Basic Research in Pediatric IBD:

Where are We Going?

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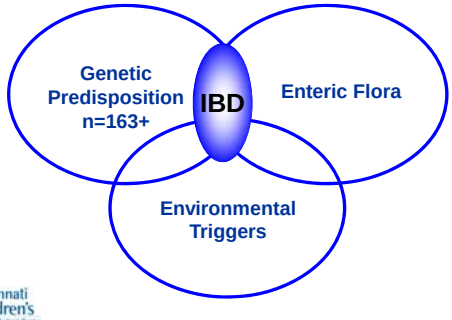
Objectives

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

Multi-factorial Pathogenesis of IBD

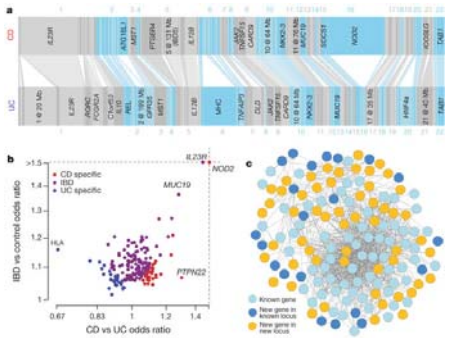


CCFA IBD Research Challenges 2013

- Define clinically relevant subsets of patients with IBD using genetic, immunologic, microbial, tissue expression, and clinical profiles (including drug metabolism and pharmacokinetics) that will predict aggressiveness of disease, complications, and response to treatment.
- Understand how environmental factors enhance the risk of IBD through effects on microbial, epigenetic, immunologic, and mucosal barrier influences.
  - A specific focus on the role of diet is warranted.
- Determine which environmental triggers initiate, perpetuate, and/or reactivate disease.
- Further understand reciprocal interactions (cross talk) between genes, microbiota, epithelial cells, and innate and adaptive immune responses that determine pathways mediating mucosal homeostasis versus inflammation.
  - Determine critical rate-limiting cell/cellular pathways for communication with the microbiota.
  - Definition of critical cell types and the functional pathways leading to further understanding of homeostasis versus inflammation, with an ultimate goal of identifying putative (therapeutic) targets.
- Determine optimal treatment approaches and strategies through comparative effectiveness studies.

Denson et al IBDJ 2013

The IBD Genome



Cho et al Nature 2012

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

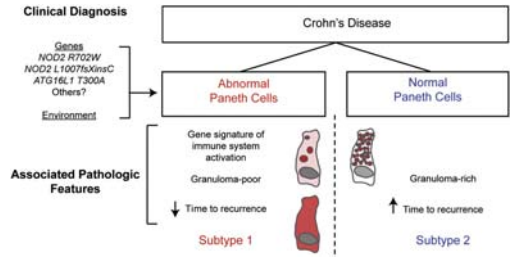
- Tested 160/163 adult-onset risk genotypes which explain ~ 20% of the genetic susceptibility
- 1047 pediatric-onset IBD cases and 1663 healthy controls from RISK study
- Replicated 88% CD and 90% UC variants
- Sequencing approaches needed for more comprehensive dissection of known risk loci and discovery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

Kugathasan et al, under review 2014
PRO-KIIDS RISK Cohort

Next Steps for Gene Discovery and Pathway Function

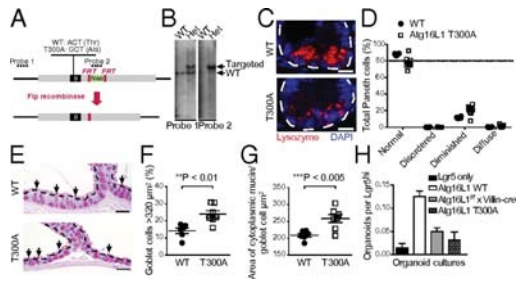
- Whole genome and exome sequencing to discover rare and highly damaging variants: NEOPICS & RISK
- Gene variant/pathway functional analyses in primary cells, mice with human knock-in mutations, and cell lines: CCFA Genetics Initiative and RISK
- eQTL analyses to define variants which increase risk via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



Stappenbeck et al Gastro 2014
CCFA Genetics Initiative
Gastroenterology 2014 146, 200-209

