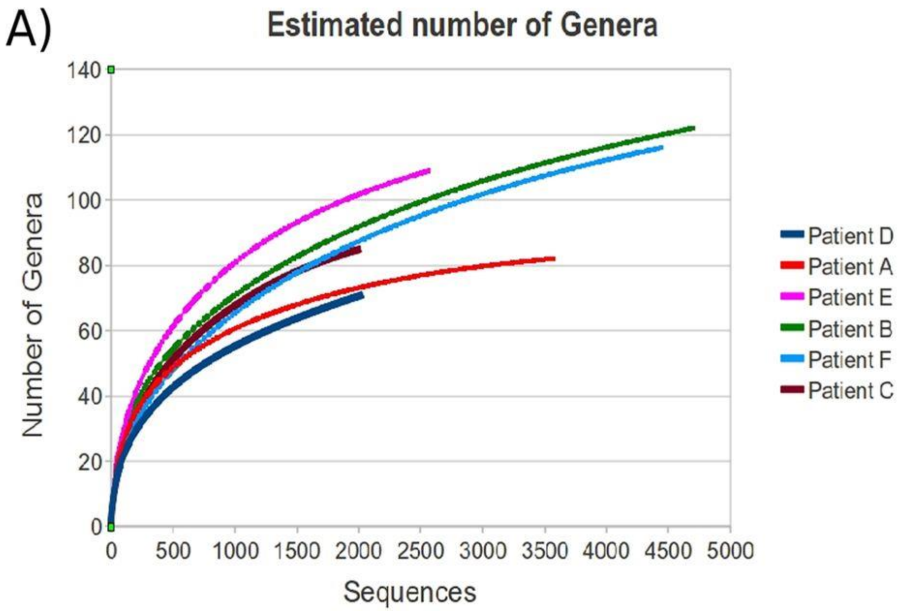
Raúl Cabrera-Rubio,[a] Marian Garcia-Núñez,[b,c] Laia Setó,[b,c] Josep M. Antó,[f,g,h,i] Andrés Moya,[a,h] Eduard Monsó,[b,c,d,e] and Alex Mira[a]

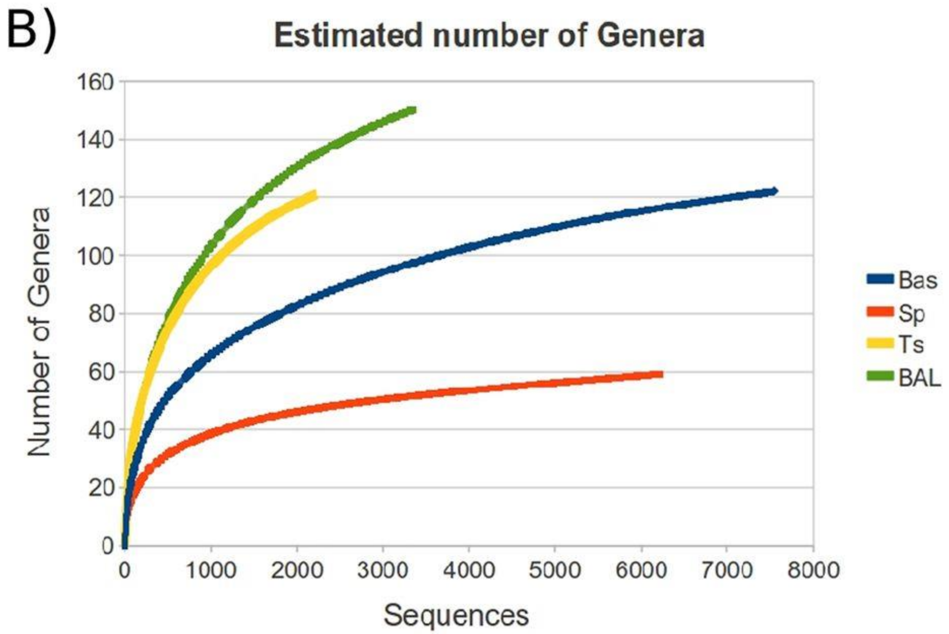
Department of Genomics and Health, Center for Public Health Research, Valencia, Spain[a]; CIBER de Enfermedades Respiratorias–CIBERes, Bunyola, Mallorca, Illes Balears, Spain[b]; Fundació Institut Universitari Parc Taulí, Sabadell, Spain[c]; Pulmonary Department, Hospital Universitari Parc Taulí, Sabadell, Spain[d]; Departament de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Spain[e]; Centre for Research in Environmental Epidemiology, Barcelona, Spain[f]; IMIM (Hospital del Mar Research Institute), Barcelona, Spain[g]; CIBER de Epidemiología y Salud Pública (CIBEResp), Barcelona, Spain[h]; and Universitat Pompeu Fabra, Barcelona, Spain[i]

