The gut microbiome in IBD: current concepts

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Objectives

• Review the body of literature about gut microbiome in health & IBD over last decade

• Understand if genetic and environmental factors determine the gut microbiome

• 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
Microbiome

- **Microbiota**
  - The microorganisms that live in an established environment

- **Microbiome**
  - The full complement of microbes, their genes, and genomes in a particular environment
History

• 1920’s Rettger et al.
  – The effect of Bacillus acidophilus in the intestinal microbiota

• 1940 Kirsner
  – Possible correlation between streptococci and UC
  – The effect of oral sulfonamide in fecal bacteria

• Late 1990’s
  – Evident association between fecal microbiota and CD
  – Recurrent inflammation after reestablishment of fecal stream in postop. CD patients

• Strong descriptive basis for IBD-microbiota relationship

The Gut Microbiota

• 100 trillion bacteria live in our GIT
• 10x the number of “human” cells in our body

- Comprised of Bacteria, Viruses, others (Archaea, Eukaryotes)
- Distinctive microbiomes at each body site (gut, lung, skin, mucosa etc.)

• 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 150-fold greater than human
• Large numbers species present, most uncultured

• 100x as many genes as there in the human genome
• Partnership has evolved > 1000 yrs.
Gut microbiota

- More than 1000 species
- Collective weight of **about 1kg**
- Four major bacterial phyla
  - Bacteroidetes
  - Firmicutes
  - Actinobacteria
  - Proteobacteria

  ~ 90% of the gut microbiota

- Individuals:
  - Distinctive pattern of gut microbiota
  - Remains remarkably stable during lifetime

Rajilic-Stojanovic M et Willem M. FEMS Microbiol Rev 2014
First 3 years of life—microbiota is highly variable “The sensitive period”

- Colonization of the GI tract begins immediately after birth
- Starts from a “germ free” intrauterine environment
  - Maternal vaginal/fecal flora (vaginal delivery)
  - Skin flora CC
  - Oral feeding (breast milk vs. formula)

- Complete adult colonization
  - 3rd year of life
Microbiota diversity increase with age

- Bacterial diversity increases with age in all populations
- Least diverse in > 50 year old men
Microbiota diversity is modified through life by diet, genetics, drugs and presence of inflammation.
Host-Microbial Mutualism

• Host benefits to bacteria
  – Provides unique niche
  – Intestinal mucus provides a source of nutrition

• Bacteria benefits the host
  – Prevents colonization by pathogens
  – Metabolic role
    - Caloric salvage, production SCFA, Vitamin K and folate
    - Participates in drug metabolism
      - Activates 5-ASA
  – Deconjugates bile acids
  – “Educates the immune system”
Association of the Gut Microbiota with Disease

- **Diabetes**
  - T1 DM (MyD88-dependent in NOD Mice); T2 DM (TLR&TLR5 Kos)
- **Atherosclerosis**
  - Oral, gut and plaque microbiota; Metabolism of choline to TMA
- **Asthma**
  - Sanitized environment
- **Colon Cancer**
  - Enterotoxigenic Bacteroides fragilis and Fusobacterium
- **Inflammatory Bowel Disease**
  - Dysbiosis
### Microbiome

- Alterations in gut microbiota linked with
  - FBD
  - Obesity
  - Celiac disease
  - Allergies
  - NAFLD/NASH
  - Autism
  - Depression
  - Metabolic diseases

<table>
<thead>
<tr>
<th>Pathogenesis involves genetic and environmental factors</th>
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</thead>
<tbody>
<tr>
<td>All associated with inflammation</td>
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<tr>
<td>Rapid increase in incidence</td>
</tr>
<tr>
<td>Geographically in more industrialized nations</td>
</tr>
<tr>
<td>Many associated with diet</td>
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</table>
Gut Microbiota and IBD

• Does the intestinal microbiota differ in patients with IBD from healthy subjects?

