The Gut Microbiome: Markers of Human Health, Drug Efficacy and Xenobiotic Toxicity Biomarkers of Toxicity and Disease

Identification of microbiome-based biomarkers and challenges associated with their application: Case studies from obesity, IBD, and cancer

25 June 2018 | Alexandria, VA

Emily Hollister
VP, Information Technology & Analytics

Background – Diversigen is a commercial microbiome service provider powered by CMMR

DIVERSIGEN

MICROBIOME SERVICES

Metagenomic sequencing pipelines | Germ-free Project consulting Bioinformatics

HEADQUARTERS

Houston, Texas - located at the heart of the Texas Medical Center

2013

YEAR FOUNDED

Operational since December, 2014

PEOPLE

12 highly talented and experienced individuals with a total of 8 PhD's

ORIGIN

TMC TEXAS MEDICAL CENTER

21	8	10	3	1	2
Renowned Hospitals	Research Institutions	Academic Institutions	Medical Schools	Dental School	Pharmacy Schools

CMMr
Alkek Center for Metagenomics and Microbiome Research

Centre for Metagenomics and Microbiome Research (CMMR)

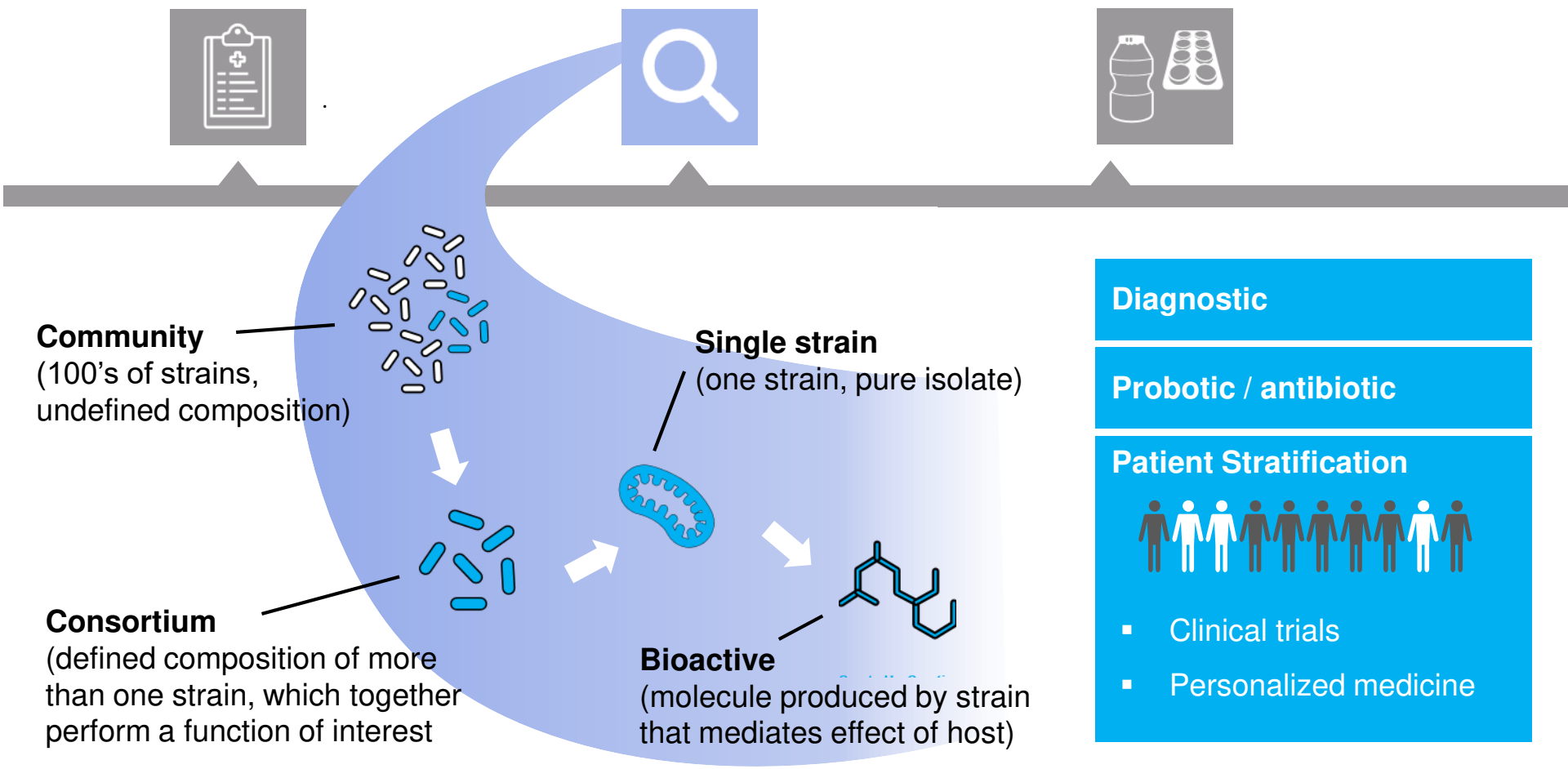
Microbiome Exploration | Microbial ecology modeling and dissection Therapeutic development | Policy and outreach | Education | Translation

Metagenomics plays a key role in therapeutic and diagnostic development

Compare microbiota in healthy and ill subjects

ID organisms / genes associated with health / disease

Develop applications / solutions



Diagnostic

Probotic / antibiotic

Patient Stratification

- Clinical trials
- Personalized medicine

Microbiome-based biomarkers

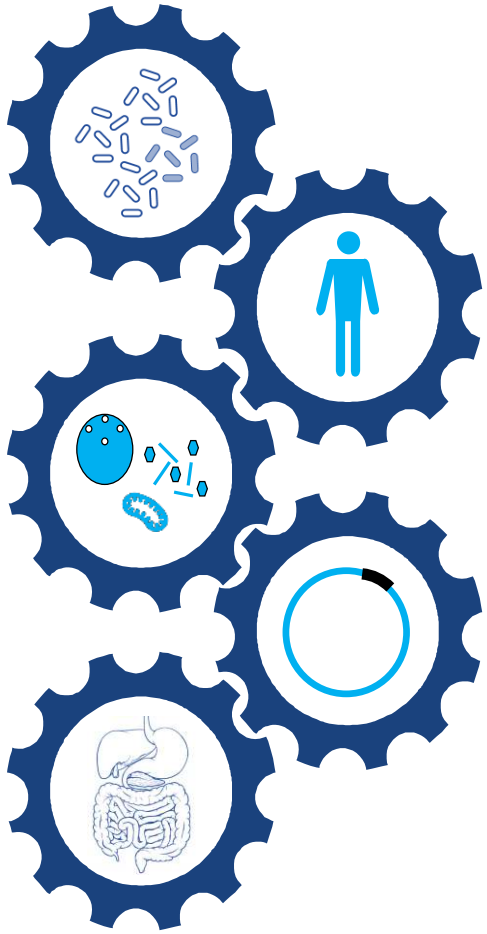
- What are they? Do they exist?

- What are the challenges associated with their application?
  - Defining **healthy** has turned out to be difficult
  - Consensus/lack of across multiple studies – sources of bias, evolution of microbiome science/understanding

- They **don't have to be bacterial** – although many purported biomarkers are

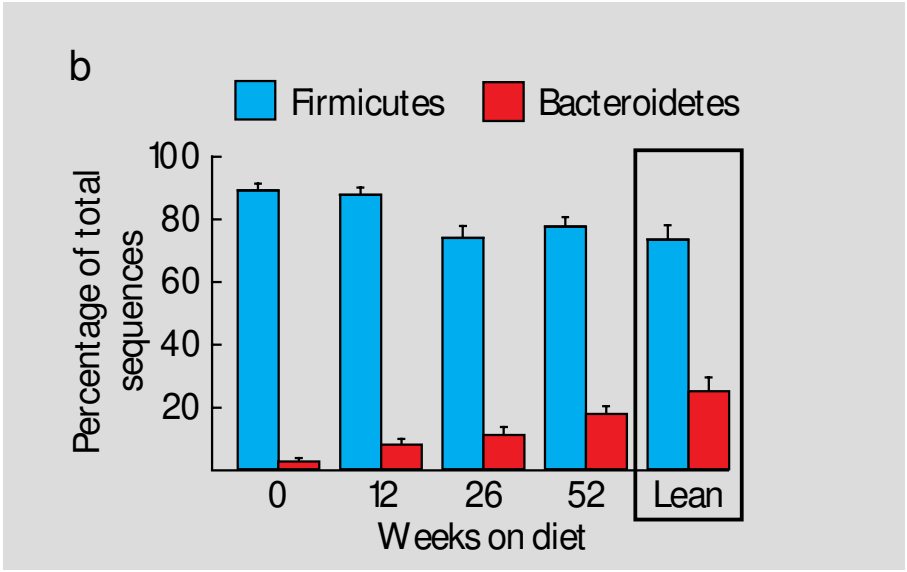
- The majority of the data are **16S-based**
  - 16S lacks the resolution that we'd prefer to have (i.e., better than genus)