J Clin Microbiol 2012;50:3562-8



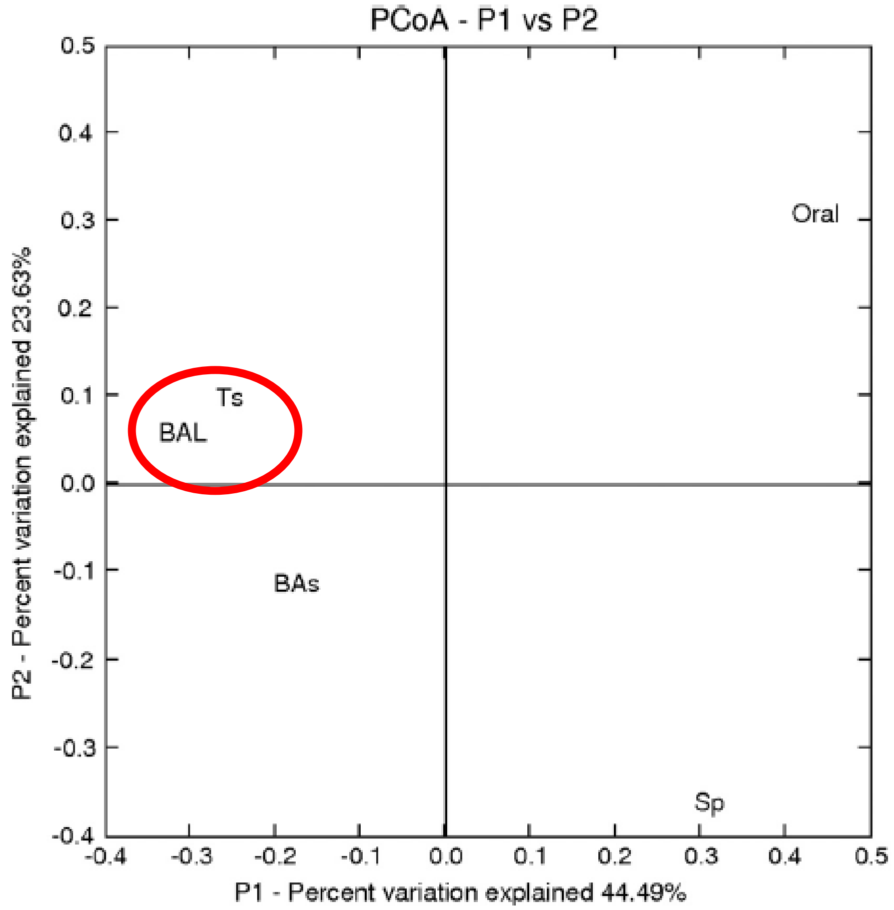
Bacterial diversity in respiratory tract samples





Cabrera-Rubio et al. *J Clin Microbiol 2012;50:3562-8*

Principal component analysis of the four respiratory tract sample types



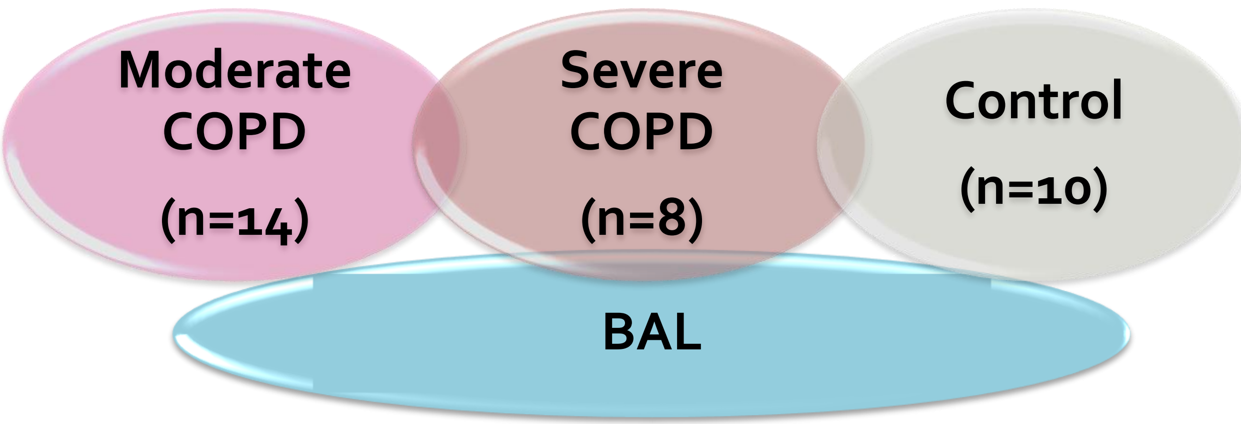
Cabrera-Rubio et al. *J Clin Microbiol 2012;50:3562-8*

The Lung Microbiome in Moderate and Severe Chronic Obstructive Pulmonary Disease

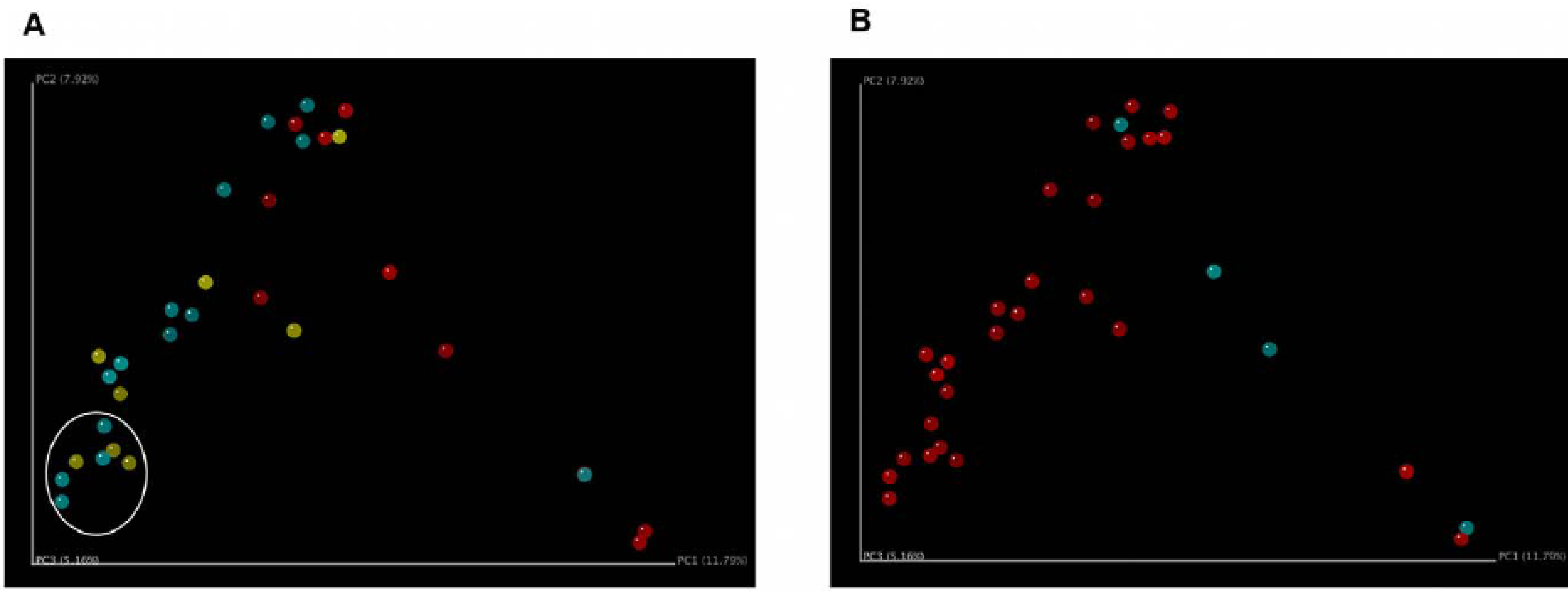
Alexa A. Pragman[1], Hyeun Bum Kim[2¤], Cavan S. Reilly[3], Christine Wendt[4], Richard E. Isaacson[2*]