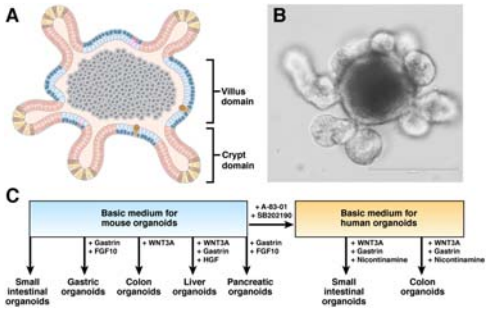
Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense



Lassen K G et al. PNAS 2014;111:7741-7746
CCFA Genetics Intiative

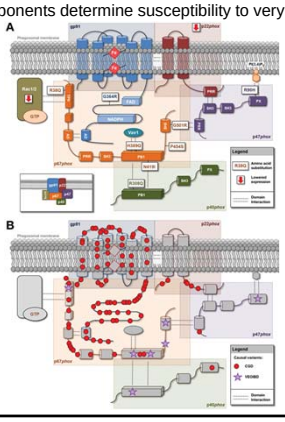
PNAS

Utilization of LGR5+ Stem Cell or Crypt-Derived Intestinal Organoids for Functional Genetic Studies of the Epithelial Compartment



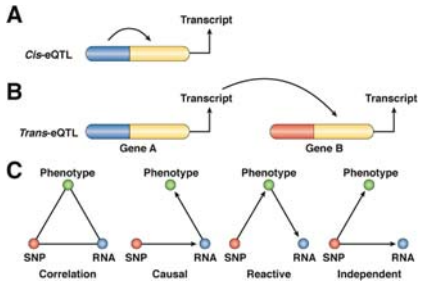
Clevers et al *Gastroenterology* 2014 147, 289-302

Variants in nicotinamide adenine dinucleotide phosphate oxidase complex components determine susceptibility to very early onset IBD



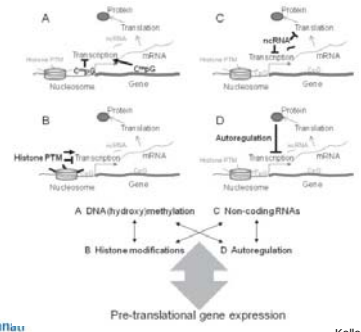
Muise et al Gastroenterology 2014
NEOPICS
Gastroenterology 2014 147, 680-689

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



Silverberg et al Gastro 2013
NIDDK IBD GC

DNA methylation-associated colonic mucosal immune and defense responses in treatment-naïve pediatric ulcerative colitis.



Kellermayer et al Epigenetics 2014
Kellermayer Can J Gastro 2012

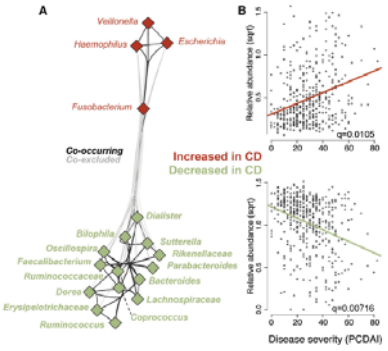
Environmental Factors

- Smoking: CD vs UC
- NSAIDs
- Vitamin D deficiency

- Perinatal & childhood infections/microbial exposures?
- Stress?
- Food or food additives?
- Genes Environment Microbes study

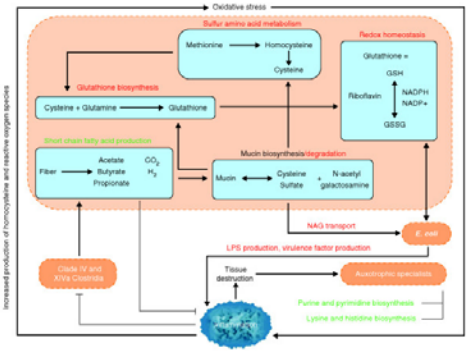
- Final measurable effect: microbial shifts

The Microbial Dysbiosis Index Characterizes CD Severity



Gevers et al Cell Host & Microbes. 2014
PRO-KIIDS RISK Cohort

Metabolic Roles of the IBD Microbiome



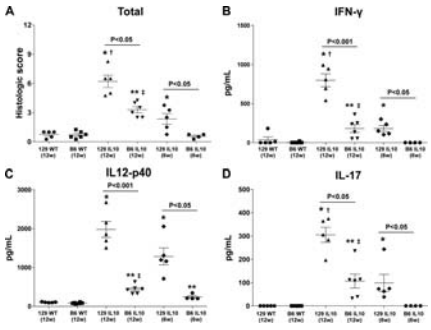
Morgan et al Genome Bio 2012

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 Consoritium

Muise et al Gastroenterology 2014

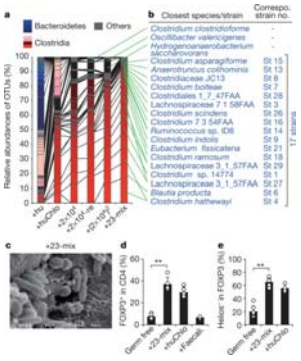
Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-10-/- mice.



