Basic Research in Pediatric IBD: Where are We Going?

Ted Denson, MD
Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine

Objectives

- Review recent research developments
- Identify knowledge gaps and next steps
- Discuss implications for clinical practice

Multi-factorial Pathogenesis of IBD

CCFA IBD Research Challenges 2013

- Define clinically relevant subsets of patients with IBD using genetic, immunologic, microbiota, tissue expression, and clinical profiles (including drug metabolism and pharmacogenetics) that will predict geographical, racial, and environmental differences in disease, complications, and response to treatment.
- Understand how environmental factors enhance the risk of IBD through effects on microbial, epithelial, immunologic, and mucosal barrier integrity. (a specific focus on the role of diet is warranted)
- Determine which environmental triggers initiate, perpetuate, and/or exacerbate disease.
- Further understand reciprocal interactions (cross talk) between genes, infections, epithelial cells, and immune and gastric immune responses that determine pathways mediating intestinal homeostasis versus inflammation.
- Define cell-essential autocrine/paracrine pathways for communication with the microbiota.
- Define critical cell types and the functional pathways leading to further understanding of homeostasis versus inflammation, with an ultimate goal of identifying preventive/therapeutic targets.
- Determine optimal treatment approaches and strategies through comparative effectiveness studies.

The IBD Genome

The allelic architecture of common susceptibility variants for pediatric IBD is similar to adult onset

- Tested 160/163 adult-onset risk genotypes which explain ~20% of the genetic susceptibility
- 1047 pediatric-onset IBD cases and 1663 healthy controls from RISK study
- Replicated 88% CD and 90% UC variants
- Sequencing approaches needed for more comprehensive dissection of known risk loci and discovery of rare damaging mutations
Next Steps for Gene Discovery and Pathway Function

- Whole genome and exome sequencing to discover rare and highly damaging variants: NEOPICS & RISK
- Gene variant/pathway functional analyses in primary cells, mice with human knock-in mutations, and cell lines: CCFA Genetics Initiative and RISK
- eQTL analyses to define variants which increase risk via regulation of gene expression: NIDDK IBD Genetics Consortium & RISK
- Epigenetic analyses to define acquired differences (e.g., DNA methylation) in genetic regulation of risk and host responses

---

Genetic variants synthesize to produce Paneth cell phenotypes that define subtypes of Crohn's disease

![Diagram showing genetic variants and associated phenotypes](image)

---

Expression quantitative trait loci analysis identifies associations between genotype and gene expression in human intestine

![Diagram illustrating the relationship between genotype and phenotype](image)
DNA methylation-associated colonic mucosal immune and defense responses in treatment-naive pediatric ulcerative colitis.

Kellermayer et al. Epigenetics 2014
Kellermayer Can J Gastro 2012

Environmental Factors

- Smoking: CD vs UC
- NSAIDs
- Vitamin D deficiency
- Perinatal & childhood infections/microbial exposures?
- Stress?
- Food or food additives?
- Genes Environment Microbes study
- Final measurable effect: microbial shifts

The Microbial Dysbiosis Index Characterizes CD Severity

PRO-KIDS RISK Cohort

The Metabolic Roles of the IBD Microbiome

Morgan et al. Genome Bio 2012

Next Steps for Microbial Community Profiling & Functional Characterization

- Longitudinal studies of intestinal and fecal microbial community in newly diagnosed IBD patients and controls: HMP2
- Transfer of human microbiota into traditional and humanized mouse models: CCFA Microbiome Consortium
- Identification of regulatory microbial metabolites: CCFA Microbiome Consortium

Morgan et al. Genome Bio 2012
Muise et al. Gastroenterology 2014

Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-10-/- mice.

Sartor et al. Infect Imm 2014
CCFA Microbiome Consortium

Morgan et al. Genome Bio 2012
Muise et al. Gastroenterology 2014
Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.

Butyrate induces the differentiation of Treg cells in the colonic lamina propria.

Chromatin modification at the Foxp3 locus by butyrate.

Clinical Forms of Inflammatory Bowel Disease

Next Steps for Patient Classification Using Microbial & Genomic Information

- Validation of microbial and gene expression panels for clinical sub-sets and predictive models using biopsy and stool samples: RISK, PROTECT, and Broad Adult-Onset Cohort
- Commercial partner to develop tests using clinical path specimens
- Test utility in clinical practice: ImproveCareNow? CCFA Clinical Research Alliance?