1 Department of Medicine, University of Minnesota, Minneapolis, Minnesota, United States of America, 2 Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota, United States of America, 3 Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota, United States of America, 4 Department of Medicine, VA Medical Center, Minneapolis, Minnesota, United States of America, and for the FORTE Study Group

PLoS ONE 2012;7:e47305



Principal Coordinate Analysis Demonstrates Clustering of COPD Samples



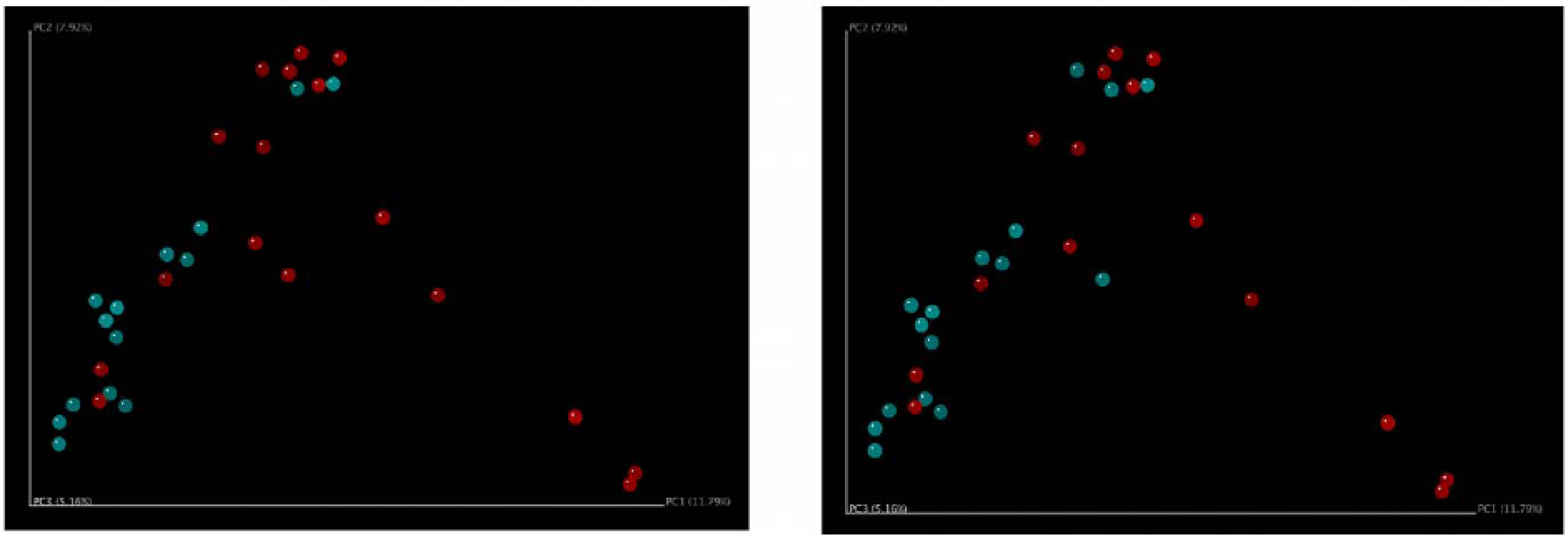
- Control
- Moderate COPD
- Severe COPD

- Non-smokers
- Smokers

Pragman et al. *PLoS ONE 2012;7:e47305*

Principal Coordinate Analysis Demonstrates Clustering of COPD Samples

C D



- ICS non-users
- ICS users

