THE ROLE OF MICROBIOME IN IBD

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Disclosures

- Janssen
- UCB

No relevance to the talk

Objectives

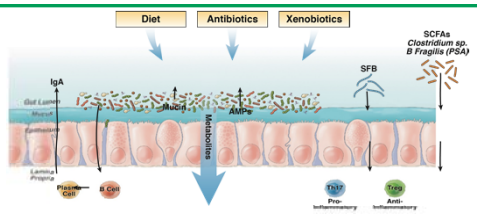
- Review the body of literature about the gut microbiome in health & IBD over the last decade
- Understand if diet and enteral nutrition determine the gut microbiome and to critically review if microbiome influences the diagnosis and treatment in IBD
- Speculate how the emerging discoveries of gut microbiome can help clinicians manage IBD in day-to-day practice

Acknowledgements for sharing slides/ideas

- Bob Baldassano
- Sandy Kim
- Ben Gold
- Konstantinos Gerasimidis
- Dirk Gevers & Ramnik Xavier

This talk serves a summary of several talks during this meeting

The Gut Microbiota in Health and Disease



- **Food, antibiotics, xenobiotics and host genetics shape the composition of the gut microbiota**
- **Diet, and the derivatives serve as substrates for the gut microbiota to produce metabolites**

The Gut Microbiota

- There are **100 trillion** bacteria that live in our GI tract
- **10x** the number of "human" cells in our body
- **100x** as many genes as there are in the human genome

We are more bacteria than we are human

The Gut Microbiota or *The Microbiome*

- There are **100 trillion** bacteria that live in our GI tract
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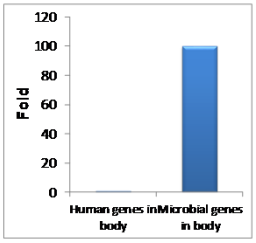
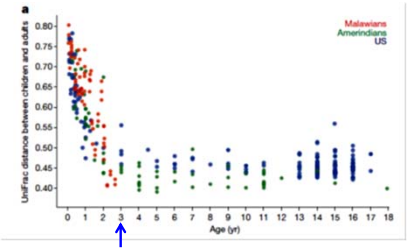


- **Microbial colonization starts at birth**
- **This partnership has evolved over thousands of years**

First 3 years of life – Microbiota is highly variable "The sensitive period"

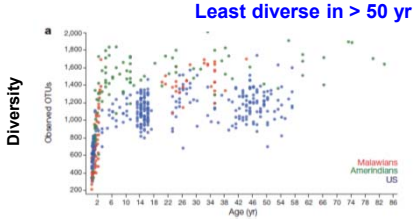


Phylogenetic composition of bacterial communities evolves toward adult composition over the first three years of life in all populations

Nature 486: 222, 2012 *Science* 331: 337, 2011

Microbiota diversity increase with age

Least diverse in > 50 yr old men

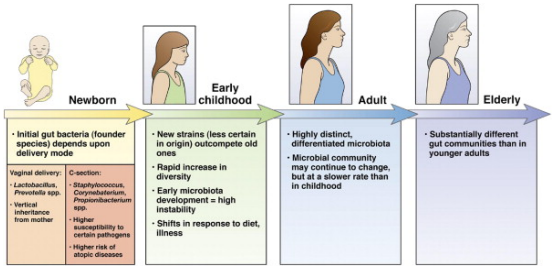


Bacterial diversity increases with age in all populations. Fecal microbiota of US adults is the least diverse.

