Model of IBD pathogenesis, 2002

Genetics
- Barrier function
- Mucosal immune response

Environment
- Antigen
  - Appropriate
  - Inappropriate
- Modifiers
  - Smoking
  - Appendectomy

IBD
Model of IBD pathogenesis, 2012

- Genetics
  - Autophagy
  - Phagosome assembly
  - ER stress
  - Inflammasome
  - IL23R pathway
  - Epithelial barrier
  - Paneth cells
  - NFkB / IRF system
  - Unknown pathways

- Immunity
  - Th1 / Th17 imbalance
  - Treg
  - Defects in innate immunity

- Microbial flora
  - Good and bad microbes and products
  - Community structure
  - Stability of flora
  - AIEC

- IBD
  - Segmental disease
  - Environmental cues / triggers
Applying an ecosystem of microbial community analysis tools

Curtis Huttenhower, Rob Knight, Dirk Gevers
The microbiome in IBD: a complex microbial disease

• The gut microbiota varies in IBD
  – Diversity is almost certainly reduced
  – Specific clades are often over/under enriched
  – IBD subsets (colitis, ileal CD, etc.) are differentially affected

  Like disease alleles:
  infectious disease ↔ one microbe
  complex disease ↔ many microbes

• Which structural changes might be causal?
  – (If any)
  – (In each subset)
  – And which are instead associated with treatment/environment?

• And **why**: what microbial functions might be involved in these changes?
The IBD microbiome in the OSCCAR and PRISM cohorts

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Healthy</th>
<th>CD</th>
<th>UC</th>
<th>M</th>
<th>F</th>
<th>&lt;18</th>
<th>18-65</th>
<th>&gt;65</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSCCAR</td>
<td>108</td>
<td>0</td>
<td>61</td>
<td>47</td>
<td>68</td>
<td>48</td>
<td>23</td>
<td>84</td>
<td>9</td>
</tr>
<tr>
<td>PRISM</td>
<td>112</td>
<td>27</td>
<td>58</td>
<td>27</td>
<td>61</td>
<td>51</td>
<td>0</td>
<td>110</td>
<td>2</td>
</tr>
</tbody>
</table>

- **Treatments:**
  - Antibiotics
  - Immunosup.
  - Mesalamine
  - Steroids

- **Location:**
  - Mucosal (biopsy)
  - Luminal (stool)

- **Genetics:**
  - ~200 loci, IBD-targeted

~1-4K 16S reads/sample
Environment and disease:
You are your microbes’ environment: age, sampling, and treatment

MaAsLin: Multivariate Analysis by Linear Models (Tim Tickle)

~200 OSCCAR+PRISM individuals

We can use linear models to tease apart partially confounded effects
What about IBD?

MaAsLin: Multivariate Analysis by Linear Models (Tim Tickle)

- Main effects agree with previous findings
  - Roseburia (Lachnospiraceae) down
  - Ruminococcus down
  - Enterobacteriaceae up
- Also correctly classify environment
  - Bifidobacterium down (age)
  - Anaerostipes down (smoking)
  - Faecalibacterium down (ileal)
- What about diet? sample handling? genetics?

• Main effects agree with previous findings
  – Roseburia (Lachnospiraceae) down
  – Ruminococcus down
  – Enterobacteriaceae up
• Also correctly classify environment
  – Bifidobacterium down (age)
  – Anaerostipes down (smoking)
  – Faecalibacterium down (ileal)
• What about diet? sample handling? genetics?
What about IBD?

MaAsLin: Multivariate Analysis by Linear Models (Tim Tickle)

- **Main effects agree with previous findings**
  - Roseburia (Lachnospiraceae) down
  - Ruminococcus down
  - Enterobacteriaceae up
- **Also correctly classify environment**
  - Bifidobacterium down (age)
  - Anaerostipes down (smoking)
  - Faecalibacterium down (ileal)
- **What about diet? sample handling? genetics?**
Reconstructing functional differences

MaAsLin: Multivariate Analysis by Linear Models (Tim Tickle)

Microbial metabolism is differentially abundant in IBD

Starch+sugar met.
Amino acid met.
DNA maint.
Sec. and invasion

IBD iCD

Relative abundance

0.08  0.10  0.12  0.14
But what about functional detail?

IBD in the MetaHIT cohort

<table>
<thead>
<tr>
<th>DNA maintenance</th>
<th>CC + growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA packaging</td>
<td>phosphorilation</td>
</tr>
<tr>
<td>reproduction</td>
<td>rRNA modification</td>
</tr>
<tr>
<td>chromosome condensation</td>
<td>tRNA depolymerization</td>
</tr>
<tr>
<td>metabolism</td>
<td>DNA mediated transformation</td>
</tr>
<tr>
<td>DNA maintenance</td>
<td>glycosaminoglycan biosynthetic process</td>
</tr>
<tr>
<td>DNA seqs.</td>
<td>MetaHIT seqs.</td>
</tr>
<tr>
<td>HUMAnN</td>
<td>MetaHIT seqs.</td>
</tr>
<tr>
<td>pathway abundances</td>
<td>pathway abundances</td>
</tr>
</tbody>
</table>

- Up in CD: DNA maintenance, CC + growth, Sugar utilization, Signaling + secretion, Iron + drug transport
- Down in CD: DNA maintenance, CC + growth, Sugar utilization, Signaling + secretion, Iron + drug transport
Not who, but why: the IBD microbiome is defined by adaptation to oxidative stress.
Dysbiosis implicated in disease in human and animal models
Commensal Bacterial metabolism modulates Inflammatory Response

Commensal bacterial metabolism

short-chain fatty acids (SCFA)

GPR43

Apoptosis

Inflammation of intestinal mucosa

Maslowski et al. Nature 2009
Experimental approach to identify education pathways

Gut metabolites bugs/bioactives → Stimulate cells → Transcriptional and metabolomic assays

SFB, Prevotella, Bioactives → T cells, Macrophages, Epithelial cells

L1000, Metabolomics

Pathways for immune education
Severe Crohn’s Colitis
IL23R isoforms and susceptibility loci implicated in Crohn's disease

Identification of autophagy in CD pathogenesis

• Among the genes conclusively associated to Crohn’s, 2 major autophagy genes have been associated
  – ATG16L1, non-synonymous coding SNP (T300A)
  – IRGM, synonymous coding SNP, in perfect LD ($r^2=1$) with promoter deletion
Why is autophagy important?

• Constitutive and conserved

• Disease-associated
  – Crohn’s Disease
  – Neurodegeneration
  – Tuberculosis, leprosy, viral infection

• Other roles
  – Inflammation initiation and resolution
  – Antigen presentation
  – Cancer
  – Longevity
Core machinery is highly conserved

- Two ubiquitin-like conjugation systems
- Atg5/12/16 membrane association is transient
- Atg16 mediates Atg5/12 membrane targeting
- Atg8 / LC3 conjugation occurs on both sides of the membrane
- LC3 is the favored autophagosome marker