Eun C S et al. Infect. Immun. 2014;82:2239-2246

Sartor et al Infect Imm 2014
CCFA Microbiome Consortium

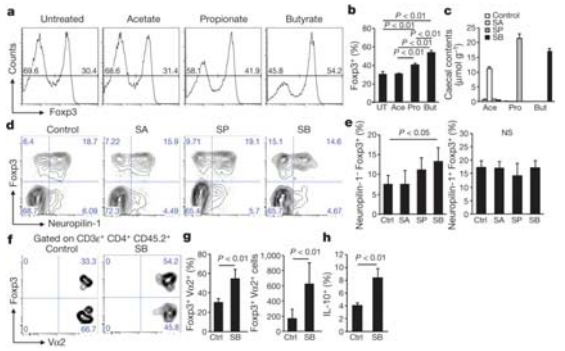
Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.



K Atarashi *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12331

nature

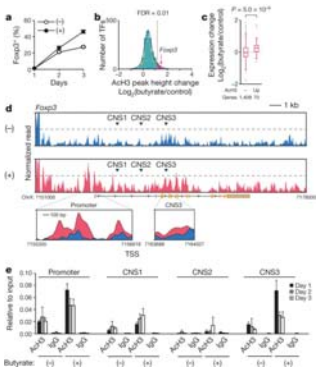
Butyrate induces the differentiation of T_{reg} cells in the colonic lamina propria.



nature

Y Furusawa *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12721

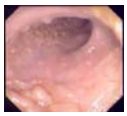
Chromatin modification at the Foxp3 locus by butyrate.



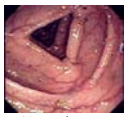
Y Furusawa *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12721

nature

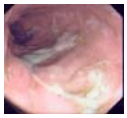
Clinical Forms of Inflammatory Bowel Disease



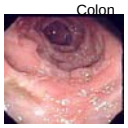
Normal Ileum



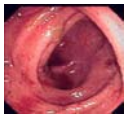
Normal Colon



Crohn's Ileitis



Crohn's Colitis



Ulcerative Colitis

Cincinnati Children's

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?

Cincinnati Children's

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

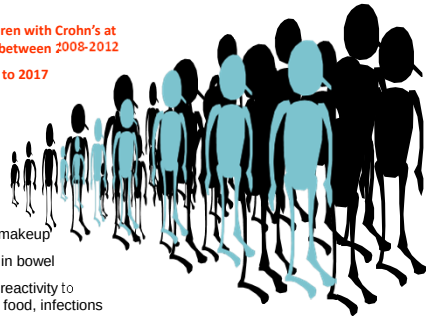
Several gene expression diagnostics for oncology
Afirma Thyroid Cancer test
56,540 thyroid cancer cases per year
Indeterminate pathology in 30%
Expression of 142 genes in thyroid biopsy
49 site validation in 3789 patients: 92% accuracy
Prevent 25,000 thyroid resections per year
Charge: covered by Medicare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

Alexander et al NEJM 2012

CCFA Sponsored Clinical Research Network: PRO-KIIDS

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

CROHN'S & COLITIS FOUNDATION OF AMERICA

PRO-KIIDS

- Enrolling sites
- Thomas D. Walters, SickKids, Toronto, Canada
- Subra Kugathasan, Emory-Children's Center, Atlanta, GA