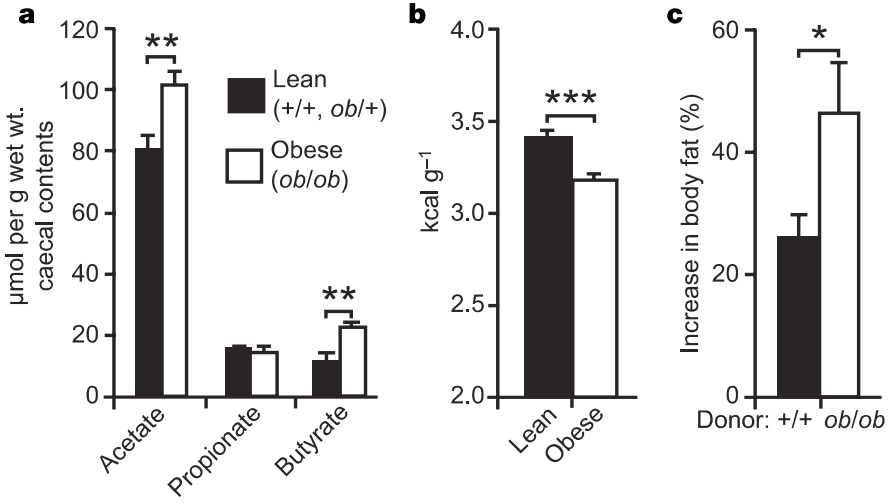
- The majority of data is **stool-based**
  - good for broad applicability and user acceptance; may not reflect biology at the **disease interface**. But…..at the end of the day, does it matter?

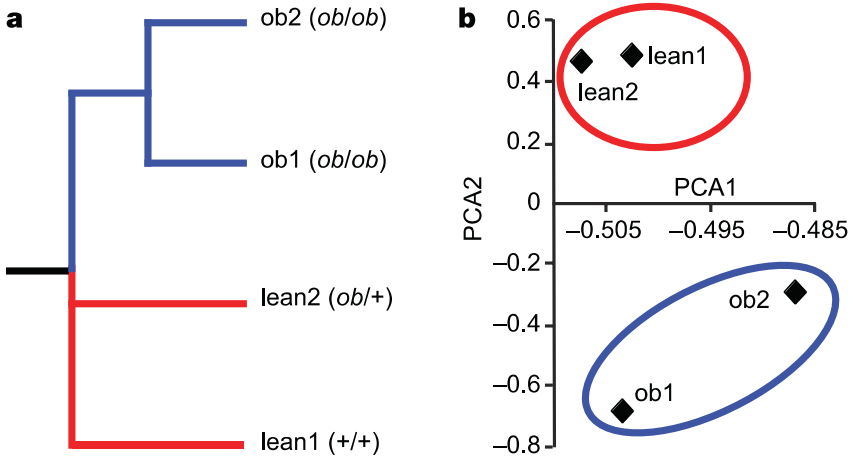


Obesity and the gut microbiome

- Summarizing the literature:
  - Data from animal studies is very compelling
  - Data from human studies is somewhat murky
- Consensus across studies is limited
  - Multiple potential pitfalls







Ley et al 2006; Turnbaugh et al 2006

Conferring obesity through FMT

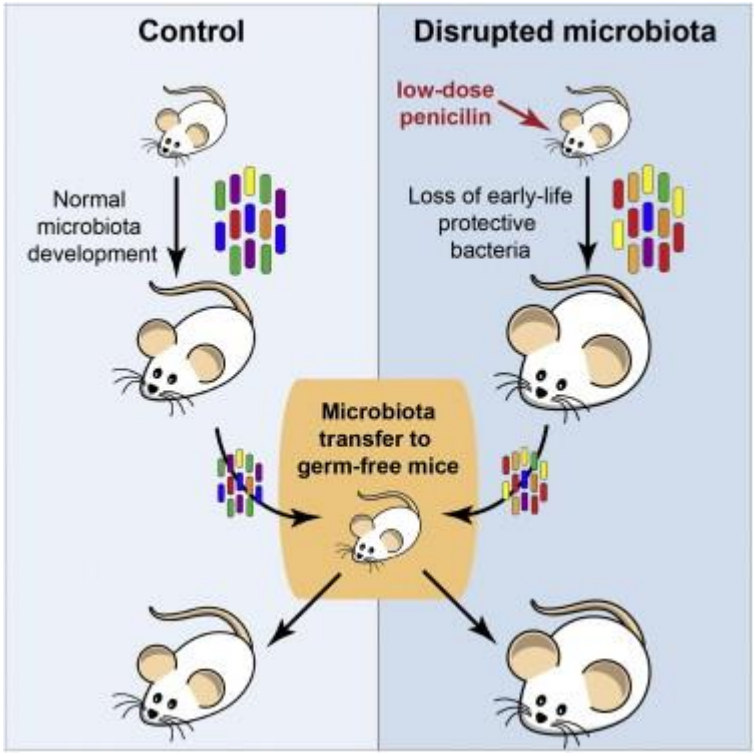
Weight Gain After Fecal Microbiota Transplantation

Neha Alang[1] and Colleen R. Kelly[2]

[1]Department of Internal Medicine, Newport Hospital, and [2]Division of Gastroenterology, Center for Women's Gastrointestinal Medicine at the Women's Medicine Collaborative, The Miriam Hospital, Warren Alpert School of Brown University, Providence, Rhode Island

Fecal microbiota transplantation (FMT) is a promising treatment for recurrent *Clostridium difficile* infection. We report a case of a woman successfully treated with FMT who developed new-onset obesity after receiving stool from a healthy but overweight donor. This case may stimulate further studies on the mechanisms of the nutritional-neural-microbiota axis and reports of outcomes in patients who have used non-ideal donors for FMT.

Keywords. *Clostridium difficile* infection; fecal microbiota transplantation; gut microbiota; obesity.



Alang and Kelly 2015; Cox et al 2014

Microbiome alterations in are subtle and only partially reproducible

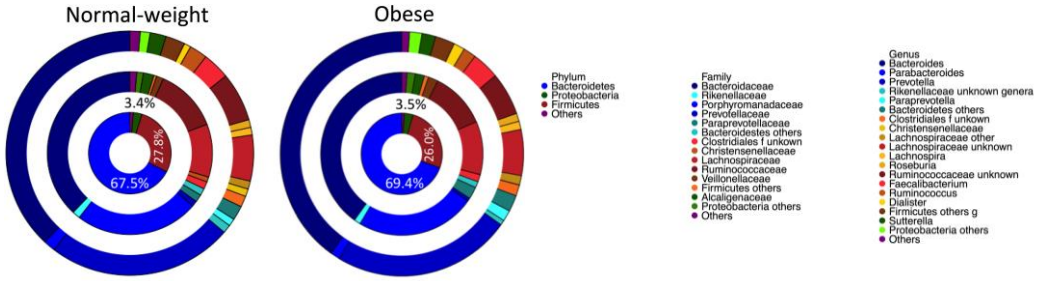
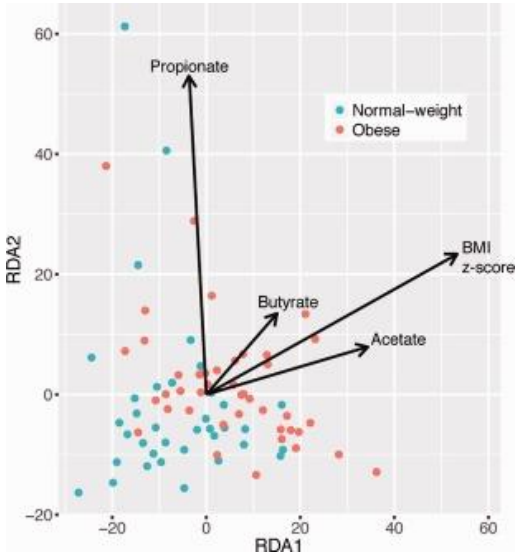


Table 2. Correlation of Operational Taxonomic Unit Abundances with Body Mass Index z-Scores Across Normal Weight ($n=22$) and Obese ($n=42$) Children