Nature 486: 222, 2012 *Science* 331: 337, 2011

Summary: Development of the Human Microbiota

•modified by diet, genetics and the environment, throughout life

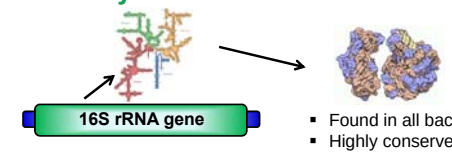


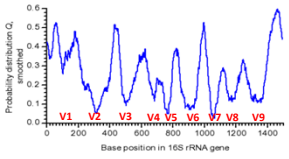
•Dominguez-Bello MG, et al. Gastro 2011;140:1713 - 1719

GUT MICROBIOME 101

Methods & Terminology for clinicians

16sRNA: Culture-Independent Sequence Analysis of the Microbiome



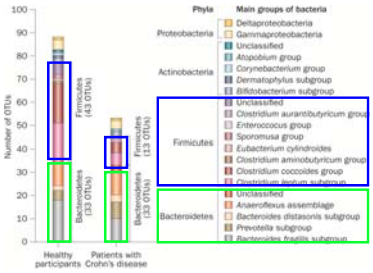


- Found in all bacteria
- Highly conserved
- Hypervariable regions provide species-specific signature sequences
- Useful for bacterial identification
  - qPCR
  - 16s gene sequencing
  - High throughput

GUT MICROBIOME - Terminology

- **Dysbiosis:** Altered microbiota composition either quantitatively, qualitatively or both
- **Alpha diversity:** Alpha diversity (intrinsic measure): a measure of species richness or diversity within an individual sample (how many types of microbial sequences in a sample)
- **Beta diversity:** Beta diversity (comparative measure): is a term for the comparison of samples to each other (how many different types are distributed between samples
- **Enterotypes of gut microbiome:** abundance of bacteria; usually one of the 3 generas - bacteroids, prevotella or ruminococcus
- **OTUs: Operational taxanomic units:**

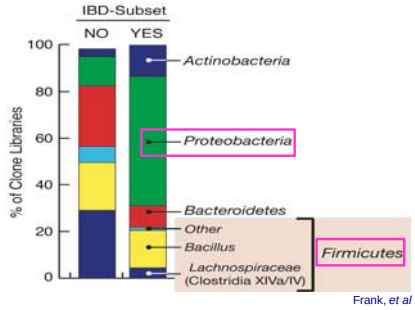
Reduction of Bacterial Diversity in Patients with Crohn's Disease
More inflammation, greater the reduction



nature REVIEWS GASTROENTEROLOGY & HEPATOLOGY

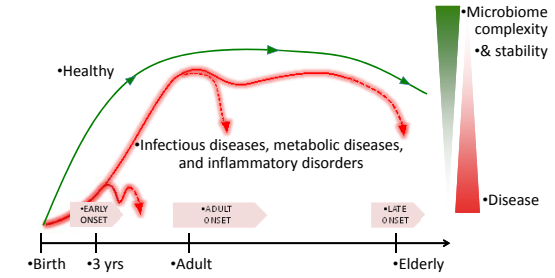
Manichanh. *et al.* 2012. *Nat Rev Gastro Hep*

Different & altered composition in IBD
IBD Subsets Characterized by ↓ *Firmicutes* and *Bacteroidetes and* ↑*Proteobacteria*



Frank, *et al* (2007). *PNAS*

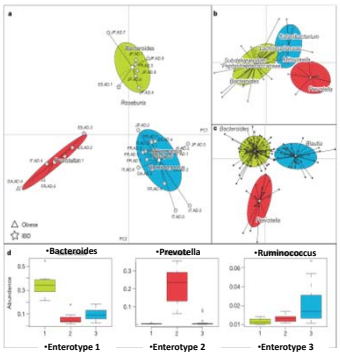
A healthy microbiome develops complexity
Dysbiosis is implicated in diseases like IBD



•Opportunity to use this information to diagnose, predict, and treat diseases

•Kostic AD, Xavier RJ, Gevers D. Gastroenterology. 2014

Enterotypes of Gut Microbiome



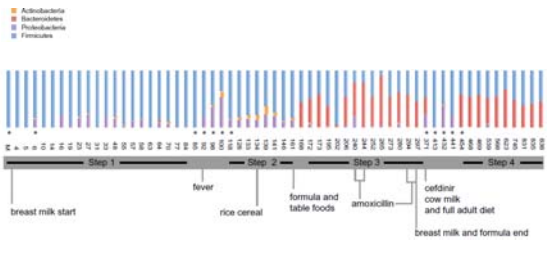
Enterotypes Abundance of one of three genera

