The predictive role of the microbiome in inflammatory bowel diseases

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Conflicts of Interest

• Scientific advisory boards for Abbvie, Gilead, Takeda, and Merck
Microbiome and IBD

What are we trying to predict?

Healthy

Clinical IBD

Complications

Treatment

Rx response
Unique challenges for prediction

- Need for:
  - Large sample sizes
  - Long duration of follow-up
  - ‘Pre-event’ samples

- Time varying external exposures:
  - Treatment
  - Diet and lifestyle
  - Disease phenotype
Microbiome and IBD

Outline – Can we predict who…

- Develops incident IBD?
  - Post-operative CD recurrence model

- Will have IBD-related complications?
  - Disease-related surgery
  - Penetrating and stricturing Crohn’s disease

- Respond to particular therapy?
  - Steroids
  - Biologics
Microbiome and IBD
Can we predict IBD from health?

- Most commonly used model is siblings/relatives of affected individuals

- Single center study from the UK:
  - 22 patients with inactive Crohn’s disease
  - 21 healthy siblings
  - 25 controls

- Stool: 16s rRNA and fecal calprotectin
- Blood: T cell phenotyping and genotyping for risk alleles
- Intestinal permeability (lactulose rhamnose absorption)

Hedin CR. Gut 2014 Oct;63(10):1578-86
Microbiome and IBD
Predicting IBD from health

Microbial changes were apparent in healthy siblings who demonstrated some aspects of a CD-like dysbiosis.

<table>
<thead>
<tr>
<th></th>
<th>Concentrations of bacteria, $\log_{10}$ copies/g median (IQR)</th>
<th>p Value</th>
<th>Between-group comparisons p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n=22)</td>
<td>Siblings (n=21)</td>
<td>Controls (n=25)</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>6.88</td>
<td>9.27</td>
<td>9.59</td>
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<td></td>
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<tr>
<td>Clostridia cluster IV</td>
<td>7.76</td>
<td>9.34</td>
<td>9.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster IV</td>
<td>7.05</td>
<td>8.75</td>
<td>9.55</td>
</tr>
<tr>
<td>Ruminococcus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roseburia spp.</td>
<td>9.19</td>
<td>9.34</td>
<td>9.92</td>
</tr>
<tr>
<td>Bacteroides-Prevotella</td>
<td>8.83</td>
<td>10.16</td>
<td>10.48</td>
</tr>
</tbody>
</table>

Hedin CR. Gut 2014 Oct;63(10):1578-86
“CD-like” dysbiosis in healthy individuals may be associated with subclinical inflammation

Single center study: Recruited 21 families:
- 26 CD patients in clinical remission
- 10 UC patients in clinical remission
- 54 healthy siblings / parents

Stool: Fecal 16s rRNA sequencing, untargeted metabolomics, and fecal calprotectin measurement

Multivariate modeling revealed 2 distinct OTU types

OTU type 2 correlated with IBD status (particularly CD)
- Low diversity
- Enriched in Blautia (ruminococcus) and bacteroideses
- Depleted in firmicutes

Microbiome and IBD
Predicting IBD from health

10 healthy individuals had OTU-type 2

Such patients had elevated fecal calprotectin levels
Microbiome and IBD

Predicting IBD: Post-op models

- Post-operative Crohn’s disease offers another attractive “disease incidence” model
  - No active inflammation after ‘curative’ resection
  - Relatively high rate of recurrence → smaller cohort may yield significant effects
Endoscopic recurrence is seen in 90% at 1 year.

Recurrence is usually at the anastomosis (or proximal to it).

Endoscopic recurrence usually precedes clinical recurrence.

Up to a third may require repeat surgery within 10 years.

Reduced *F. prausnitzii* at surgery was associated with increased risk of endoscopic recurrence at 6 months.
Microbiome and IBD

Predicting IBD: Post-op models

- Small study: 6 CD patients undergoing resection, 40 CD controls, healthy controls.
- Patients who remained in remission were more similar to controls.