- Inhaled bronchodilator non-users
- Inhaled bronchodilator users

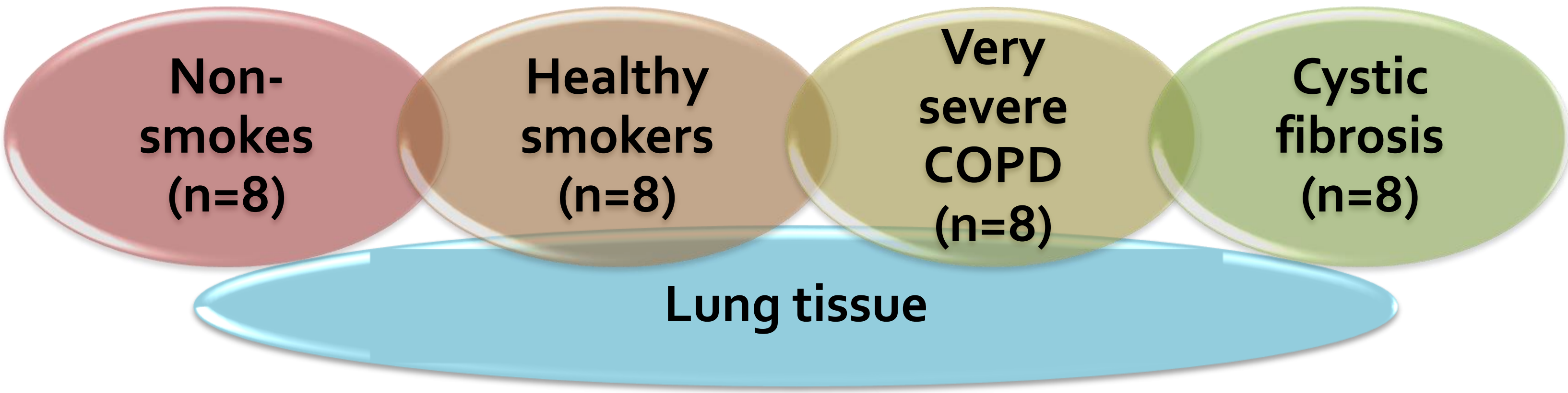
Pragman et al. *PLoS ONE 2012;7:e47305*

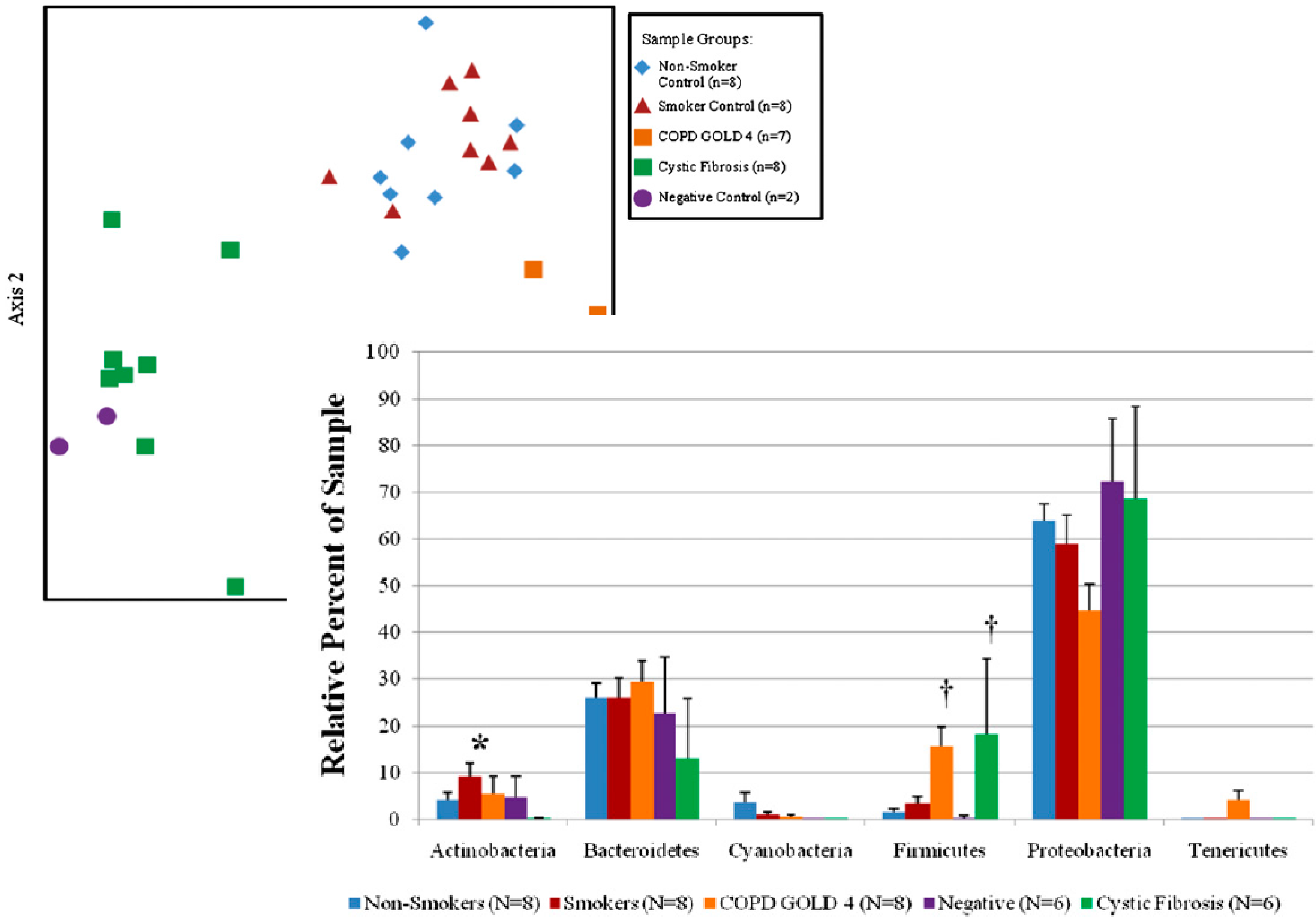
The Lung Tissue Microbiome in Chronic Obstructive Pulmonary Disease

Marc A. Sze[1], Pedro A. Dimitriu[2], Shizu Hayashi[1], W. Mark Elliott[1], John E. McDonough[1], John V. Gosselink[1], Joel Cooper[3], Don D. Sin[1], William W. Mohn[2], and James C. Hogg[1]

[1]The James Hogg Research Centre, Providence Heart-Lung Institute at St. Paul's Hospital, Departments of Medicine and Pathology and Laboratory Medicine, and [2]Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada; and [3]Department of Cardiovascular and Thoracic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania

Am J Respir Crit Care Med 2012;185:1073-1080





Sze et al. *Am J Respir Crit Care Med* 2012;185:1073-1080

A Persistent and Diverse Airway Microbiota Present during Chronic Obstructive Pulmonary Disease Exacerbations

Yvonne J. Huang,[1] Eugenia Kim,[2] Michael J. Cox,[2] Eoin L. Brodie,[3]
Ron Brown,[2] Jeanine P. Wiener-Kronish,[4] and Susan V. Lynch[2]

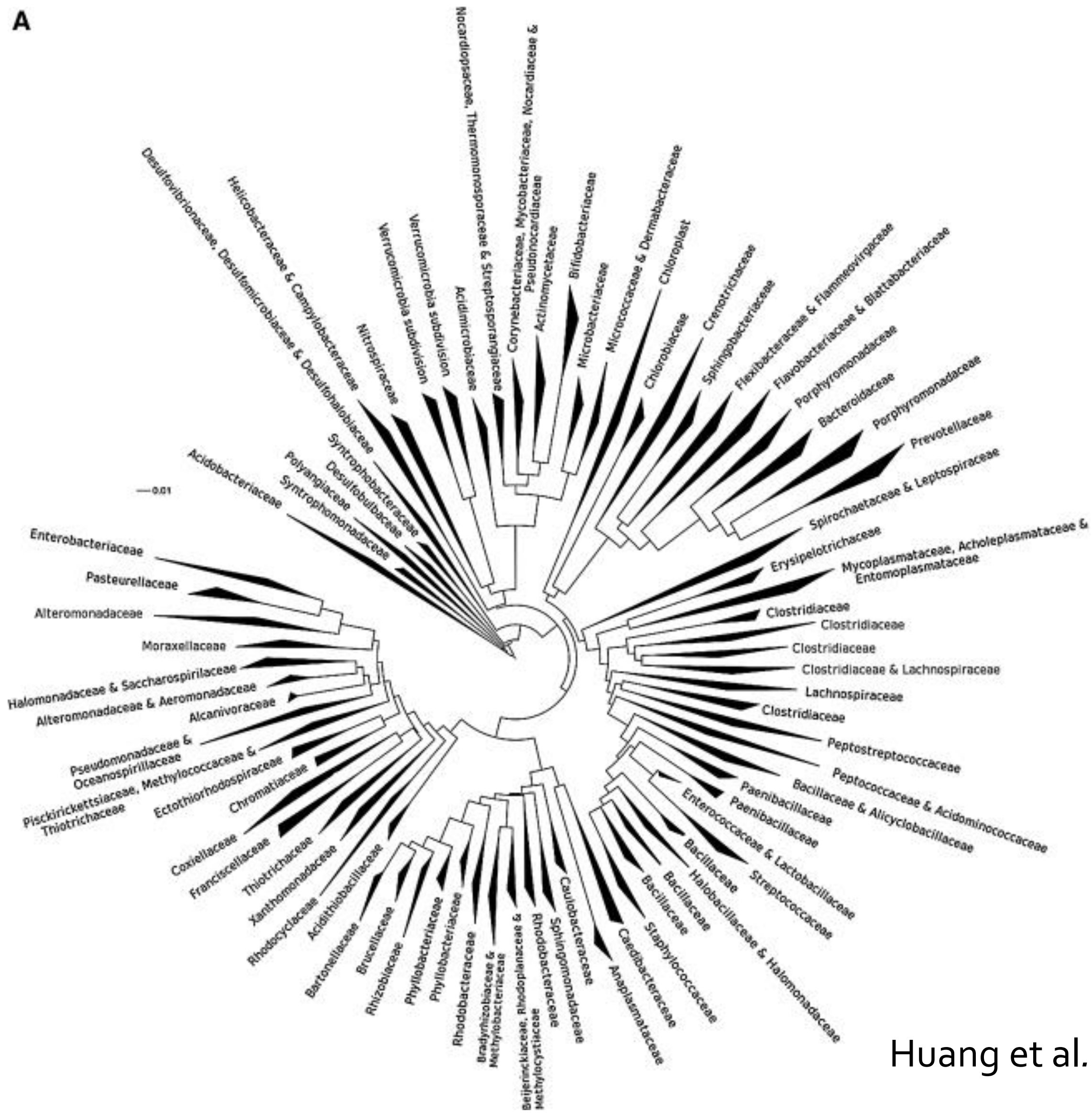
OMICS 2010;14:9-59

TABLE 1. CLINICAL CHARACTERISTICS OF SUBJECTS AND SAMPLES

Patient	*Age*	*Gender*	*Intubation days at sample collection*	*Antimicrobial therapy received within the past month*	*Days of active antimicrobial therapy at time of sample collection*	*Culture results*[a]
1	63	M	16	ceftazidime	16	*PA*[b*]
2	69	F	6	vancomycin, tobramycin, levofloxacin	5	*PA*[b*]
3	78	M	1	vancomycin, piperacillin/tazobactam, levofloxacin	1	*PA*[b*], *KP*[b*], *AF*
4	78	M	21	piperacillin/tazobactam	31	*PA*[b*], *SM*[b*]
5	86	F	17	levofloxacin	17	*PA*[b*]
6	85	F	16	doxycycline, moxifloxacin, vancomycin	1	*PA*[b*]
7	61	M	5	vancomycin, piperacillin/tazobactam	7	*PA*[b*], *SA*[b]
8	73	M	3	piperacillin/tazobactam	3	*PA*[b], *EA*[b*]