- **Bacteroides**
- **Prevotella**
- **Ruminococcus**

The basis for enterotype clustering is unknown but appears to be independent of:

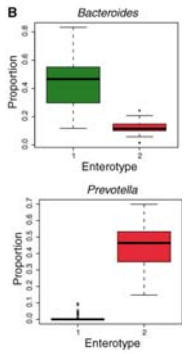
- **Nationality**
- **Gender**
- **Age**
- **Body Mass Index (BMI).**

Early Diet Affects Microbiome



•Koenig J E et al. PNAS 2011;108:4578-4585

Clustering of gut microbiome into enterotypes is associated with long-term diet

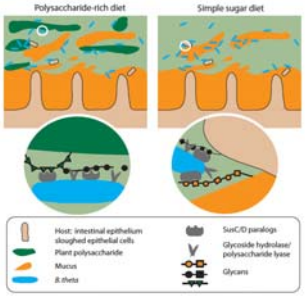


- The *Bacteroides* enterotype
  - highly associated with animal protein and saturated fats, which suggests meat consumption as in a Western diet
- The *Prevotella* enterotype,
  - high values for carbohydrates and simple sugars, indicating association with a carbohydrate-based diet more typical of agrarian societies

•Wu G, et al. Science. 2011 Oct 7;334(6052):105-8

Dietary components regulate bacterial gene transcription

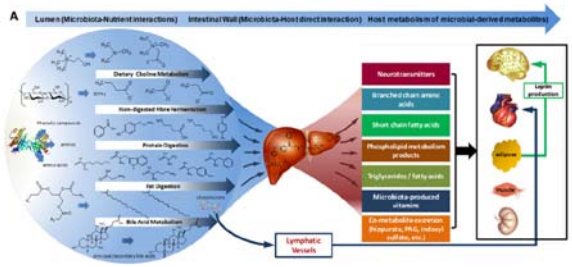
- Important function of the intestinal microbiome is metabolism of glycans (complex carbohydrates and polysaccharides)
- *Bacteroides thetaiotaomicron*
  - Highly abundant obligate anaerobe in the microbiota of most adults
  - Known for its ability to metabolize polysaccharides



•Sonnenburg et al. *Science*. 2005.

Diet, the Gut Microbiome, Metabolome, and Disease

•Diet serves as a substrate for the microbiota to produce certain metabolites



•Holmes et al. *Cell Met*. 2012;16:559

MICROBIOME in treatment naïve Crohn's disease

CCFA Sponsored Pediatric Clinical Research Network: PRO-KIIDS RISK Study

•1112 children with CD at diagnosis between 2008-2012

• Follow-up to 2017

Paris Classification
Genotype
Environmental Exposures
Serology
Microbial Community/Gene Expression

Define patients with complication / surgery

The RISK cohort: a unique inception cohort for pediatric Crohn's disease

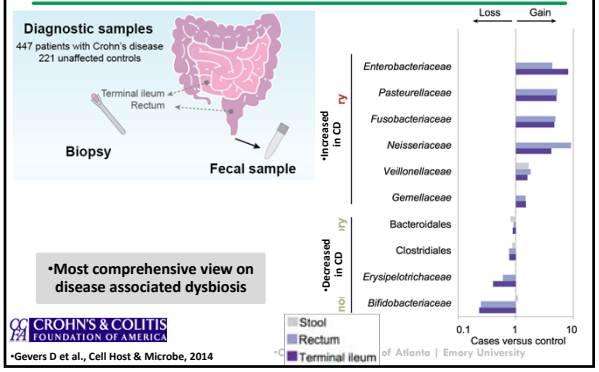
Challenges with previous studies
- *Small cohort size*
- *Established disease*
- *Not treatment-naïve*
- *Inconsistent sampling*

RISK investigators (steering committee & 28 sites) and
- •Ramnik Xavier (MGH/Broad)
- •Dirk Gevers (Broad)
- •Rob Knight (Colorado University)