• If microbiota does differ, is it an important factor in the pathogenesis of the disorder?
Gut Microbiota and IBD

• The (convincing) evidence- germ free mouse models:
  – chronic intestinal inflammation develops after colonization with commensal gut bacteria
  – remain disease free in bacteria-free conditions

• Primary role of non-pathogenic enteric bacteria in the pathogenesis of IBD

• Current theory: “no bacteria, no IBD”

Macpherson and Harris, Nat rev Immunol., 2004
The gut microbiota: The proximate environmental risk factor for IBD

• Tissue damage?
  – An abnormal immune response to a normal microbiota or
  – Normal immune response against abnormal microbiota

• Twin studies
  – The concordance rate (UC <20%; CD~ 50%) in monozygotic twins
  – Healthy siblings have altered microbial profiles associated with IBD, distinct from their genotype-related risk
  – The environment has a greater effect (especially in UC)

• Migrant studies

Gut Microbiota and IBD

• In general
  – Overall decrease in microbial diversity and stability of the intestinal microbiota in IBD patients—*Dysbiosis*
  – On average, 25% fewer genes could be detected in the fecal samples from IBD patients vs. healthy persons

GUT MICROBIOME- Terminology

• **Dysbiosis:**
  – Altered microbiota composition quantitatively, qualitatively or both

• **Alpha diversity** (intrinsic measure):
  – measure of species richness or diversity **within an individual sample**

• **Beta diversity** (comparative measure):
  – comparison of samples to each other

• **Enterotypes of gut microbiome:**
  – abundance of bacteria;

Dysbiosis of Gut Microbiota in IBD

- Inflammation leads to tissue hyperemia and bleeding into the lumen of the GI tract
- Increases in Proteobacteria and Actinobacteria
  - Generally aerotolerant
  - Organisms able to manage oxidative stress

<table>
<thead>
<tr>
<th>Potentially injurious species in susceptible hosts</th>
<th>Protective species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides vulgatus, B. thetaiotaomicron</td>
<td>Lactobacillus species</td>
</tr>
<tr>
<td>Escherichia coli (adherent/invasive)</td>
<td>Bifidobacterium species</td>
</tr>
<tr>
<td>Enterococcus faecalis (nonpathogenic)</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Bacteroides thetaiotaomicron</td>
</tr>
<tr>
<td>Fusobacterium varium</td>
<td>Faecalibacterium prausnitzii</td>
</tr>
<tr>
<td>Helicobacter hepaticus and other intestinal species</td>
<td></td>
</tr>
<tr>
<td>Sartor PNAS 2008;105:16413</td>
<td></td>
</tr>
</tbody>
</table>
Dysbiosis of Gut Microbiota in IBD

Mucosal bacterial numbers
Adherent-Invasive *E.coli*
*Enterobacteriaceae*
*Fusobacteriaceae*
*Mycobacterium avium paratuberculosis*
*Clostridium difficile*

Diversity
*Bacteroides*
*Clostridia*
*Bifidobacteriaceae*
*Ruminococcaceae*

Gevers et al. Cell Host Microbiome 2015
Advances in sequencing technology-Dysbiosis

• Different microbiota composition
  – Healthy individuals vs. IBD patients
  – Different phenotype groups

• General reduction in diversity of luminal microbiota
  – Depletion in commensal bacteria (Bacteroidetes and Firmicutes)
    • Bifidobacterium, Lactobacillus & Faecalibacterium prausnitzii
  – Decreased Clostridium abundance (diversity of clusters IV and XIVa)

  – Concomitant over-expression of pathogenic bacteria
    • Actinobacteria and Proteobacteria

Miquel S et al. Mbio 2015;6:300-15
Quevrain E et al. Gut 2015
Advances in sequencing technology-Dysbiosis

- **Ileal CD patients vs. healthy controls**
  - Lower abundance of *Firmicutes* (*Faecalibacterium prausnitzii*)
  - Increased abundance of Fusobacteria
  - Increased abundance of Proteobacteria

- **Ileal vs. Colonic CD patients**
  - Ileal CD: a prevalence of Adherent-invasive E. coli (AIEC)
  - Colonic CD:
    - Increased abundance of Firmicutes
    - Decreased abundance of Fusobacteria

