The Gut Microbiome in Health and Disease

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The Human Microbiome

- Comprised of Bacteria, Viruses, others (Archaea, Eukaryotes)

- Distinctive microbiomes at each body site (gut, lung, skin, mucosa etc.)

The Gut Microbiome

- Human gut is home to ~ 100 trillion bacterial cells

- Density of $10^{11}$ to $10^{12}$ per gram in the colon

- Genome size of microbiota at least 100-fold greater than human

- Large numbers species present, most uncultured

Host-Microbial Mutualism of the Gut

**Host benefits to the bacteria**
- Provides a unique niche
- Intestinal mucus provides a source of nutrition

**Bacteria benefits to the host**
- Fermentation of indigestible carbohydrates to assist digestion
- Biotransformation of conjugated bile acids
- Urease activity participates in nitrogen balance
- Synthesis of certain vitamins
- Metabolize drugs
- Education of the mucosal immune system
Gut Microbiome Development

Colonization of the gut begins at birth

Transition to the highly distinct, highly differentiated adult microbiota

Factors that affect gut microbiome composition

- Environment
- Genetics
- Other Host Factors
- Antibiotics
- Inflammation
- Diet

Science. 2011 May 20;332(6032):970-4
Science. 2011 Jul 1;333(6038):101-4
Science. 2011 Oct 7;334(6052):105-8
Elements of Modern Lifestyle Lead to Changes in Gut Microbiota

- Improved sanitation
- Less crowded living conditions
- Decline in parasite and *H. pylori* infections
- Vaccinations
- Increased antibiotic use
- Sedentary lifestyles
- Caesarean section
- Refrigeration
- Food processing
- Diet changes
Diet and the Gut Microbiota
Greatest change occurs with introduction of solid foods

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 that meat consumption is associated with 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
**Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children from Europe and Rural Africa**

African Diet: High Fiber and carbohydrate, low animal fat and protein

European Diet: High animal fat and protein, low fiber

De Filippo C, et al. PNAS 2010: 14691-96
HUP/CHOP Microbiome Project: Longitudinal analysis of microbiome under controlled feeding

Changes detectable within 24 hours!

Each color represents a different subject

Methods
454 pyrosequencing of 16S rDNA

Day 1 is different than all other days!!
Diet, the Gut Microbiome, Metabolome, and Disease

Holmes et al. *Cell Met.* 2012;16:559
Gut Microbiome and Disease

- Diabetes: Type 1 DM and Type 2 DM
- Obesity: dysbiosis?
- Atherosclerosis: Oral, gut and plaque microbiota; Microbial metabolism of choline to TMA
- Asthma: Sanitized environment
- Colon Cancer: Enterotoxigenic *Bacteroides fragilis* and *Fusobacterium*
- Inflammatory Bowel Disease: Dysbiosis
Environment + Host Genotype = Disease

• Increased Incidence

• Geographic distribution
  – Clustering in industrialized nations

• Immigration studies
  – Adoption of disease risk of the host country within 1 or 2 generations

• Genomic advances
  – Contribution of host genetics to the risk of disease development is significantly less than 50%
IBD and the Gut Microbiome
IBD Susceptibility

Adapted from Inflamm Bowel Dis 2010:16;152
Trends in Incidence of IBD

N. America/Europe

Trend not explained by genetics, but environmental changes (Westernized diet, gut microbiome)
Associations of Environmental Factors with New Onset IBD

- Infectious gastroenteritis
- Early antibiotic use has been associated with IBD
  - Tetracycline
  - Oral antibiotics for ear infection
Clinical Evidence Implicating a Role of Bacteria in the Pathogenesis of IBD in Humans

• Inflammation occurs predominantly in the terminal ileum and colon, where the greatest concentrations of bacteria are found

• Antibiotics are effective treatment for IBD

• Surgical diversion of the fecal stream is an effective treatment for Crohn disease
  • Inflammation is known to recur upon restoration of the fecal flow
The gut microbiome is different in people with IBD.
Role of Inflammation, antibiotics?
Inflammation, Antibiotics and Diet as Environmental Stressors of the Gut Microbiome in Pediatric Crohn’s Disease

Example of a large-scale shotgun metagenomic study

Response of the bacterial microbiota

Two clusters, one overlapping the healthy controls

Strong association with antibiotic use

Dysbiotic cluster associated with high levels of human DNA in stool

Lewis et al., Cell Host & Microbe 2015
Therapy and microbiome

• Medications
• Antibiotics
• Probiotics
Enteral Nutritional Therapy

TO BE DISCUSSED LATER!
Trichuris suis (whipworm)

- Several small observational studies suggested possible effect in Crohn disease
- Recent phase 2 trial was discontinued after interim analysis
  - Treatment did not outperform placebo
  - No safety concerns
  - High placebo response
Fecal Transplant

Provide donor stool to affected patient in effort to alter microbiome

Effective and safe treatment for *C. diff*

Efficacy in IBD *not established yet*
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Identification of Disease Associated Pathways