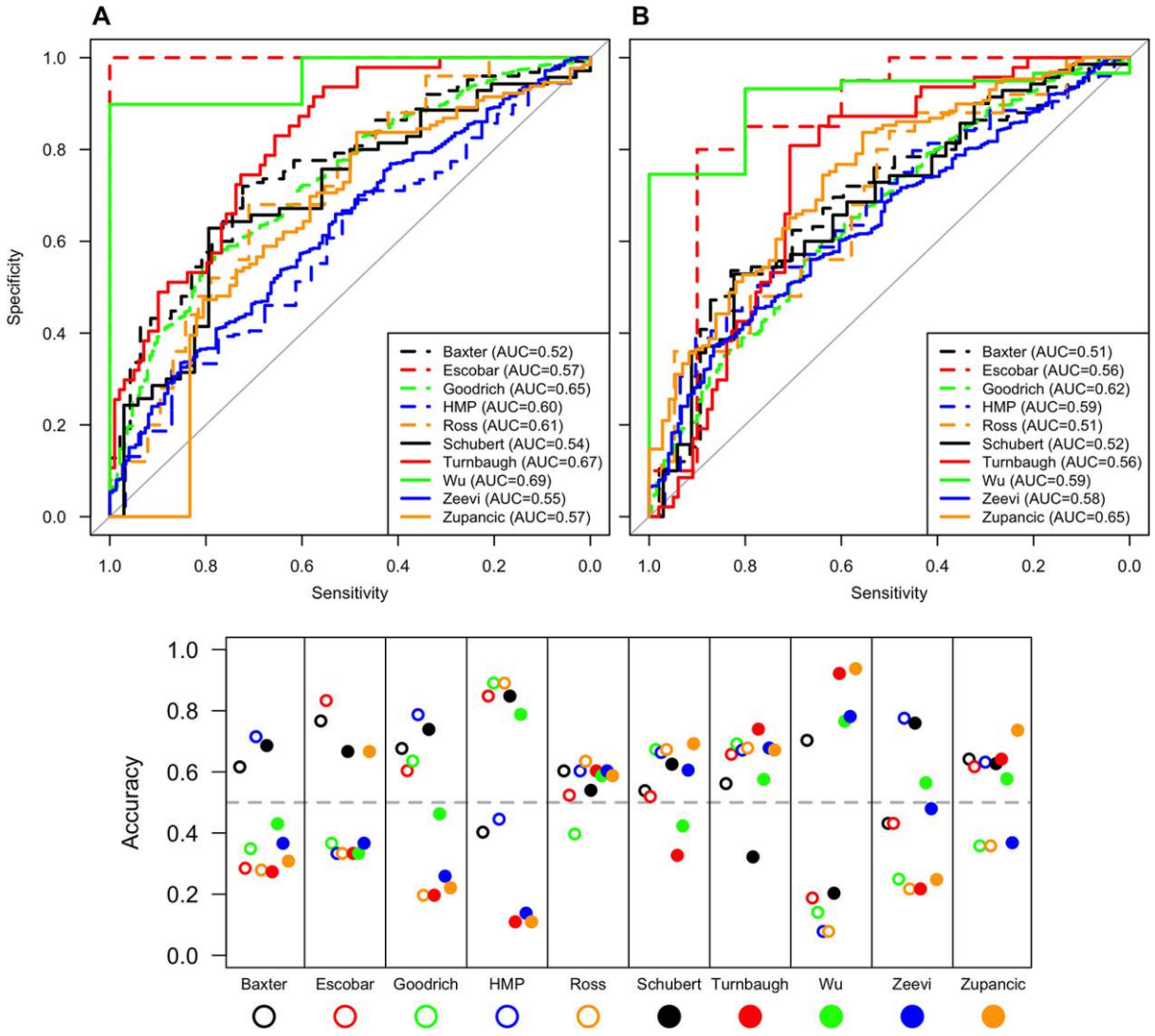
OTU ID	Taxonomic identify	Spearman rho	q-value
OTU_86	Unclassified proteobacteria	–0.59	0.0004
OTU_161	Paraprevotella clara	–0.55	0.0018
OTU_515	Unclassified bacterium	–0.52	0.0050
OTU_59	Bacteroides plebeius	–0.51	0.0054
OTU_216	Bacteroides thetaiotaomicron	–0.47	0.0170
OTU_476	Fusicatenibacter saccharivorans	–0.47	0.0170
OTU_103	Prevotella stercorea	0.45	0.0282
OTU_107	Phascolarctobacterium succinatutens	0.45	0.0282
OTU_330	Unclassified bacterium	0.45	0.0282
OTU_98	Bacteroides salyersiae	0.44	0.0312
OTU_409	Clostridium bolteae	–0.43	0.0407
OTU_355	Unclassified firmicutes	–0.43	0.0442
OTU_21	Unclassified bacteroidales	0.42	0.0566
OTU_13	Bacteroides massiliensis	0.40	0.0995

Spearman correlations were used to identify relationships between variables, and the Benjamini-Hochberg procedure was used to apply false discovery rate corrections.



Lopez-Contreras et al 2017 Pediatric Obesity; Hollister et al 2018 Childhood Obesity; Riva et al 2017 Environmental Microbiology

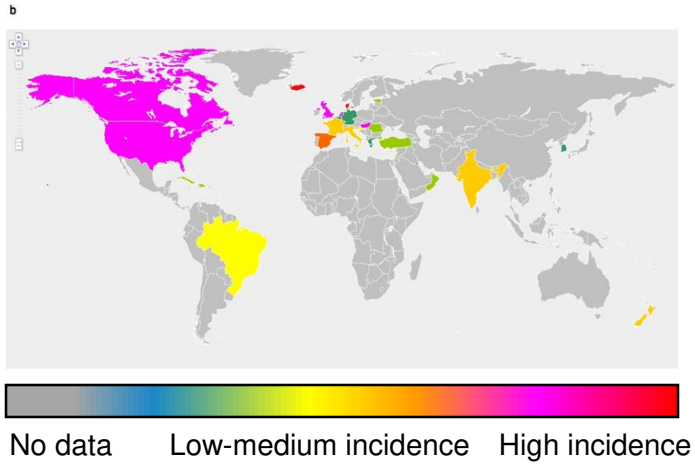
Microbiome alterations in obesity are inconsistent

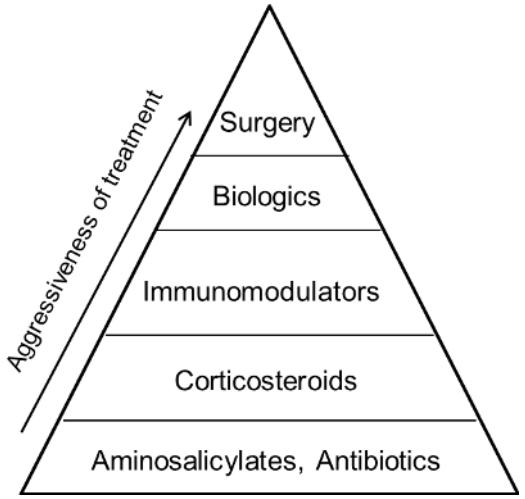


Sze and Schloss 2016

IBD: The interaction of genetics, the immune system, and environment

- IBD includes Crohn's Disease and Ulcerative Colitis
- IBD affects 1.4M Americans, with increasing incidence in recent decades
- Greater incidence in Western nations and countries experiencing demographic/economic development
- Observed at greater rates in children immigrating to high(er) prevalence countries
- Concordance <50% in monozygotic twins
- Direct cost of treatment in the US represents an economic burden of ~$4 billion annually (UC)
- Greater risk for developing colorectal cancer