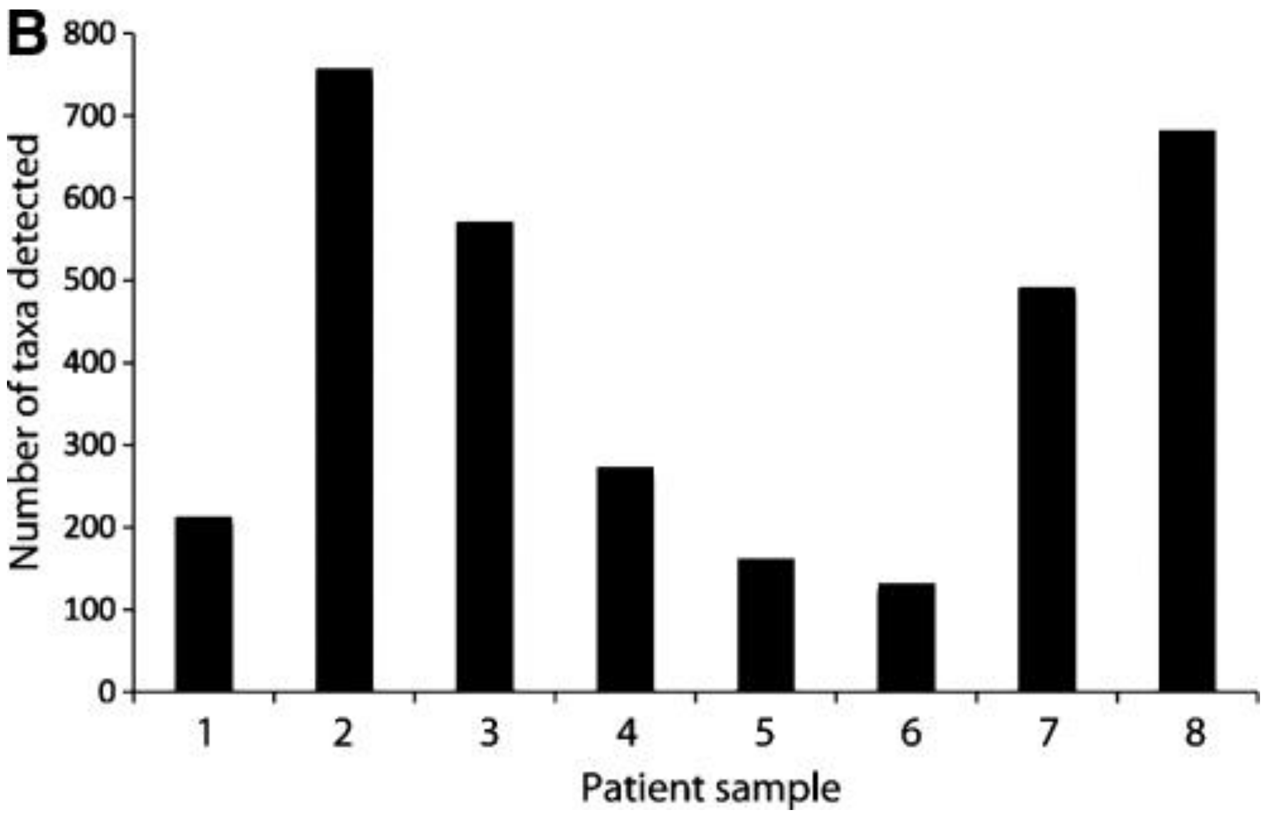
[a]mini-BAL, minibronchoalveolar lavage clinical culture. The most recent, available culture data were obtained from within 1–5 days prior to the endotracheal aspirate sample analyzed by PhyloChip.
[b]Detected by PhyloChip; *≥10,000 colony-forming units on quantitative mini-BAL culture.
PA, *Pseudomonas aeruginosa;* KP, *Klebsiella pneumoniae;* SA, *Staphylococcus aureus;* EA, *Enterobacter aerogenes;* SM, *Stenotrophomonas maltophilia;* AF, *Aspergillus fumigatus.*

family level bacterial diversity detected in COPD airways despite antimicrobial administration