CROHN'S & COLITIS FOUNDATION OF AMERICA

- Involves 28 pediatric IBD centers in USA/CAN
- Inception cohort (diagnostic sample)
- Sampled before treatment initiation
- Total of 1,100 children with CD (1,700 participants)
- Samples: blood, ileal + rectal biopsies, stool
- Host genetics, mucosal gene expression, serology, & microbiome

The Microbiome shifts in pediatric Crohn's disease

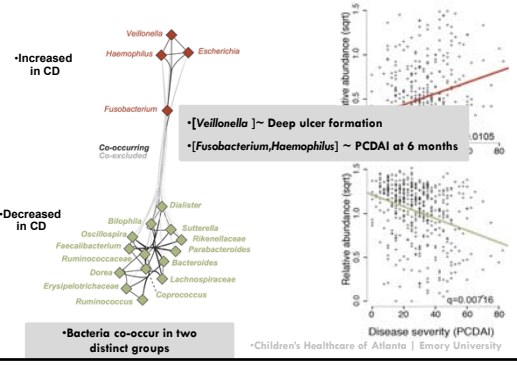


•Most comprehensive view on disease associated dysbiosis

CROHN'S & COLITIS FOUNDATION OF AMERICA

•Gevers D et al., Cell Host & Microbe, 2014

The Microbiome shifts in pediatric Crohn's disease

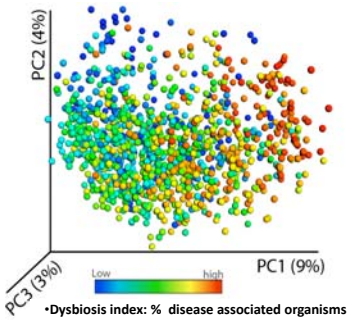


•[*Veillonella*]~ Deep ulcer formation

•[*Fusobacterium,Haemophilus*] ~ PCDAI at 6 months

•Bacteria co-occur in two distinct groups

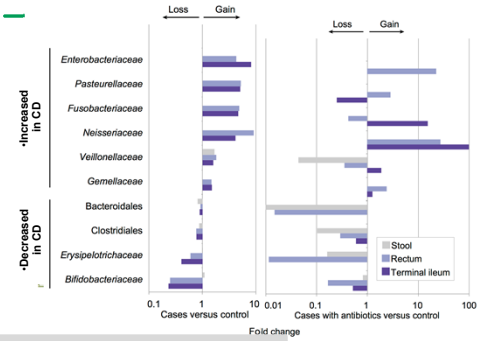
The dysbiosis presents itself as a gradient across the patient population



•Dysbiosis index: % disease associated organisms

•Gevers D et al., Cell Host & Microbe, 2014

Antibiotic exposure amplifies the microbial dysbiosis



•Loss of beneficial microbes may trigger proliferation of less beneficial organisms

•Gevers D et al., Cell Host & Microbe, 2

Determining the key players of microbial dysbiosis in new-onset pediatric CD – RISK study

- We identified microbial organisms associated with subject's disease phenotype (controlled for confounding variables, such as past antibiotic use, age, gender, and race)
- Several taxa were reported before, including Enterobacteriaceae, Bacteroidales, and Clostridiales.
- We identified additional taxa as significant biomarkers for disease, including members of the Pasteurellaceae, Veillonellaceae, Neisseriaceae, and Fusobacteriaceae.
- Antibiotic exposure amplifies the microbial dysbiosis, by further loss of *Bacteroides*, *Clostridiales*, and *Erysipelotrichaceae*, and increase in *Fusobacteriaceae* and *Enterobacteriaceae*

Relationship between Gut Microbiota and Enteral Nutrition

Decline in Presumptively Protective Gut Bacterial Species and Metabolites Are Paradoxically Associated with Disease Improvement in Pediatric Crohn's Disease During Enteral Nutrition

Konstantinos Gerasimidis, PhD,*† Martin Bertz, MSc,*‡ Laura Hanske, PhD,‡ Jana Junick, PhD,‡ Olga Biskou, MSc,* Margarita Aguilera, PhD,§ Vikki Garrick, BSc,† Richard K. Russell, PhD,*† Michael Blaut, PhD,‡ Paraic McGrogan, PhD,*† and Christine A. Edwards, PhD*