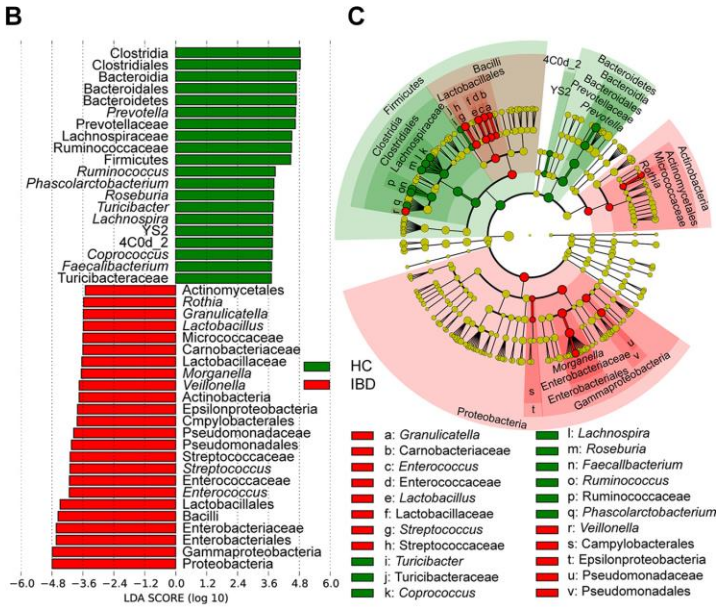


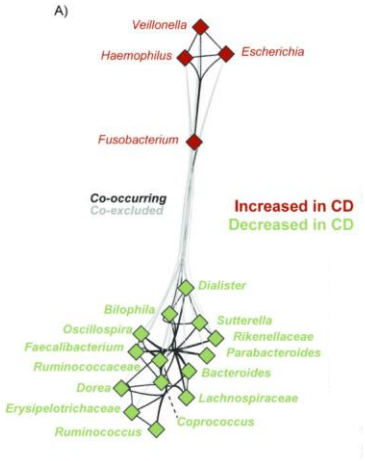
Danese, S. & Fiocci, C. (2011). NEJM; Kornbluth A, Sachar DB (2004). Am. J. Gastroenterol; Cohen et al. (2010). Aliment Pharmacol Ther; Etchvers et al. 2008. World J Gastroenterol; Talley et al. (2011). Am J. Gastroenterol

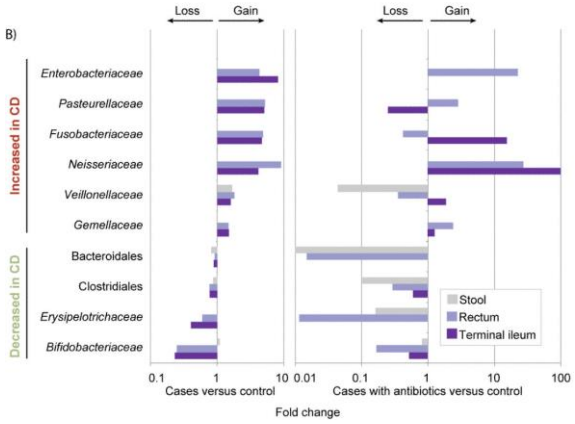
IBD: Taxonomic patterns and trends

Taxonomic patterns:
Imbalances/dysbiosis commonly described in the literature – largely 16S based:

- CD: ⇩ Roseburia, ⇩ Faecalibacterium prausntizii, ⇧ Ruminococcus gnavus
- UC: not as clearly defined
  - ⇧ sulfate-reducing Proteobacteria have been reported
  - ⇩ Roseburia, ⇩ Ruminococcaceae,





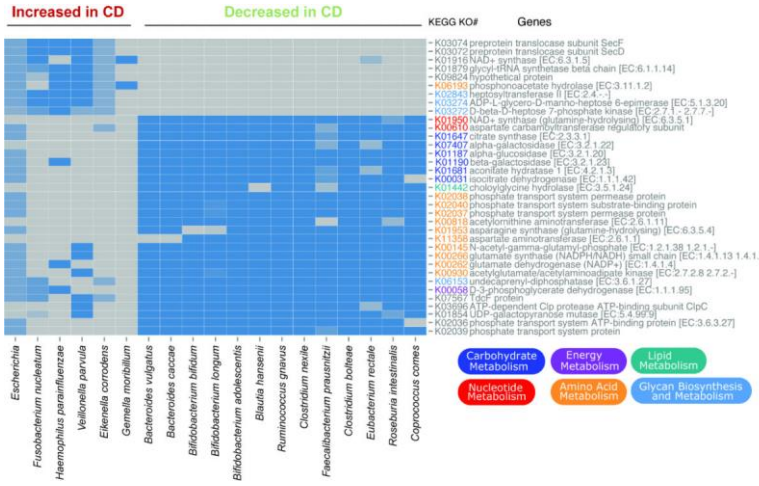


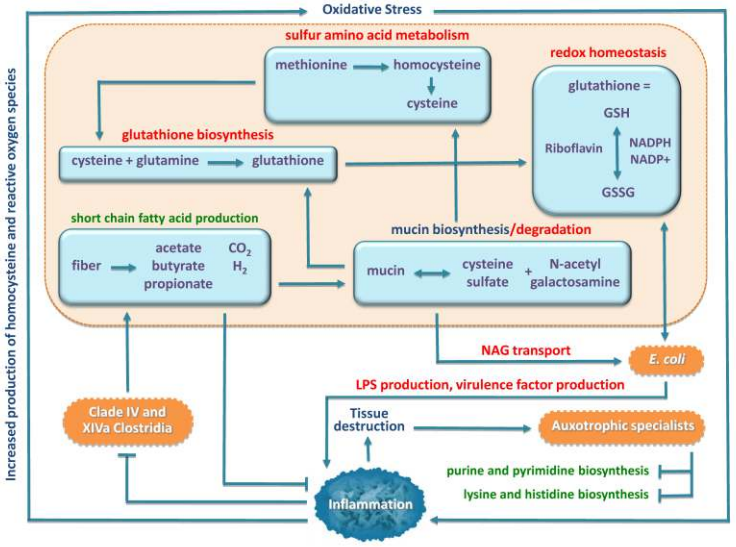
Gevers et al 2014 Cell Host Microbe (CD)
Zhou et al 2018 mSystems

IBD: Functional metagenomic patterns and and trends

Functional patterns:
Fewer studies have used functional metagenomics.

- Gevers et al 2014: Species increased in CD contributed enrichment of glycerophospholipid and lipopolysaccharide metabolism
- Morgan et al 2012: Increased metabolism of sulfur-containing amino acids in IBD; shifted metabolism toward the degradation of mucin
- Alterations suggest an environment characterized by inflammation and oxidative stress





Morgan et al (2012) Genome Research
Gevers et al 2014 Cell Host Microbe

Does consensus exist with respect to IBD biomarkers?

Consensus?
To some degree, yes:

- Studies have repeatedly identified many of the same taxa (16S, WGS)
  - ⇧ Enterobacteriaceae
  - ⇧ Pasteurellaceae
  - ⇧ Veillonellaceae
  - ⇧ Fusobacteriaceae
  - ⇩ Faecalibacterium
  - ⇩ Roseburia
  - ⇩ Clostridiales
- Unclear roles: Causal, contributory, coincident, consequential

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RESEARCH ARTICLE
Host-Microbe Biology

Gut Microbiota Offers Universal Biomarkers across Ethnicity in Inflammatory Bowel Disease Diagnosis and Infliximab Response Prediction

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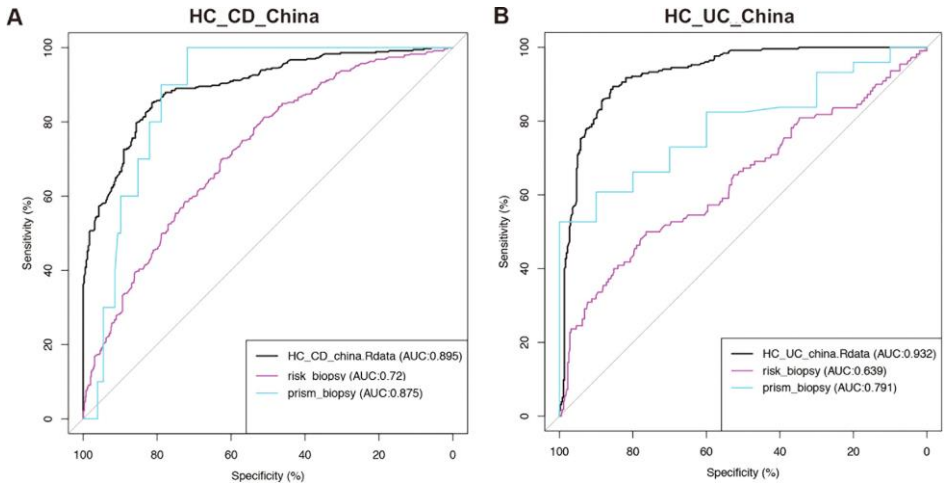