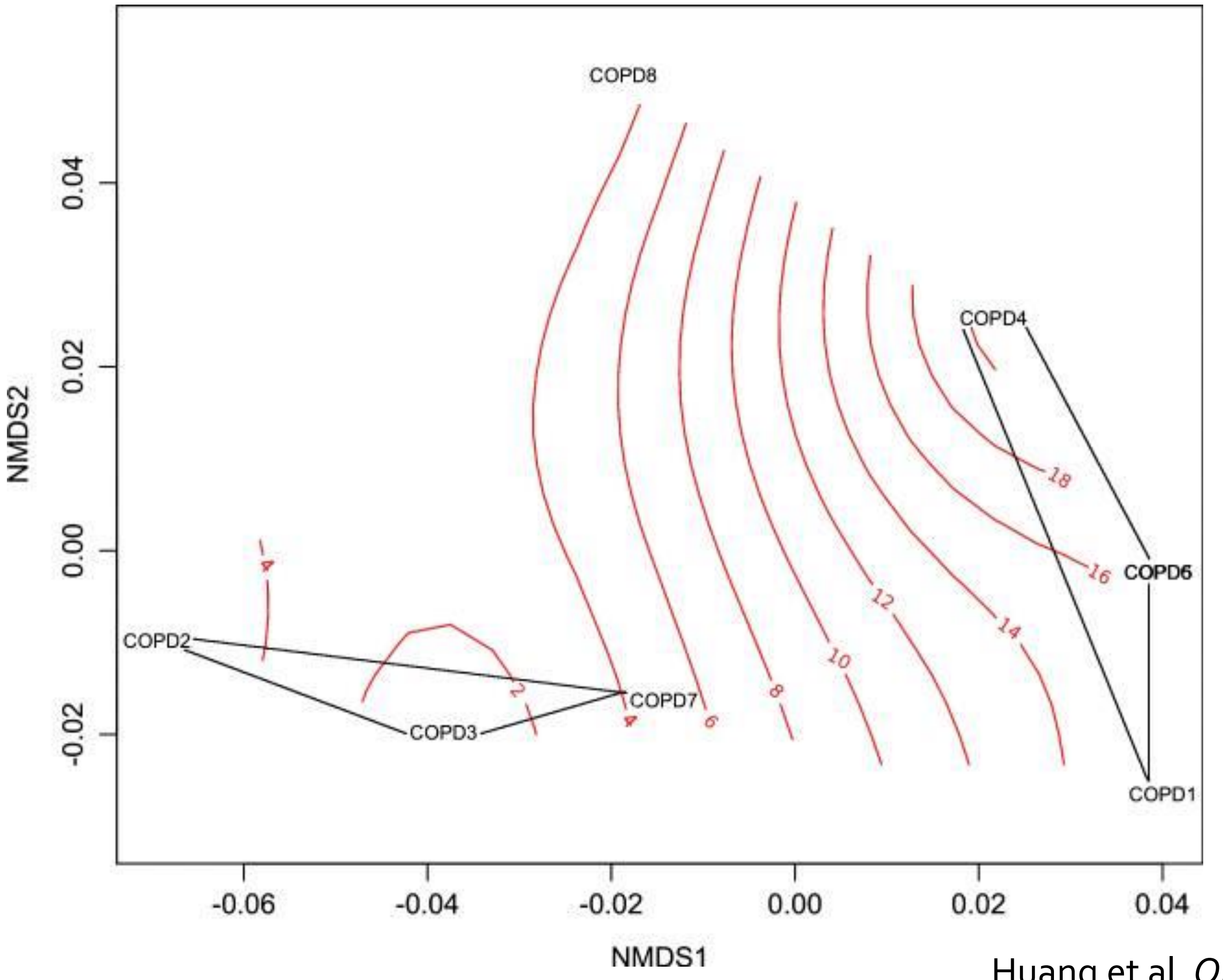
Huang et al. *OMICS 2010;14:9-59*

Bacterial richness in individual patient samples



Huang et al. *OMICS 2010;14:9-59*

Bacterial community composition is highly influenced by the duration of intubation



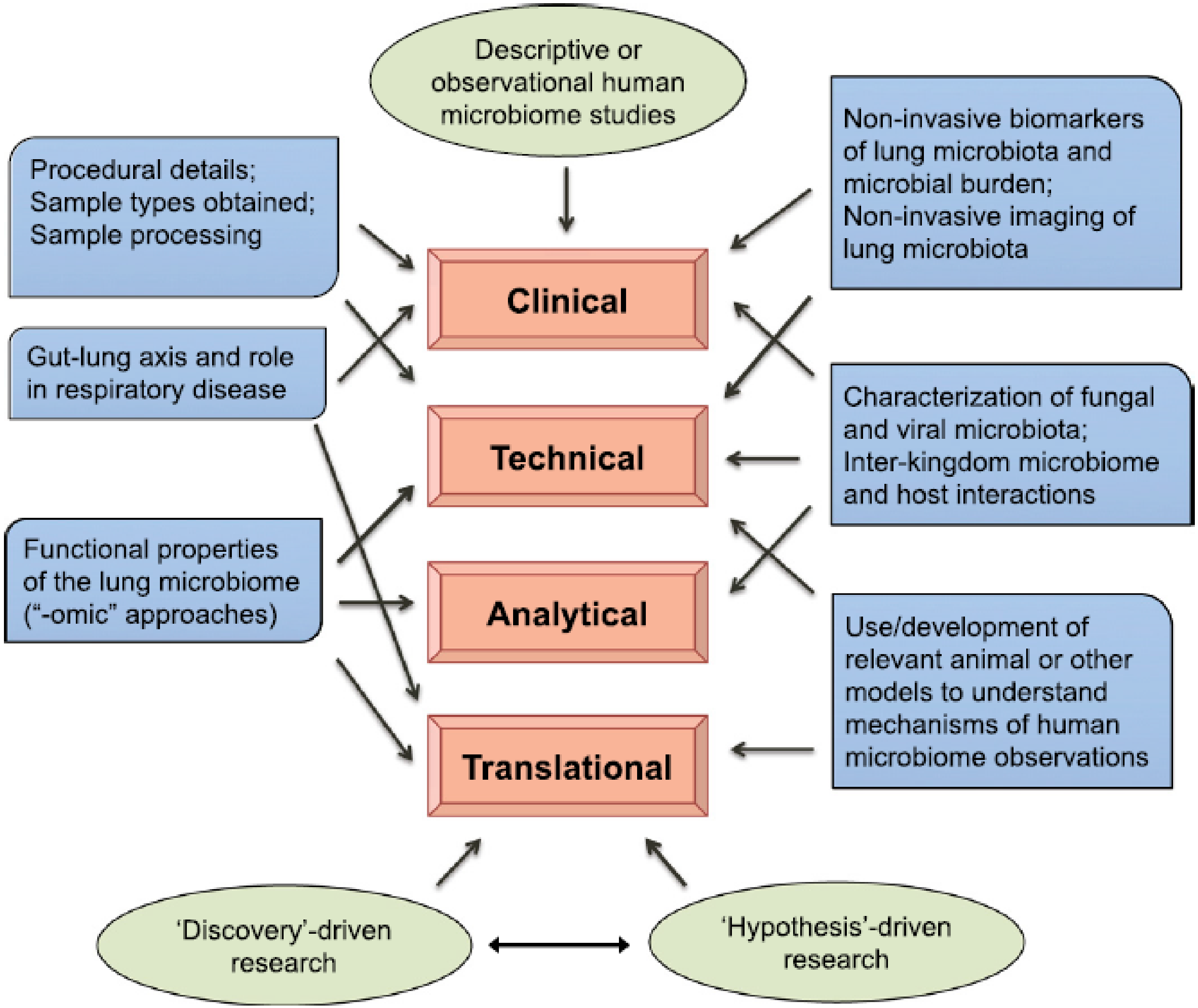
Huang et al. *OMICS 2010;14:9-59*

FUTURE DIRECTIONS

Ultimate Goals

- To understand whether specific aspects of the lung microbiome relate to **disease progression** and whether manipulating the bacterial community might **preserve lung function**

- To define how bacterial communities change around the time of **exacerbation**, and even whether changes in the bacterial community structure might predict exacerbation, possibly allowing early or preemptive intervention



Huang YJ et al. *Am J Respir Crit Care Med* 2013;187:1382-1387

In the coming years..