Recurrence vs. Remission:

Lower

Lachnospiraceae ($p < 10^{-2}$)
Erysipelotrichachaea ($p < 0.05$),
clostridia genus ($p < 120.2$),
firmicutes

Higher

rhodobacteraceae
unknown proteobacteria ($p < 10^{-2}$)
Microbiome and IBD

Predicting IBD: Post-op models

- 12 CD patients undergoing surgery: Microbiome characterized using pyrosequencing
- Several species separated out those with recurrence from those remaining in remission

De Cruz P. J Gastroenterol Hepatol 2015 Feb;30(2):268-78.
913 patients with CD recruited at diagnosis (RISK)
- Clinical information
- Serologies
- Genetics
- Ileal gene expression
- Microbiome

Outcome: 9% of patients developed complications (B2/B3)

Microbiome and IBD
Predicting complicated disease

Clinical data predicted complications but were inadequate

<table>
<thead>
<tr>
<th></th>
<th>Stricturing behaviour (B2)</th>
<th>Penetrating behaviour (B3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Competing-risk model (n=913)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>1.07 (0.97–1.17)</td>
<td>0.16</td>
</tr>
<tr>
<td>African American race</td>
<td>1.08 (0.52–2.22)</td>
<td>0.84</td>
</tr>
<tr>
<td>Isolated ileal location (L1)</td>
<td>1.60 (0.88–2.91)</td>
<td>0.12</td>
</tr>
<tr>
<td>ASCA IgA positive</td>
<td>1.69 (0.94–3.07)</td>
<td>0.0816</td>
</tr>
<tr>
<td>CBir1 positive</td>
<td>2.30 (1.26–4.20)</td>
<td>0.0070</td>
</tr>
</tbody>
</table>

Microbiome and IBD

Predicting complicated disease

- Microbiome data analyzed using MaAsLin (FDR $\alpha < 0.2$)
- Certain genera predicted risk of complicated B2 or B3 disease

Microbiome and IBD

Predicting complicated disease

Dysbiosis index (MDI) = \( \log \) [increased in CD] / \( \log \) [decreased in CD]

- Correlated with clinical disease severity and inversely with species richness

Can relapse be predicted?

- 33 patients with CD from an IFX withdrawal trial (STORI)
- Fecal samples at baseline, 2 and 6 months
- Microbiome compared to 29 healthy subjects

Outcome: 19 patients relapsed

Findings:
- Relapsers demonstrated greater dysbiosis at baseline
- Findings in relapsers were similar to the CD-healthy control difference

Microbiome and IBD
Predicting relapse


<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Baseline HS (n = 29)</th>
<th>CD at Baseline (n = 33)</th>
<th>P</th>
<th>Relapsers at Baseline (n = 19)</th>
<th>P</th>
<th>Non Relapsers Baseline (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All bacteria</td>
<td>11.7 (11.5 to 11.8)</td>
<td>11.7 (11.3 to 11.9)</td>
<td>0.9</td>
<td>11.5</td>
<td>0.3</td>
<td>11.7</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>-1.1 (-1.3 to -0.9)</td>
<td>-1.0 (-1.3 to -0.8)</td>
<td>0.4</td>
<td>-1.1</td>
<td>0.2</td>
<td>-0.7^a</td>
<td>0.006</td>
</tr>
<tr>
<td><em>C. coccoides</em></td>
<td>-0.7 (-0.8 to -0.5)</td>
<td>-1.3 (-2.0 to -1.1)^a</td>
<td>0.0003</td>
<td>-1.9^a</td>
<td>&lt;0.0001</td>
<td>-0.9</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Bifidobacteria</em></td>
<td>-2.6 (-2.9 to -2.5)</td>
<td>-2.9 (-3.3 to -2.2)</td>
<td>0.5</td>
<td>-2.7</td>
<td>0.6</td>
<td>-2.7</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>-4.0 (-4.2 to -3.7)</td>
<td>-4.1 (-4.3 to -3.5)</td>
<td>0.9</td>
<td>-3.6</td>
<td>0.8</td>
<td>-4.5</td>
<td>0.3</td>
</tr>
<tr>
<td><em>C. leptum</em></td>
<td>-1.7 (-1.7 to -1.7)</td>
<td>-2.6 (-3.6 to -2.4)^a</td>
<td>&lt;0.0001</td>
<td>-2.4^a</td>
<td>&lt;0.0001</td>
<td>-2.4^a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>F. prausnitzii</em></td>
<td>-1.4 (-1.6 to -1.1)</td>
<td>-2.5 (-2.8 to -1.4)^a</td>
<td>0.003</td>
<td>-2.5^a</td>
<td>&lt;0.0001</td>
<td>-1.1</td>
<td>0.6</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-3.9 (-4.5 to -3.7)</td>
<td>-3.5 (-4.1 to -3.2)</td>
<td>0.09</td>
<td>-3.2</td>
<td>0.3</td>
<td>-3.5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