IBD-related processes
- Epithelial barrier
  - GNA12, HNF4A, CDH1, ERRF1, MUC19, ITLN1*
- Restitution
  - REL, PTGER4, NKX2-3, STAT3, ERRF1, HNF4A, PLA2G2A/E
- Solute transport
  - SLC9A4, SLC22A5, SLC22A4*, AQP12A/B, SLC9A3, SLC26A3
- Paneth cells
  - ITLN1*, NOD2*, ATG16L1*, XBPI*
- Innate mucosal defence
  - NOD2*, ITLN1*, CARD9*, REL, SLC11A1, FCGR2A/B
- Immune cell recruitment
  - CCL11/CCL2/CCL7/CCL8, CCR6, IL6R/IL8R, MST1*
- Antigen presentation
  - ERAP2*, LNPED, DENND1B
- IL-23/T_H17
  - IL23R*, JAK2, TYK2*, STAT3, ICOSLG, IL21, TNFSF15*
- T-cell regulation
  - NDFIP1, TNFSF8, TAGAP1, IL2, IL2R*, TNFSF9, PIM3, IL7R*, IL12B, IL23PRD1, ICOSLG, TNFSF8, IFNG, IL2*
- B-cell regulation
  - IL5, IKZF1, BACH2, IL7R*, IRF5
- Immune tolerance
  - IL10, IL27*, SBN02, CREM, IL1R1/IL1R2, NOD2*

Cellular responses
- Autophagy
  - ATG16L1*, IRGM, NOD2*, LRRK2, CUL2, PARK7, DAP
- Apoptosis/necroptosis
  - FASLG, THADA*, DAP, PUS10, MST1*
- Carbohydrate metabolism
  - GCKR*, SLC2A4RG
- ER stress
  - CPEB4, ORMDL3, SERINC3, XBPI*
- Intracellular logistics
  - VAMP3, KIF21B, TTL8, FGFR1OP, CEP72, TPPP
- Oxidative stress
  - PRDX5, BACH2, ADO, GPX4, GPX1*, SLC22A4, LRRK2, NOD2*, CARD9*, HSPA6, DLD, PARK7, UTS2*, PEX13
- Cell migration
  - ARPC2, LSP1, AAMP

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Xavier 2011
“Bacterially”-Generated Phenotypes

Role of Genomics Differs

Kugathasan, IBD 2014

- Very early onset IBD
- Early onset IBD
- Adult onset IBD

Genetic defects
- Maximal (monogenic)
- Multiple (polygenic)

Environmental factors

Primary Immunodeficiencies
Very Early-Onset IBD

- Diagnosed ≤5 years of age
- Frequently different phenotype and more severe disease presentation
- Often unresponsive to conventional therapy
- Rare genes
Candidate Causative Variants in VEO-IBD?

Buchanan, New studies failing to explain the genetics of common disease, 2012
Whole Exome Sequencing
Whole Exome Sequencing

Protein coding regions: 85% of the disease-causing mutations
Joint VEO-IBD Clinic

- Monthly clinic
- Gastroenterology and Immunology
- Integrative biology-based multidisciplinary approach
- Radically changed our method of evaluation
- Led to targeted therapy
VEO-IBD Pathways

Epithelial Barrier

ADAM17, IKBKG, COL7A1, FEMT1, TTC7A, GUCY2

Phagocyte Defects

NADPH Complex

Immuno-regulation

IL10, IL10RA, IL10RB, FOXP3, CTLA

T & B Cell Defects

RAG1/2, IL7R, PTEN, WASP

Hyper-inflammatory

XIAP, STXBP2, LYST, RAGB27a

VEO-IBD
Gut Microbiota Development

Factors affecting the microbiome
- Genetics
- Birth route
- Geography
- Hygiene
- Stress
- Diet/nutrition
- Drugs

Microbiome complexity and stability

Disease

Healthy

Perturbation

Infectious diseases, metabolic diseases, and inflammatory disorders

- Protect against pathogens
- Train/stimulate immune function
- Supply nutrients, energy, vitamins, SCFA

- Inflammation (local > systemic)
- Oxidative stress
- Increase in Gram negative bacteria
- Infection (opportunistic/pathogenic)
- Altered metabolite production

Early onset

Adult onset

Late onset

Birth 3 years Adult Elderly

Kostick et al, Gastro 2014
The microbiome shapes the innate immune response and vice versa.
Inflammation, Antibiotics, and Diet as Environmental Stressors of the Gut Microbiome in Pediatric Crohn’s Disease


Alters of the Subgingival Microbiota in Pediatric Crohn’s Disease Studied Longitudinally in Discovery and Validation Cohorts

Judith Kelsen, MD, Kyle Bittinger, PhD, Helen Pauly-Hubbard, BA, Leah Posivak, MA, Stephanie Grunberg, BA, Robert Baldassano, MD, James D. Lewis, MD, MSCE, Gary D. Wu, MD, and Frederic D. Bushman, PhD.

Fungal Signature in the Gut Microbiota of Pediatric Patients With Inflammatory Bowel Disease

Christel Chehoud, AB, Lindsey G. Altenberg, DO, Colleen Judge, AB, Christian Hoffmann, PhD, Stephanie Grunberg, BA, Kyle Bittinger, PhD, Robert N. Baldassano, MD, James D. Lewis, MD, Frederic D. Bushman, PhD, and Gary D. Wu, MD.
CHOP IBD Center Projects

- Genetics
- Microbiome
- Very early-onset IBD