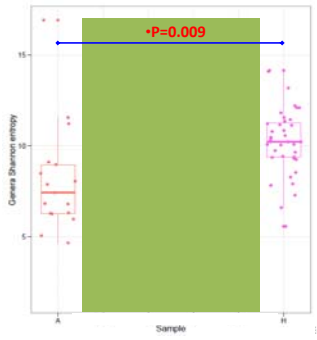
Gerasimidis et al. Inflamm Bowel Dis 2014; 20: 861-71

Subjects-Methods

- 19 active CD and 21 healthy controls
  - four were excluded (antibiotics)
- 8 weeks on EEN (Modulen®, Nestle)
  - 4 samples during EEN (0, 15, 30 60 d) and one when back on free diet (2-4 months post EEN)[1,2]
- 12/15 clinically improved & faecal calprotectin decreased[1,2]
- Next generation sequencing (Illumina, MiSeq & HiSeq)
  - 16S rRNA (n=127) for taxa assignments (Genera, OTU, Oligotypes[3,4])
  - Whole genome sequencing (n=96) (metabolic pathways-KEGG modules)

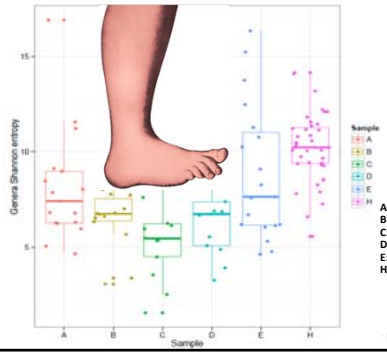
[1] Gerasimidis et al JCG 2011; [2]Gerasimidis et at IBD 2014; [3]Eren et al PNAS 2014; [4]Eren et al Methods Ecol Evol 2014

Crohn's Disease VS Controls

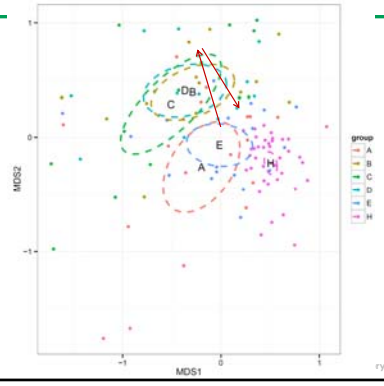


A: CD Before EEN
H: Healthy controls

Impact of EEN on microbial diversity

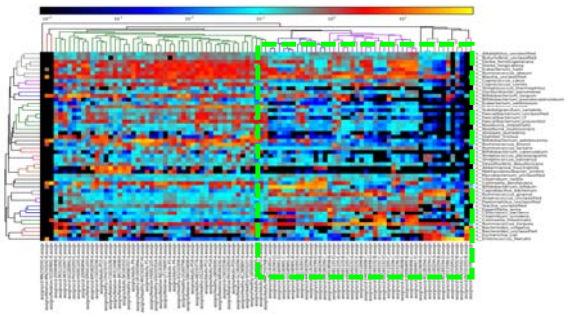


A: Before EEN
B: 15 d on EEN
C: 30 d on EEN
D: 60 d on EEN
E: Back on free diet
H: Healthy controls

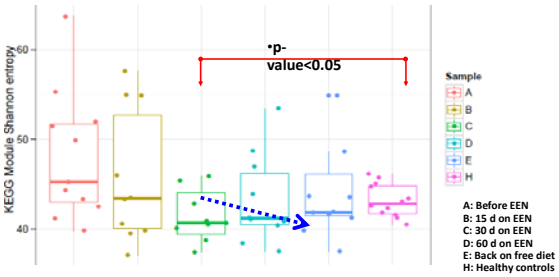


A: Before EEN
B: 15 d on EEN
C: 30 d on EEN
D: 60 d on EEN
E: Back on free diet

EEN clearly differentiated before and after treatment



Genetic Metabolic Capacity (WGS)