^aResults are given as median with IQR for healthy subjects and patients with CD, as median for relapsers and nonrelapser patients with CD. Significant difference from healthy subjects for total CD, relapsers, and nonrelapsers (P < 0.05).
Pediatric IBD patients: age 9 – 18 years
11 patients initiated anti-TNF therapy

Diversity increase in responders (blue) but not in non-responders (pink)

Microbiome and IBD
Response to therapy: Anti-TNF

Microbial diversity at baseline (and similarity to healthy controls) predicted fecal calprotectin levels at 3 months

Microbiome and IBD

Response to therapy: Anti-TNF

Current abundance of *C. sphenoides* and *haemophilus* were associated with calprotectin at 3 months.

Microbiome and IBD

Response to therapy: Steroids

- Acute severe UC affects 25% of patients with UC

- Multi-center prospective cohort of children 2-18 years of age, hospitalized for IV steroids

- Stool microbiome collected at baseline

- **Steroid failure**: Need for colectomy or second-line medical therapy with infliximab or cyclosporine

Non-responders had significantly less microbial diversity than children who were steroid responsive.
Microbiome and IBD

Response to therapy: Vedolizumab

- Prospective single center cohort of patients with refractory CD or UC initiating vedolizumab
- At each visit:
  - Disease activity (HBI or SCCAI)
  - Concomitant medications
  - Blood for C-reactive protein, hemoglobin, albumin
  - Stool for microbiome sequencing

Outcome: Clinical remission
- HBI < 4 (CD)
- SCCAI < 2 (UC)
Community alpha diversity was significantly higher in CD patients who achieved remission at week 14 at the species level.
Two species (butyrate producers) demonstrated significant different abundances in remitters and non-remitters.
13 pathways in CD and 5 in UC were differentially distributed (q < 0.1) in remitters and non-remitters.

Pathway codes: A, super-pathway of arginine and polyamine biosynthesis; B, super-pathway of branched amino acid biosynthesis; C, Calvin-Benson-Bassham cycle; D, L-citrulline biosynthesis; E, dTDP-L-rhamnose biosynthesis I; F, super-pathway of N-acetyleglucosamine, N-acetylmannosamin and N-acetyleneuraminate degradation; G, super-pathway of β-D-glucuronide and D-glucuronate degradation; H, super-pathway of hexitol degradation; I, L-isoleucine biosynthesis I; J, super-pathway of polyamine biosynthesis I; K, L-histidine degradation III; L, GDP-mannose biosynthesis; M, acetyl-CoA fermentation to butanoate II; N, colonic acid building blocks biosynthesis; O, lipid IVA biosynthesis; P, N10-formyl-tetrahydrofolate biosynthesis; Q, pentose phosphate pathway; R, pyruvate fermentation to acetate and lactate II.
In CD, 5 taxa were different in their relative abundance between baseline and week 14 (all decreased in abundance in those achieving remission)

- *Bifidobacterium longum*
- *Eggerthella*
- *Ruminococcus gnavus*
- *Roseburia inulivorans*
- *Veillonella parvula*

17 pathways were significant reduced on follow up at week 14 compared to baseline

- Decrease in TCA pathways
- NAD salvage pathway

Decreased oxidative stress with remission
13 patients provided samples at baseline, weeks 14 and 54.

Patients achieving remission at week 14 demonstrated highly significant persistency in microbiome composition at week 30 (p=0.00039) and week 54 (p=0.019).
A neural-network model with manually curated list of 40 microbiome variables provided highest classifying power.
The microbiome appears to be a promising tool to predict disease onset and relapse, complications, and response to therapy in IBD.

Unanswered questions:

- Relative predictive contribution of microbiome to other predictors
- Impact of diet, environment and geography on microbial predictors
- Can this be used to inform ‘next-generation’ probiotics as therapeutic options?