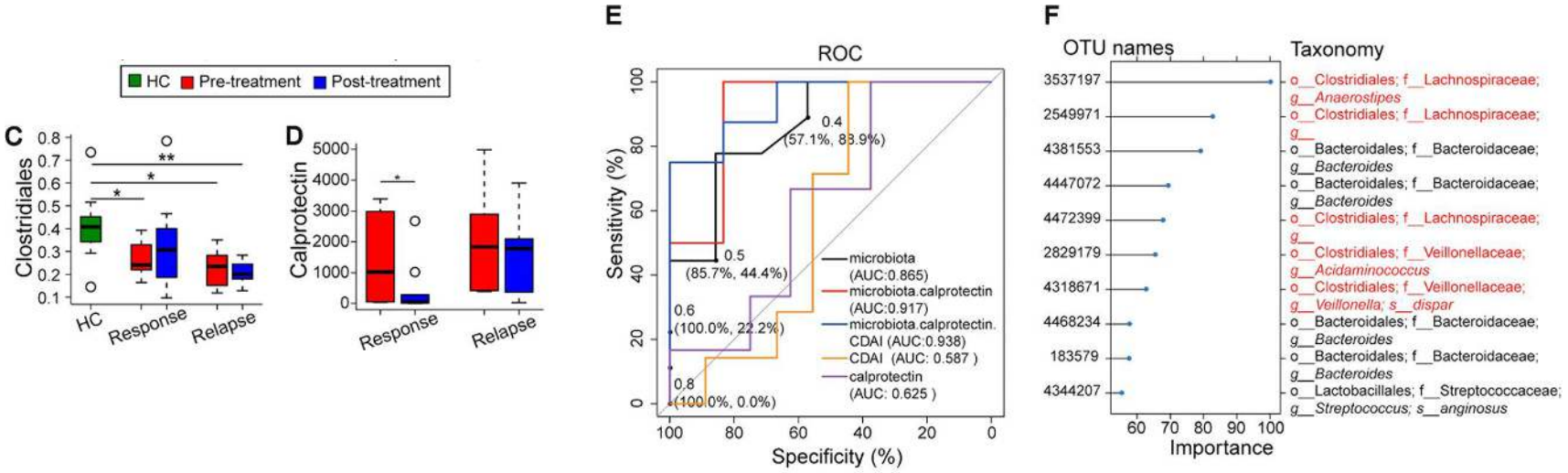
FIG 3 Gut microbiotas distinguish diseases from health similarly across cohorts. The black ROC curve indicates the accuracy of the classification model built on the Chinese cohort for classifying HC versus CD (A) and HC versus UC (B). Colored curves are the classification accuracies when these models are applied to the other cohorts.

Zhou et al 2018 mSystems

IBD: How might microbiome-based biomarkers be used?

How might biomarker be used?

1. Early diagnosis
2. Monitoring of disease status/severity
3. Evaluating treatment success
4. Monitoring progression otherwise (e.g., toward relative health or other disease states)



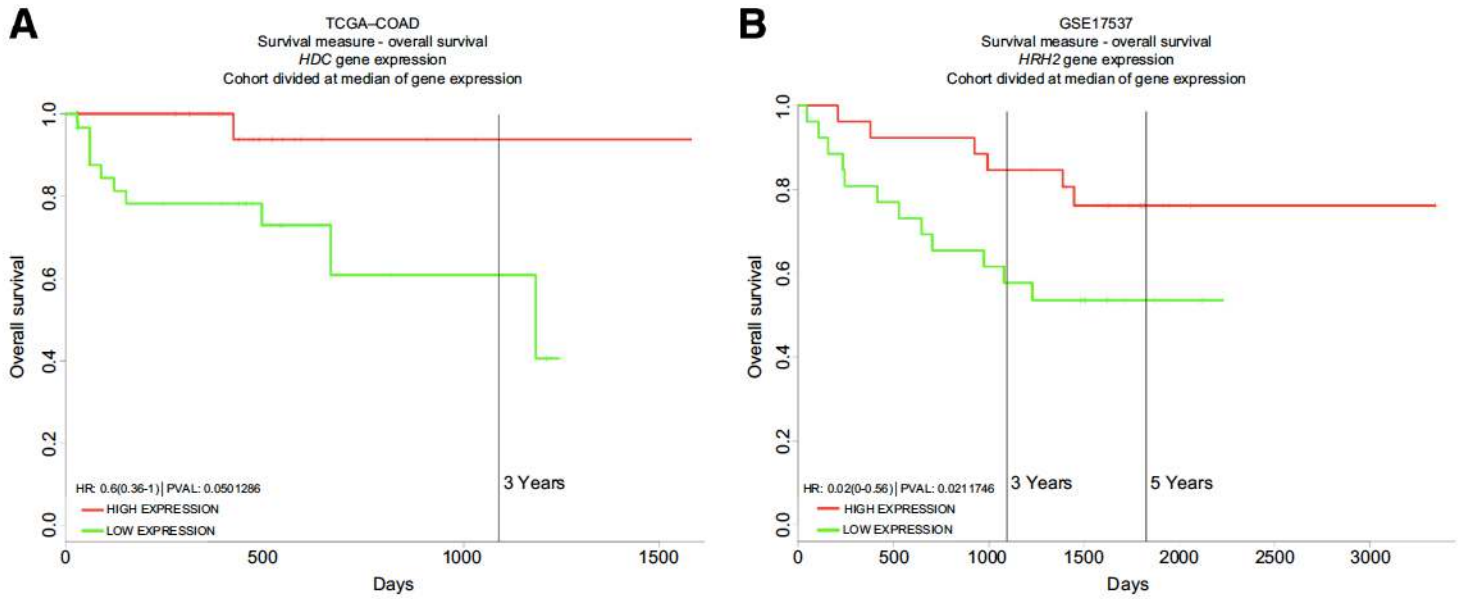
Zhou et al 2018 mSystems

CRC & IBD: Risk association

- Increased risk of CRC in IBD recognized in late 80s
- Reported risk factors for CRC include
  - extensive disease
  - young age at diagnosis
  - family history of CRC
  - co-existing primary sclerosing cholangitis
  - **persistent inflammation of the colon**
- CRC in IBD carries a 2-fold increase in mortality compared with sporadic cancers.
  - No major differences between UC and CD except for a more advanced tumor stage at the time of diagnosis in CD patients.
- With IBD, the presence of CRC is often not known prior to surgery

Inflammation in CRC

Overall survivorship influenced by gene expression



Histidine decarboxylase

Histamine H2 receptor

Gao et al 2017 American Journal of Pathology

Inflammation in CRC

Microbiome attenuation by addition of one organism

Model without chemical trigger

Model with chemical trigger

Model with chemical trigger and microbe

Model with chemical trigger and ko mutant microbe

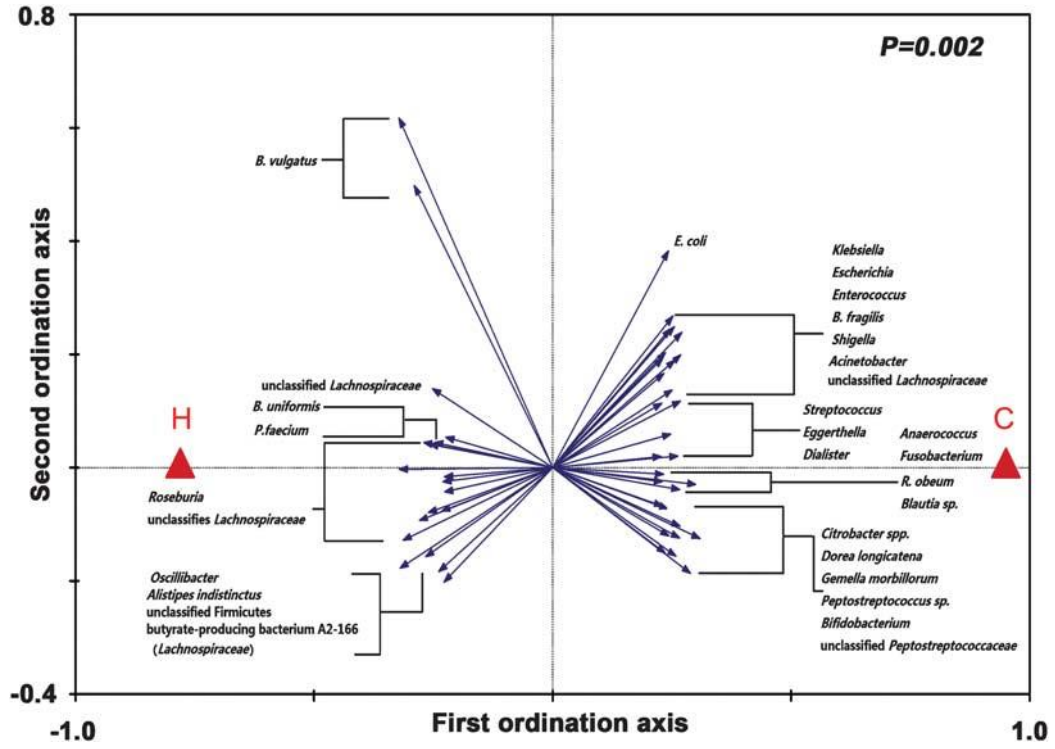
Gao et al 2017 American Journal of Pathology