Advances in sequencing technology-Dysbiosis

• Treatment-naïve pediatric IBD patients with deep ulcers
  – Increased *Pasteurellacaea*, *Veillonellaceae* & *Rothia mucilaginosa*
• CD vs. UC
• Speculations (CD)
  – *Mycobacterium avium paracellulare*, *Listeria*, *Measles virus*

Advances in sequencing technology

*No concrete evidence*

*Single pathogen is the cause of the disease*
Proposed causes of dysbiosis of the microbiota

Increased incidence of immune-mediated disorders in developed countries could be due to alterations in the microbiota
Etiological Theories in Inflammatory Bowel Disease

- Genetic predisposition
- Environmental Triggers
- Mucosal Immune System (Immuno-regulatory defect)
- Gut Microbiota
### Overlapping genetic and environmental risk factors for IBD

**Key IBD risk gene pathways:**

- Sensing (recognition) of the microbiota
- Regulators of the response to microbiota and
- Barrier function

<table>
<thead>
<tr>
<th>Genes</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD2/CARD15</td>
<td>CD-linked intracellular sensor of bacterial peptidoglycans</td>
</tr>
<tr>
<td>ATG16L1, IRGM</td>
<td>CD risk autophagy genes involved in intracellular processing of bacteria</td>
</tr>
<tr>
<td>IL23R, JAK2, IL12B</td>
<td>IL-23-Th17 pathway linked to IBD and autoimmune conditions psoriasis and ankylosing spondylitis</td>
</tr>
<tr>
<td>IL10</td>
<td>Recessive mutation linked to very early onset of IBD</td>
</tr>
<tr>
<td>MUC19</td>
<td>Involved in mucus production and mucosal barrier function</td>
</tr>
<tr>
<td>SLC22A5, GPR35</td>
<td>Immune response to bacterial-derived ligands and metabolites</td>
</tr>
<tr>
<td>IL27</td>
<td>Maintenance of epithelial barrier against commensal bacteria</td>
</tr>
<tr>
<td>ECM1</td>
<td>Glycoprotein that interacts with BM, inhibits matrix metalloproteinase 9 and activate NF-kB. UC risk gene</td>
</tr>
<tr>
<td>PTPN22</td>
<td>CD risk gene with role in autoimmunity. Protective effect in UC</td>
</tr>
<tr>
<td>IKBL</td>
<td>MHC gene associated with severe UC</td>
</tr>
</tbody>
</table>
Genes

- **GWAS**
  - Over 160 single nucleotide polymorphisms (SNPs) associated with IBD
  - Many modulate host response to microbial stimuli

- **Nucleotide-binding oligomerization domain containing protein 2 (NOD2)-caspase recruitment domain-containing protein 15 (CARD15)**
  - First gene identified- link between gut microbiome and IBD
  - Chromosome 16
  - Expressed in monocytes/Paneth cells in the terminal ileum
  - Encoding an intracellular receptor for the bacterial peptidoglycan (MDP)

Genes-microbe interaction

- **CD/carrying NOD2 risk alleles mutations**
  - Decrease in the Clostridium clusters IV and XIVa
  - Increase of Actinobacteria and Proteobacteria
  - Could trigger a decrease in the immunomodulatory cytokine, IL-10

- **NOD2 associated with more severe disease outcomes**
  - Higher rates of stricturing, fistulizing disease, and recurrent surgeries

Gene-environment interaction

- Genetic predisposition + commensal microbiota
- Response to environmental and/or nutritional exposures
- During critical periods of life
- Development of IBD

- Smoking
  - Smoking alters NOD2 expression in CD
  - Smoking cessation associated with
    - increase in abundance of *Firmicutes* and *Actinobacteria*
    - decrease in abundance of *Bacteroidetes*

Environmental Factors

- polyunsaturated fats (PUFAs)
- short chain fatty acids (SCFAs)
- refrigerated foods
- dietary fiber
- vitamin D
- food coloring and emulsifiers
- prior exposure to antibiotics or helminthic infections
- etc.
Diet-microbe-gene interaction

Diet: major environmental risk factor for changes of microbiota
  – processed food (high fat and high sugar content)
  – dietary-fat-induced taurocholic acid
  – growth of the *Biophila wadsworthia*
    • induces colitis in *Il10*−/− mice.
    • in humans with genetic defects in IL-10 and/or its receptor
  – use of food additives
    • Emulsifiers: carboxymethyl-cellulose and polysorbate 80