Clinical use of Gene Expression Panels to Improve Diagnostic or Prognostic Accuracy

Several gene expression diagnostics for oncology
Afirma Thyroid Cancer test
56,540 thyroid cancer cases per year
Indeterminate pathology in 30%
Expression of 142 genes in thyroid biopsy
49 site validation in 3789 patients: 92% accuracy
Prevent 25,000 thyroid resections per year
Charge: covered by Medicare and third party

Alexander et al. NEJM 2012
CCFA Sponsored Clinical Research Network: PRO-KIDS

1100 children with Crohn’s at diagnosis between 2008-2012
Follow-up to 2017

Study:
Genetic makeup
Bacteria in bowel
Immune reactivity to bacteria, food, infections etc
Environmental Exposures

3 years
160 – 200 patients with complication / surgery

Study Design

Whole RRS cohort

RRS + cohort

Age matched representative sub-cohort

Analysis

Methods

Study Design

Outcomes

Month 0 Steroid Free Remission (CD vs. IBD)

Multivariate Analysis by Linear Models (MaAsLin)

Between:
- Genes from the APOA1 module (APOA1, CXCL9)
- Genes from DUOX2 module (DUOXA2, MUC4, LCT)
- Clinical phenotype (OI, UC, CD)
- Endoscopic severity (ileal deep ulcers)
- Clinical severity (PCDAI)

Controlling for: age, gender, body mass index (BMI), and NOD2, FUT2, and ATG16L1 risk allele carriage.

- 70 significant microbial taxa and genes associations.
- 34 significant microbial taxa and clinical associations.

Covariation of the Ileal Microbial Community Stricture with Ileal Gene Expression and Clinical Phenotype and Severity

Haberman et al. JCI 2014
PRO-KIDS RISK Study
Pathogenesis of ileal IBD

- Normal endoscopic appearance of the ileum
- Inflamed ileum

*Alteration of the APOA1 co-expression module and reduction of Firmicutes in the ileum defines a CD specific signature.

*Gradual alteration of DUOX2 host co-expression signature and expansion of Proteobacteria is detected in the ileum of both UC and CD.

A multi-omic model is superior in predicting surgery and steroid free remission in comparison to clinical factors alone.

The relative goodness of fit of the models, \( P < 0.0043 \)

<table>
<thead>
<tr>
<th>Clinical variables only</th>
<th>Clinical, expression and microbial</th>
</tr>
</thead>
<tbody>
<tr>
<td>C statistics (AUC)</td>
<td>0.705</td>
</tr>
<tr>
<td></td>
<td>0.760</td>
</tr>
</tbody>
</table>

Multiple regression analysis including clinical, gene expression, and microbial variables.

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( \geq 10 ) vs. (&lt;10 )</td>
<td>0.8868</td>
<td>0.944</td>
<td>0.430, 2.075</td>
</tr>
<tr>
<td>Ileal DU vs. no DU</td>
<td>0.0348</td>
<td>0.771</td>
<td>0.271, 2.388</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-TNF therapy</td>
<td>0.0020</td>
<td>5.181</td>
<td>1.838, 14.706</td>
</tr>
<tr>
<td>APOA1 expression level &gt; 80th percentile</td>
<td>0.0152</td>
<td>3.058</td>
<td>1.241, 7.576</td>
</tr>
<tr>
<td>Blautia Abundant (&gt;70th percentile) vs non-abundant</td>
<td>0.0028</td>
<td>0.231</td>
<td>0.089, 0.604</td>
</tr>
<tr>
<td>Veillonella abundant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veillonella Abundant (&gt;70th percentile) vs non-abundant</td>
<td>0.0156</td>
<td>3.456</td>
<td>1.187, 10.448</td>
</tr>
</tbody>
</table>

A mucosal gene expression panel can be used to differentiate cCD from UC.

- A regression model including ileal host/microbial profiles more accurately predicts remission six months after diagnosis than one using clinical factors alone.
- Clinical “deep remission” might be obtained only when addressing both restoration of altered genes and microbes.