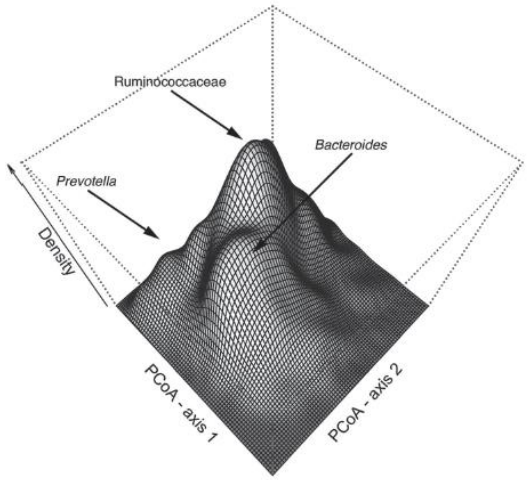
Colorectal cancer and the gut microbiome

- First CRC microbiome associations: adherent-invasive *Escherichia coli* is associated both with IBD and CRC
- Modern metagenomic techniques have yielded conflicting results
  - Most studies find associations
  - Different taxa are associated with CRC presence/absence, severity, disease progression, and survivorship
- Will microbiome be able to answer the question alone?
  - What about in high risk populations?
- CRC and the gut microbiome — Where are we with respect to describing it and identifying actionable biomarkers?

Rubin et al (2012) Front Immunol

Disparate CRC associated microbiota





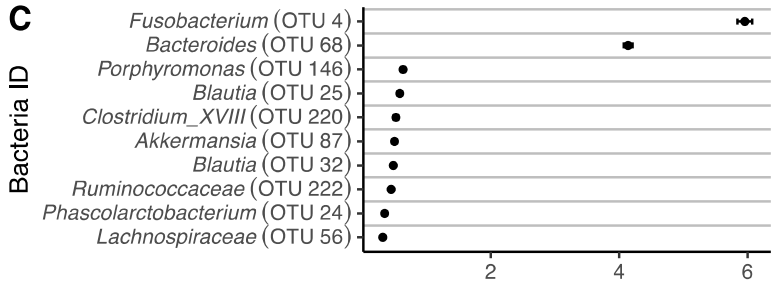


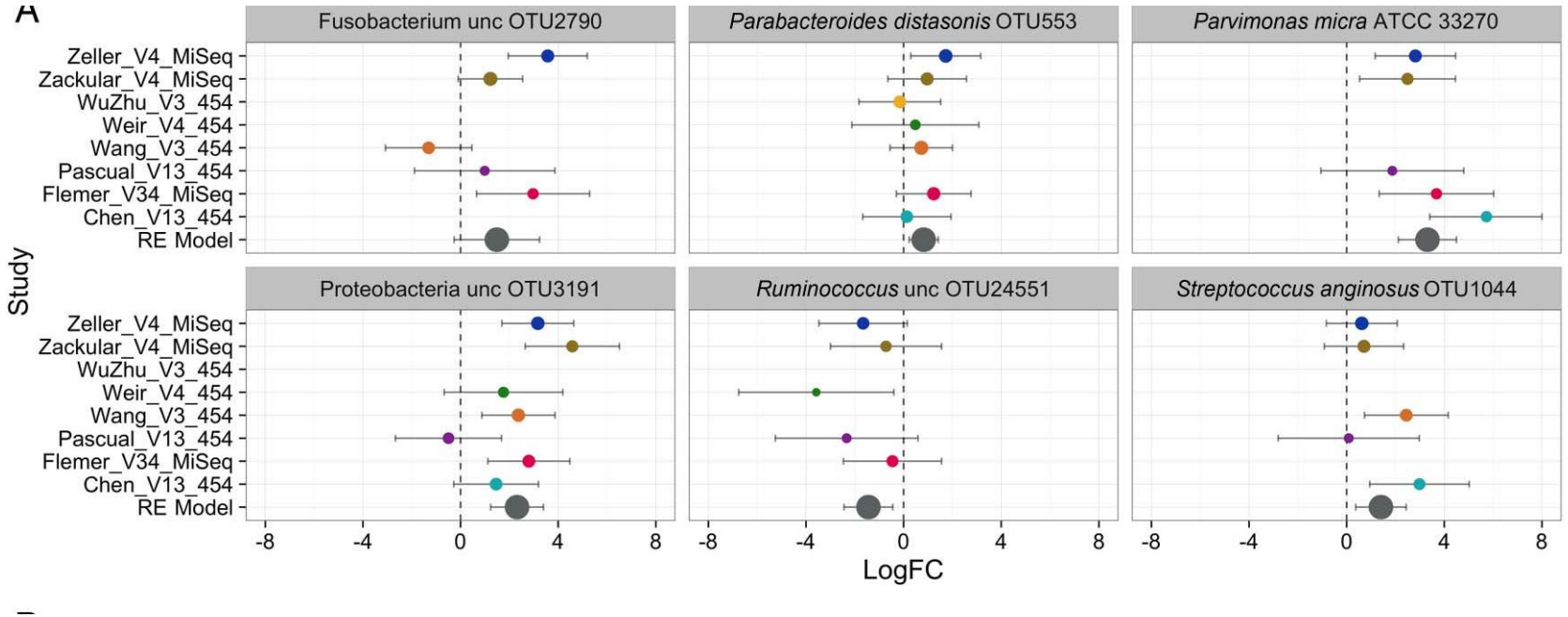
Table 3. Bacterial species significantly over-represented in CRC stool samples.

Bacterial Species	Avg. Healthy (%)	Avg. CRC (%)	Fold Change	p value
Acidaminobacter unclassified	0.05	0.39	7.7	0.0045
Phascolarctobacterium unclassified	3.31	11.0	3.2	0.0000
Citrobacter farmeri	0.08	0.37	4.6	0.0050
Akkermansia muciniphila	3.54	12.8	3.6	0.0032

doi:10.1371/journal.pone.0070803.t003

Weir et al (2013) PLoS One; Zeller et al (2014) Molecular Systems Biology; Wang et al (2014) ISME; Hannigan (2017) bioRxiv

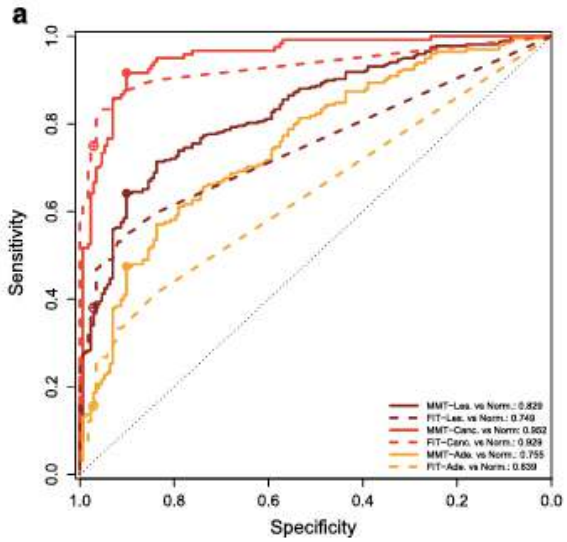
When processed similarly concordance in associations

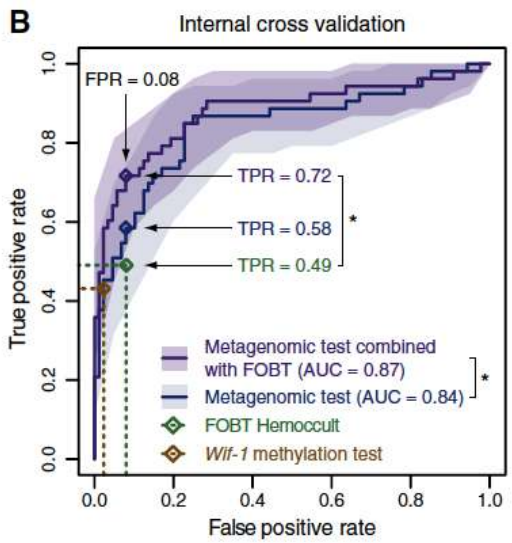


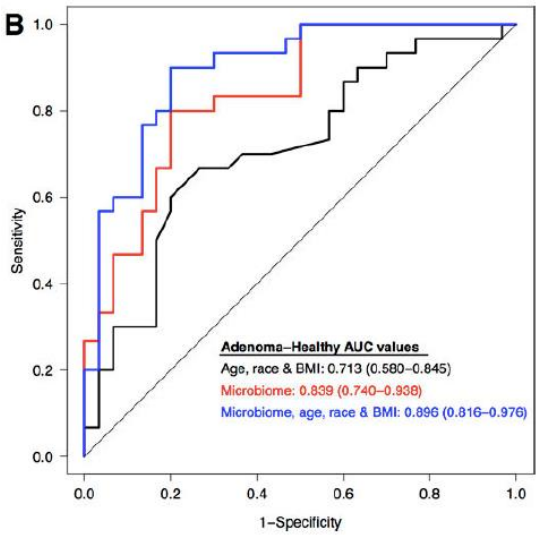
…*mostly*.