Alters microbiome and/or its interaction with the host

Clinical Relevance of Diet in IBD

• CCFA receives >14 000 inquiries/year;
• 65% ask for dietary advice
• Patients desire Tx. that do not suppress the immune system

Diet is associated with new onset IBD

• Systematic review
  – High dietary intake of total fats, PUFAs, Ω-6 FA and meat is associated with increase risk IBD
  – High fiber and fruit intakes is associated with decreased CD risk
  – High vegetable intake is associated with decrease UC risk

Hou JK et al. AJG 2011;106:563-73
Is There a Relationship Between Diet, the Gut Microbiota and IBD?

Who becomes sick and the timing of onset

• Genetically susceptible + commensal bacteria
• Intact gut immunity
• Exposure to an environmental factors
  – Infections may determine the timing of disease onset
• Disruption of the mucosal barrier (infection)
  – Host immune system exposed to the resident microbiota
  – Proliferation of pathogen-specific and commensal-specific T(h) cells
  – T cells migrate to other mucosal sites
    • react with the commensal microbiota
    • tip the balance off from physiologic to pathologic inflammation

Hand TW et al. Science 2012;337:1553-6
IBD: immunological dysregulation

mucosal homeostasis

- cytokine production by regulatory (T\textsubscript{Reg}) T cells supresses pro-inflammatory responses

\[ T\textsubscript{H}1, T\textsubscript{H}2, T\textsubscript{H}17 \rightarrow T\textsubscript{Reg} \]

mucosal inflammation

- increased production of pro-inflammatory cytokines by T helper (T\textsubscript{H}) cells

\[ TNF, IFN\gamma, IL-17 \]

\[ T\textsubscript{Reg} \text{ transfer can prevent the induction of experimental colitis} \]

Defects in innate and adaptive immune responses

Therapeutic interventions

• **Fecal diversion**
  – Benefit perianal CD and terminal ileal disease
  – Six months after re-anastomosis
    • recurrence of disease
  – Fecal stream (bacteria?) itself can trigger inflammation

• **Response to AbTx**

• **Modulation of the microbiome is a key goal of therapy**

  Curr Gastroenterol Rep 2016;18:5-13
Therapeutic interventions-AbTx, Probiotics

- **Antibiotics**
  - Antimycobacterial th, macrolides, fluoroquinolones, rifaximin
  - Some benefit in certain populations in active disease
- **IBD = dysbiosis; Use of probiotics**
  - Probiotics theoretically may interfere with possible pathogens in IBD
  - E. coli Nissle and the product VSL #3: some benefit in active UC
  - No studies have shown any benefit of treatment with probiotics in CD to date

- *More sustained or repeated ecological pressure ???*

VSL#3: mixture of *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus salivarius*
Therapeutic interventions- Probiotics
Induction of remission

Shen et al. IBD 2014;20:21-35
Therapeutic interventions- Probiotics
Remission maintenance

Shen et al. IBD 2014;20:21-35
Therapeutic interventions-FMT

- FMT in IBD: The evidence from case series
- Initial report: physician self-treated UC with fecal enemas
- Borody et al.
  - 6 pts. with ASC
  - Complete clinical, endoscopic and PH response
- Systematic review
  - 17 cases/case series
  - 41 pts.
  - 78% improved IBD symptoms
- Limitations
  - Incomplete reporting of IBD data
  - FMT protocol info incomplete and variable
  - Outcomes poorly defined; not standardized

Bennet JD. Lancet 1989
Anderson JL et al. APT 2012
Borody et al. AJG 2012
## Therapeutic interventions-FMT effective