Cincinnati Children's

CCHMC - GI
- Lee Denson
- Yael Haberman

CCHMC Bioinformatics core
- Bruce J Aronow
- Phillip Dexheimer

Cincinnati DHC Digestive Health Center

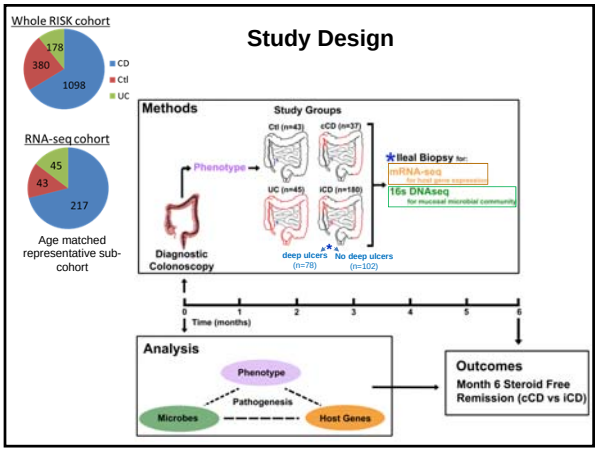
BROAD INSTITUTE

Ramnik J Xavier
Dirk Gevers

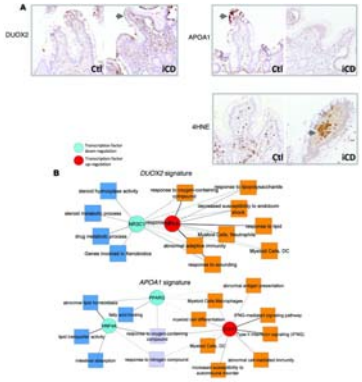
HARVARD SCHOOL OF PUBLIC HEALTH

Curtis Huttenhower
Timothy L Tickle

Study Design



A Core CD Ileal Gene Expression Signature Contains *DUOX2* and *APOA1* Co-expression Signatures



Haberman et al JCI 2014
PRO-KIIDS RISK Study

Multivariate Analysis by Linear Models (MaAsLin)

Between:

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

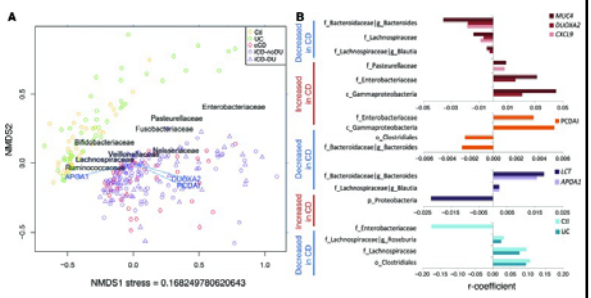
&

- Ileal microbial community

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

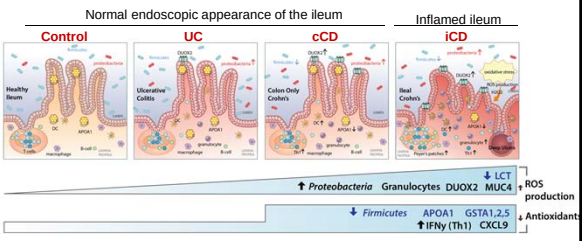
- **70 significant microbial taxa and genes associations.**
- **34 significant microbial taxa and clinical associations.**

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



Haberman et al JCI 2014
PRO-KIIDS RISK Study

Pathogenesis of Ileal IBD



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

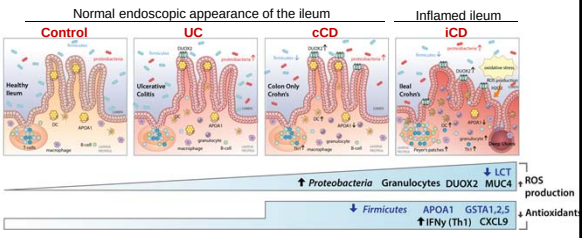
*Alteration of the APOA1 co-expression module and reduction of *Firmicutes* in the ileum defines a CD specific signature.

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		
	Clinical variables only	Clinical, expression and microbial
C statistics (AUC)	0.705	0.760

Multiple regression analysis including clinical, gene expression, and microbial variables.				
		p-value	OR	CI
Age≥10 vs. <10		0.8868	0.944	0.430, 2.075
Ileal DU vs. no DU	PCDAI>30	0.6244	0.771	0.271, 2.188
	PCDAI≤30	0.0029	4.713	1.701, 13.057
Anti-TNF therapy		0.0020	5.181	1.828, 14.706
APOA1 expression level > 80th percentile		0.0152	3.058	1.241, 7.576
Blautia Abundant (>70th percentile) vs non-abundant	Veillonella abundant	0.5183	1.634	0.368, 7.25
	Veillonella non-abundant	0.0028	0.231	0.089,0.604
Veillonella Abundant (>70th percentile) vs non-abundant	Blautia abundant	0.1350	0.454	0.187,1.104
	Blautia non-abundant	0.0816	3.201	0.696,14.723

Clinical Implications



- 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.