Shah et al 2017 Gut

Improvement in sensitivity by microbiome + risk factors



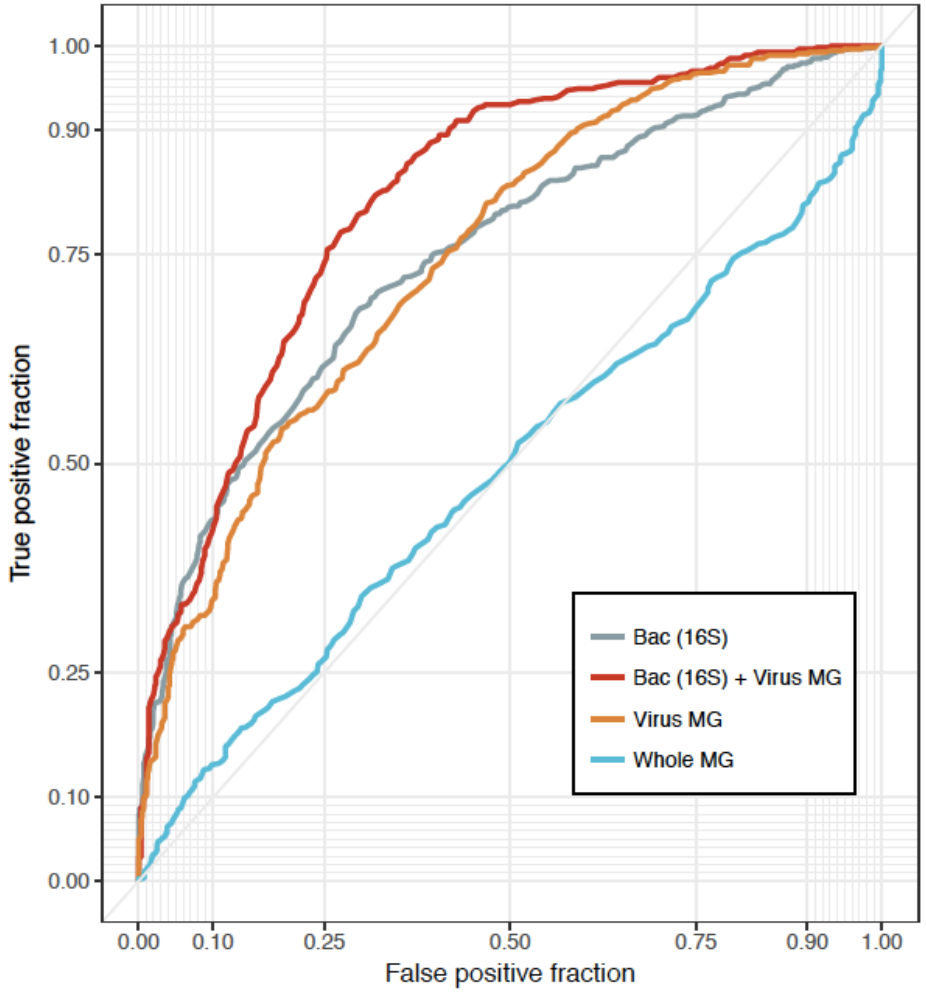




gFOBT, BMI, Wif1 methylation, fecal immunochemical test, viral clades…

Baxter et al (2016) Genome Medicine; Zellar et al (2014) Molecular Systems Biology; Zackular et al (2014) Cancer Prev Res