- **Standardization of techniques** for sample acquisition, quantification of bacterial biomass and data analysis

- Ongoing identification of **novel pathogens**, development of methods for confirming **pathogenicity**, and development of accurate, rapid and affordable point-of-care **diagnostic tools** for respiratory infection

In the coming years..

- **Longitudinal studies** of samples from individual subjects, both diseased and control, to characterize the stability and robustness of lung microbial communities over time as well as the influence of external factors such as antibiotics, immunosuppression and acute illness

- Study of **nonbacterial lung microbes** such as viruses and fungi

In the coming years..

- Development of **animal models** of lung microbiome states to test mechanistic hypotheses

- Exploration of **microbe-microbe and microbe-host signaling mechanisms**

- Clinical trials of **microbiome-targeted interventions**

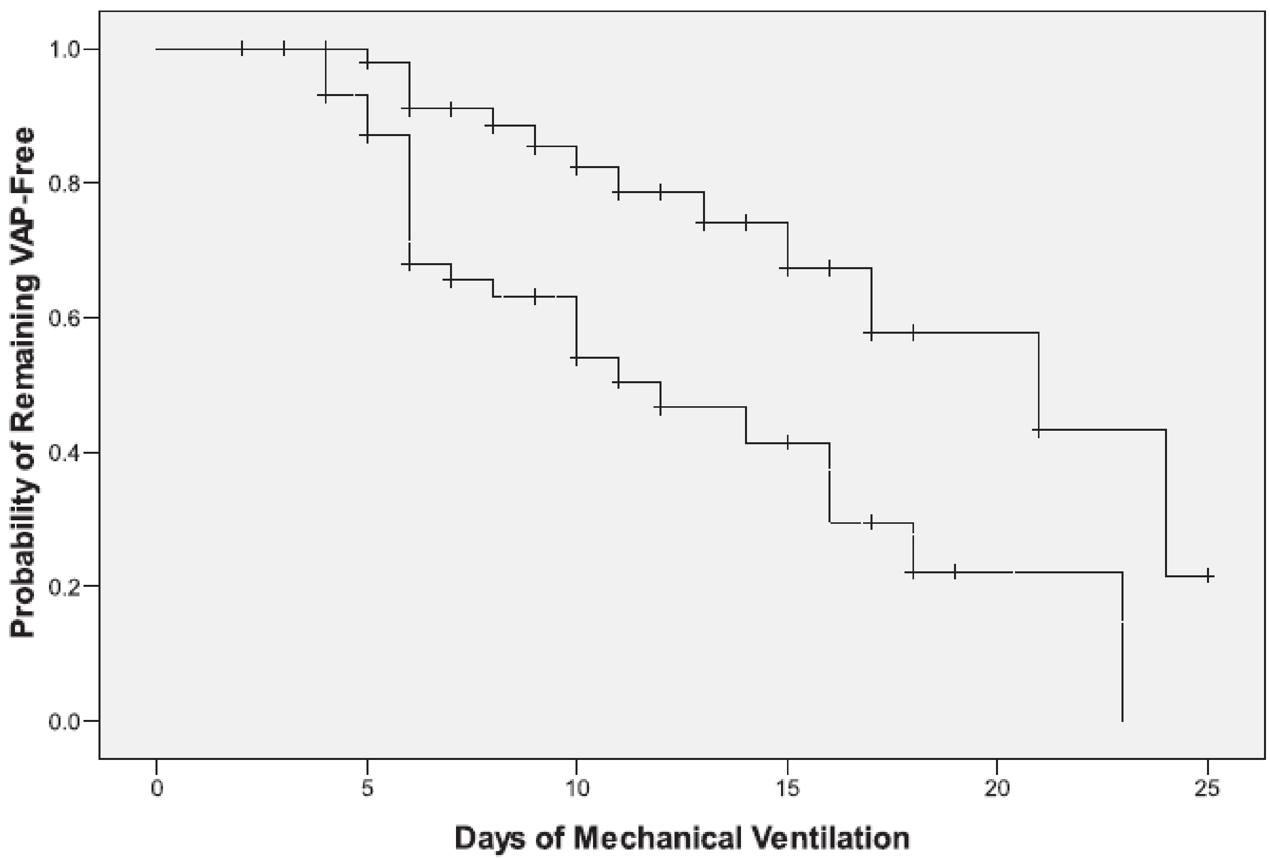
Probiotic Prophylaxis of Ventilator-associated Pneumonia

A Blinded, Randomized, Controlled Trial

Lee E. Morrow[1], Marin H. Kollef[2], and Thomas B. Casale[3]

[1]Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, Creighton University School of Medicine, Omaha, Nebraska; [2]Department of Medicine, Division of Pulmonary and Critical Care Medicine, Washington University in St. Louis, School of Medicine, St. Louis, Missouri; and [3]Department of Allergy and Immunology, Creighton University School of Medicine, Omaha, Nebraska

PLoS ONE 2010;5:e8578



Take Home Message

- Novel culture-independent techniques have recently demonstrated that the lower respiratory tract, historically considered sterile in health, contains diverse communities of microbes: the lung microbiome.

- Increasing evidence supports the concept that a distinct microbiota of the lower respiratory tract is present both in health and in respiratory diseases, although the biological and clinical significance of these findings remains undetermined.