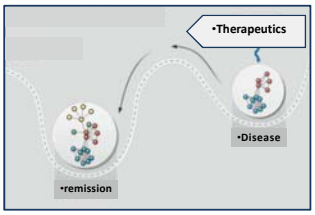
- **Higher level of metabolic capacity in CD**
- **Tendency to decrease during EEN**

Conclusion: EN and Microbiota

- CD microbiota less diverse but with a wider functional capacity
- Several genera/OTU and metabolic pathways changed during EEN
  - Some correlated with calprotectin (*Atopobium*) and other not (*F. Prausnitzii*)
- EEN may works by suppressing
  a) The entire microbiota in CD, thus inducing a lower antigenic effect to the gut
  b) Bacteria associated with CD but also other sensitive to EEN composition
- Causative association or collateral effect?
  - Maintenance of these changes may prolong disease remission?

Relationship between Gut Microbiota and FMT

We need to capture the dynamics of the microbiome in transition between health states

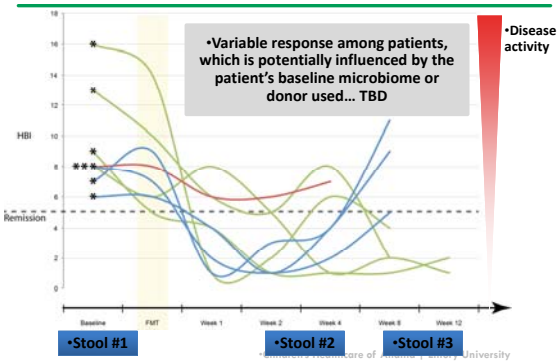


•Fecal Microbiota Transplantation Induces Early Improvement in Symptoms in Patients With Active Crohn's Disease

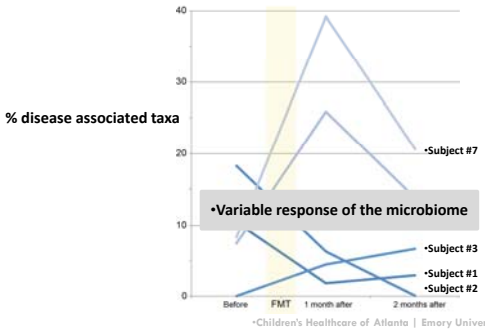
•Byron P. Vaughn, Dirk Gevers, Amanda Ting, Joshua R. Korzenik, Simon C. Robson, Alan C. Moss

Presented as poster DDW 2014

Clinical evaluation indicates a promising outcome

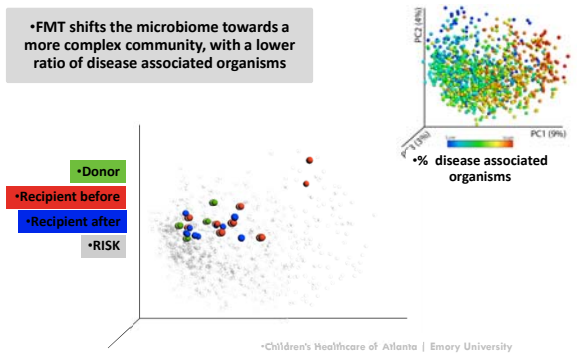


Fecal Microbiota Transplant has the potential to shift the disease associated taxa

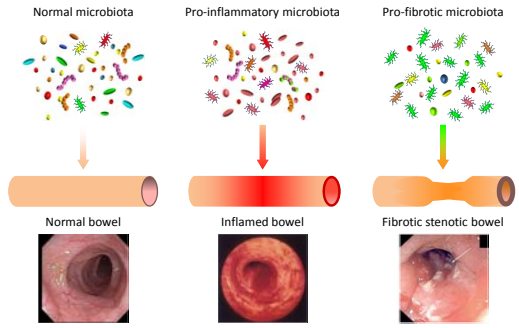


Fecal Microbiota Transplant shifts the Microbiome

•FMT shifts the microbiome towards a more complex community, with a lower ratio of disease associated organisms



Hypothesis revisited
Different Microbiota in IBD: different clinical outcome?



ACKNOWLEDGEMENT

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