How Many IBDs Are There?: Identifying and Characterizing Functional Regulatory Elements in IBD

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Genetics and IBD

IBD Explained by Genetics

- UC (F1)
- CD (F2)
- CD (L3)

Not Explained by Genetics

- UC (L3)
- UC (F2)
- UC (F3)

Inherited determinants of Crohn’s disease and ulcerative colitis phenotypes: a genetic association study


Genetics and phenotypes in inflammatory bowel disease

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ENCODE: Functionally annotate the human genome

Scientists in the Encyclopedia of DNA Elements Consortium have applied 24 experiment types (across) to more than 150 cell lines (down) to assign functions to as many DNA regions as possible — but the project is still far from complete.

**EXPERIMENTAL TARGETS**

**DNA methylation**: regions layered with chemical methyl groups, which regulate gene expression.

**Open chromatin**: areas in which the DNA and proteins that make up chromatin are accessible to regulatory proteins.

**RNA binding**: positions where regulatory proteins attach to RNA.

**RNA sequences**: regions that are transcribed into RNA.

**ChIP-seq**: technique that reveals where proteins bind to DNA.

**Modified histones**: histone proteins, which package DNA into chromosomes, modified by chemical marks.

**Transcription factors**: proteins that bind to DNA and regulate transcription.

**CELL LINES**

**Tiers 1 and 2**: widely used cell lines that were given priority.

**Tier 3**: all other cell types.

So far, scientists have examined 13 of about 60 known histone modifications and 120 of about 1,800 transcription factors.

Many more cell types are yet to be interrogated.

Every shaded box represents at least one genome-wide experiment run on a cell type.
Molecular subtyping of Crohn’s disease

Matt Weiser

ORIGINAL ARTICLE

Molecular classification of Crohn’s disease reveals two clinically relevant subtypes


Gut 2016
CD patients segregate into two distinct clusters based on gene expression profiles.

Weiser, Simon, et al., 2016, Gut
Colon-like and ileum-like gene expression subclasses in colonic CD
CD Classes Associated with Clinical Characteristics

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Ileum-like (n = 10)</th>
<th>Colon-like (n = 11)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Location</td>
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<tr>
<td>Ileum-only</td>
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<td>Ileum+Colon</td>
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Epigenetics, Regulation and Transcription

DNA Binding Proteins

“Open” Chromatin

FAIRE-seq

ATAC-seq

DNA Methylation

Histone Tail Modifications

nucleosome

Gene Transcription

RNA-seq

microRNA

IncRNA

tDRs

Small RNA-seq
Cellular Identity

- Cellular identity - what cell does
- Defined by gene expression patterns
- Gene regulation determined by epigenetic state and small RNA
- Cellular (re-)programming leads to altered identity
Open chromatin regulatory landscape altered
Long, non-coding RNAs (IncRNAs), but not tRNA-derived RNAs (tDRs) stratify CD into colon-like and ileum-like subclasses.
miRNAs also reveal same CD subtypes

PC1: 58.5% Variance

PC2: 14.1% Variance

- Black circles: Non-IBD (n=11)
- Blue circles: Colon-like (n=11)
- Red circles: Ileum-like (n=10)

miRNA expression

Ben Keith
miR-31 levels explain the stratification

Colon-like (n=11)
Ileum-like (n=10)
Non-IBD (n=11)

P < 0.02
P < 0.0001
Independent Replication

miR-31 expression

- Ileum-like
- Colon-like

Non-IBD (N=24)  CD (N=22)

\( \log_{10} \text{RQV (a.u.)} \)
miR-31 expression is increased in serum of CD patients compared to healthy controls

Shruti Saxena
Index biopsies from FFPE samples from treatment-naïve, pediatric Crohn’s patients

All microRNAs

- ▲ Non-IBD
- ● CD
Index biopsies from FFPE samples from treatment-naïve, pediatric Crohn’s patients.

All microRNAs
miR-31 elevated in epithelial cells

P < 0.0001

Nev Kazgan
Patient-derived colonoids/enteroids

**Functional Readouts**
- **miR-31**
- **Cell types**
- **Proliferation**
- **Junctional proteins**

**qRT-PCR**
- **miR-31**
- **Cell types**
- **Proliferation**
- **Junctional proteins**

**miR-31 overexpression**

**miR-31 knockdown**

STEM → DIFFERENTIATION
basal condition || Immune cell Co-culture || TNF-alpha

**TEERs**
- Barrier function

**Immunohistochemistry**
- **miR-31**
- **Cell types**
- **Proliferation/Edu assay**

Jasmine Barrow
miR-31 expression kinetics in patient-derived colonoids

![Graph showing miR-31 expression kinetics in patient-derived colonoids over crypts and days 2 and 6. The graph indicates a significant difference in expression levels, with P < 0.05.]
Summary

• Two molecular subtypes
  – mRNA, chromatin, IncRNAs, tDRs, microRNAs
  – Clinically relevant
  – Adult and pediatric patients

• miR-31 stratifies subtypes
  – Serum, FFPE samples
  – Intestinal epithelial cells
  – Patient-derived colonoids
Future Directions - subtypes

• More patient samples, molecular assays to define more subtypes
  – NIDDK funded R01 - adults
  – Tissue and individual cell types

• Microbial sequencing

• Pediatric patients

• Mechanistic studies
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