<table>
<thead>
<tr>
<th>Author</th>
<th>IBD</th>
<th>Population</th>
<th>N</th>
<th>FMT method</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kunde et al. 2013</td>
<td>UC</td>
<td>Pediatric</td>
<td>9</td>
<td>Enema</td>
<td>7/9 (78%) response 3/9 (33%) remission at 1 wk. 6/9 (67%) maintained response at 1 month</td>
</tr>
<tr>
<td>Suskind et al. 2014</td>
<td>CD</td>
<td>Pediatric</td>
<td>9</td>
<td>NG</td>
<td>8 CR 1 improved</td>
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<tr>
<td>Zhang et al. 2013</td>
<td>CD</td>
<td>Adult</td>
<td>16</td>
<td>Gastroscope</td>
<td>12 Achieved CR</td>
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</tbody>
</table>
### Therapeutic interventions-FMT Mixed results

<table>
<thead>
<tr>
<th>Author</th>
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<th>Population</th>
<th>N</th>
<th>FMT method</th>
<th>Response</th>
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<tbody>
<tr>
<td>Kump PK et al. 2013</td>
<td>UC</td>
<td>Adult</td>
<td>6</td>
<td>Colonoscopy</td>
<td>2 improved; None achieved CR</td>
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<tr>
<td>Angelberger S et al. 2013</td>
<td>UC</td>
<td>Adult</td>
<td>5</td>
<td>NJ+enema</td>
<td>1 CR 2 Worsened</td>
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<tr>
<td>Damman et al. 2014</td>
<td>UC</td>
<td>Adult</td>
<td>7</td>
<td>Colonoscopy</td>
<td>1 Worsened 4 No improvement</td>
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<tr>
<td>Vaughn et al. 2014</td>
<td>CD</td>
<td>Adult</td>
<td>9</td>
<td>Colonoscopy</td>
<td>4 CR 2 Improvement 3 No improvement</td>
</tr>
</tbody>
</table>
### Therapeutic interventions-FMT No improvement

<table>
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<tr>
<th>Author</th>
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<th>FMT Method</th>
<th>Response</th>
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<tbody>
<tr>
<td>Vermeire S et al. 2012</td>
<td>CD</td>
<td>Adult</td>
<td>4</td>
<td>NJ</td>
<td>NS at 8 weeks</td>
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<tr>
<td>Landy et al. 2013</td>
<td>UC</td>
<td>Adult</td>
<td>5</td>
<td>NG tube</td>
<td>No improvement</td>
</tr>
</tbody>
</table>
Therapeutic interventions - FMT

**Meta-analysis**

- 36.2% achieved clinical remission (95% CI 17.4%-60.4%)
  - 60.5% of CD patients (95% CI 28.4%-85.6%)
  - 64% of younger patients (95% CI 10.6%-96.4%)
  - 22% of UC patients (95% CI 10.4%-40.8%)

Forest plot of all cohort studies’ overall pooled estimate of clinical remission

Colman RJ, JCC 2014
Therapeutic interventions-FMT

Moayyedi et al.
- Via enema
- Once weekly
- 6 weeks
- YES, it works

Rossen et al.
- Via nasoduodenal tube
- Two procedures
- 3 weeks interval
- NO, it doesn’t work

Therapeutic interventions - FMT

• Much work remains
• Is FMT effective and safe in IBD?
  – Adequately powered trials
• Ideal recipient?
  – Genotype
  – Phenotype:
    • Pouchitis, Proctitis, Post-op prophylaxis, ileal CD, maintenance of remission
  – Concomitant Tx
  – Can FMT make some patients worse?
Therapeutic interventions-EN

PLEASE: Pediatric Longitudinal Study of Elemental Diet and Stool Microbiota Composition

**Hypothesis:** Elemental diet-induced alterations in the gut associated with therapeutic efficacy in the treatment of disease.

**EN may work by suppressing**
- The entire microbiota in CD, thus inducing a lower antigenic effect to the gut
- Bacteria associated with CD but also other sensitive to EEN composition

Longitudinal Cohort Study-UPENN
https://github.com/chvlyl/PLEASE
Conclusions

• The microbiota has a role in experimental IBD
• Dysbiosis is present in humans suffering from IBD
• However, targeting the microbiota presently has only a limited value in IBD except for superinfections and pouchitis
• Combo therapy with other disease modifiers and using long-term ecologic pressure is rational
Gut Microbiome

“All disease begins in the gut”

Hippocrates 460 BC – 370BC

“Health is determined by the microbiota in our gut”

Hippocrates