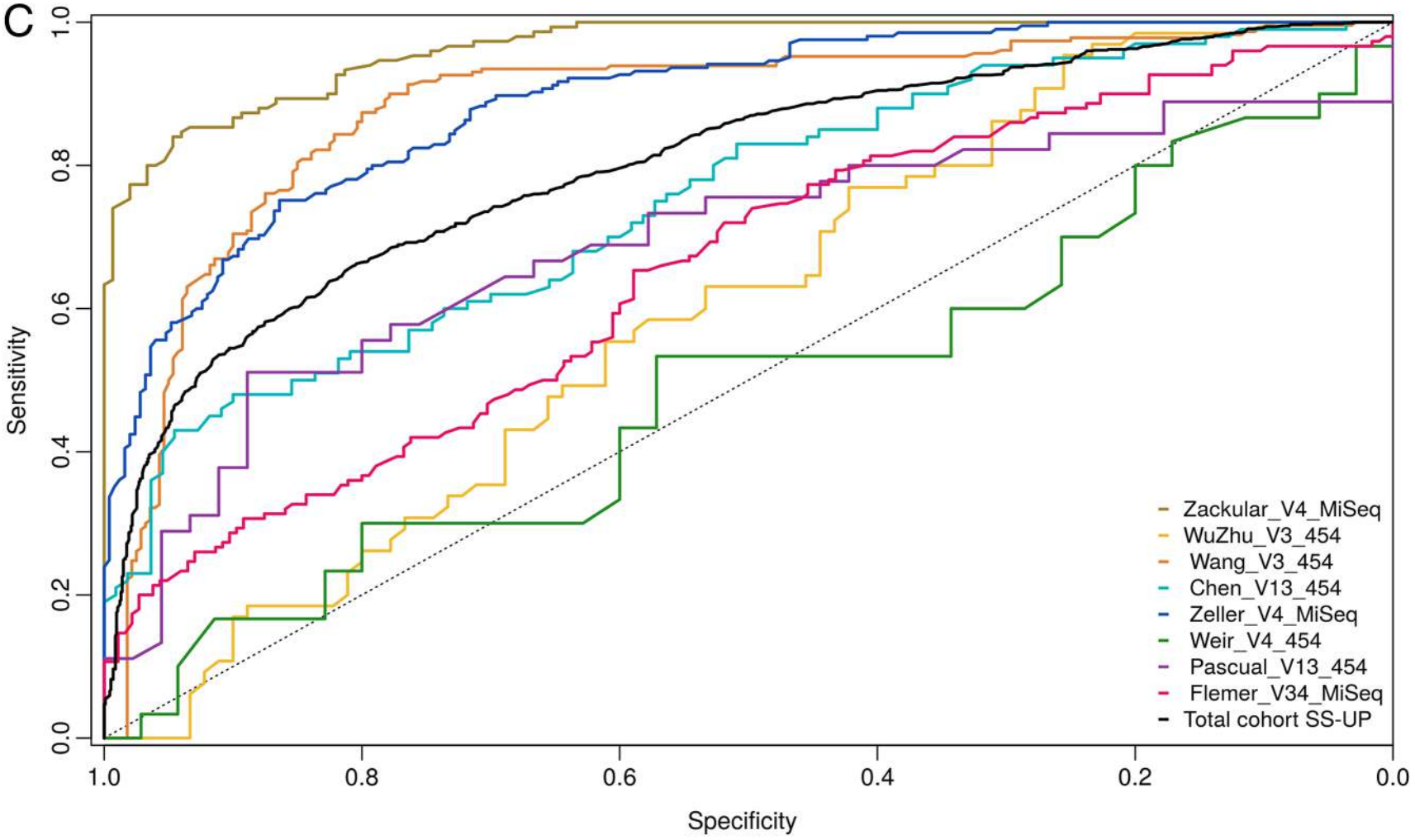
Addition of metagenomic data



- 16S
- WGS
  - Bacterial
  - Viral
- Depth of sequencing

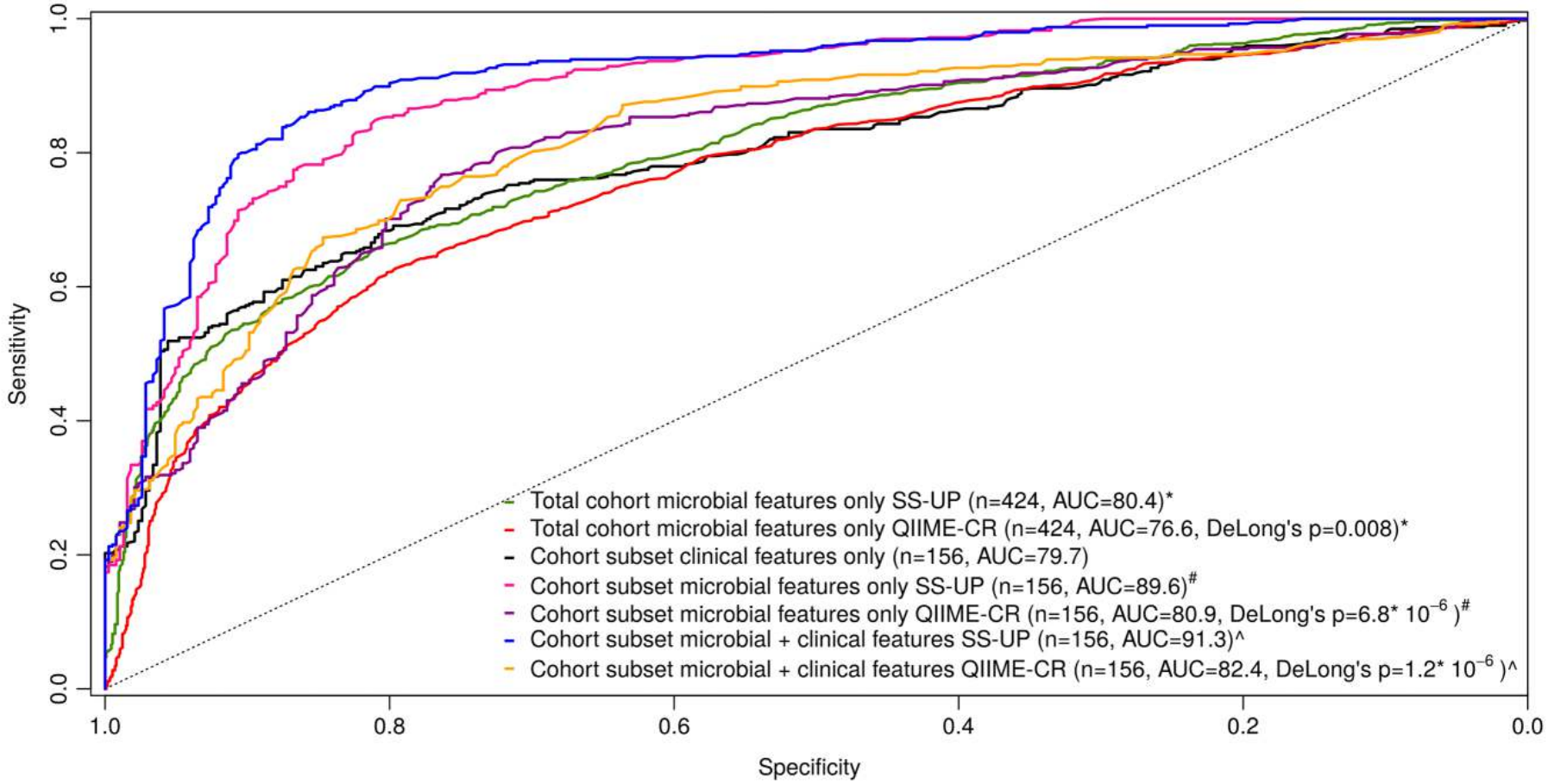
Hannigan (2017) bioRxiv

Multiple studies processed separately



Shah et al 2017 Gut

Multiple studies processed together



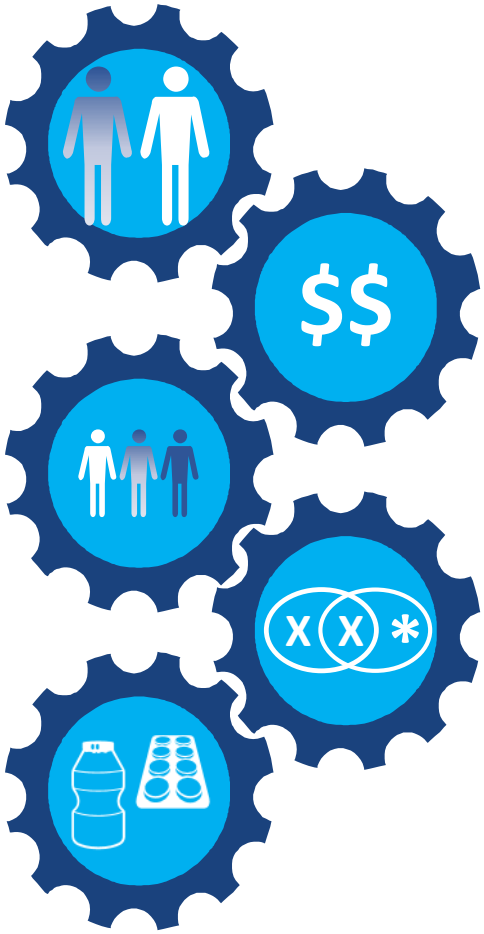
Shah et al 2017 Gut

Microbiome-based biomarkers

Where do we go from here?

Ideal biomarkers should be able to:

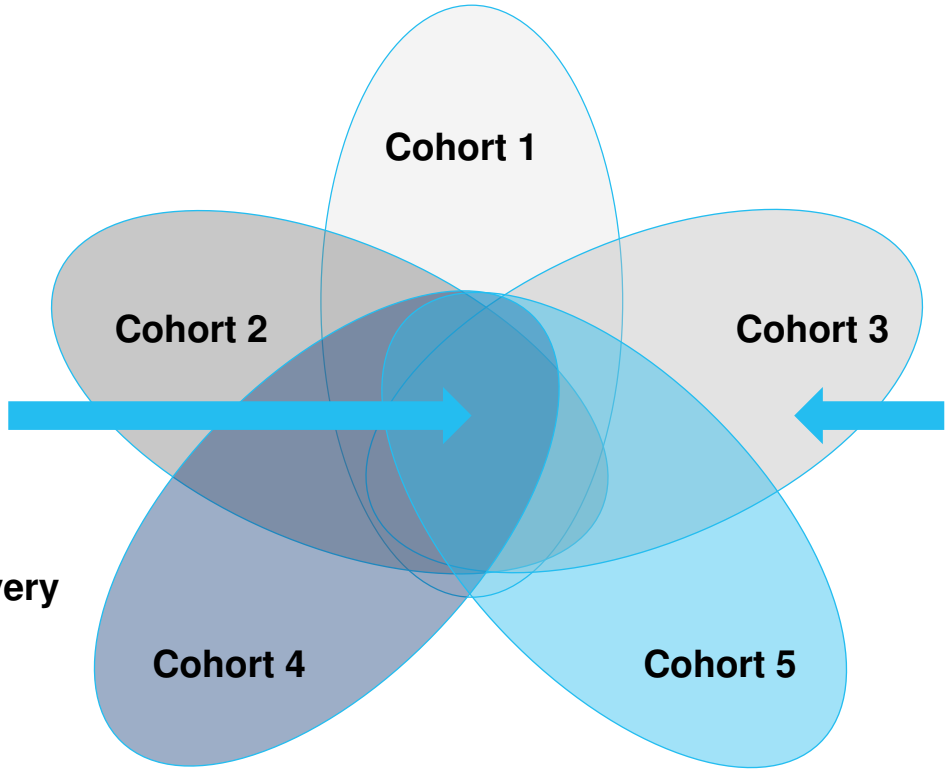
- **Identify** disease at/prior to onset; **differentiate** between stages of disease (i.e., disease progression and/or response to treatment)
- Be **accessible and affordable** to facilitate broad acceptance for large scale screening
- **Work in high-risk populations** (e.g., T2DM, IBD are risk factors for CRC)
- **Distinguish** between disease of interest and other potentially microbiome-mediated conditions
- **Be robust** to lifestyle factors such as OTC or prescription drug consumption



Performing metagenomics at scale has many advantages

Studying multiple disease cohorts can **facilitate dissection of disease mechanisms and lead to better diagnostics and therapeutics**

Universal factors:
- Common exposures
- Common genetic risks



Unique regional factors:
- Diet
- Race/ethnicity
- Access to health care
- Environment

*** Universal target discovery**

*** Personalized target discovery**

Future outlook – efforts to accelerate and improve microbiome drug development

Access to sequencing resources and **comprehensive platforms**

Incorporate machine learning for predictive and association analysis

Utilize libraries of microbiome **samples and data**

Adopt industry standards e.g. CLIA, GLP

Improve study design and share clinical knowledge & insights

Grow functional data (WGS, imputed analysis, IgASeq, RNAseq, etc)

Increase Black Box Methods to identify/classify microbiomes

Facilitate the shift from **association to causality**

Thank You