Genetics and Inflammation – a Paradigm for Complex Disease

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Can we develop models for diagnosis?

- **Classical diagnostic tools**
  - Clinical phenotype
    - history
    - investigations – chemistry, haematology
  - Endoscopy – appearances, mapping sites of disease
  - Biopsy - histopathology

- **New diagnostic tools**
  - Immunology - Biopsy – subtle and unusual inflammation
  - Genetics
  - Microbiome
  - Serology & Faecal Biomarkers
Chronic Gut Syndromes

- Inflammation is key to symptom generation in complex chronic gut syndromes
- IBS/PI-IBS
- MC
- IBD
- DD
- Common symptoms
- Recognising overlap
- But different inflammatory responses...
- Phenotypes may depend on Genotype, Epigenetics and Environment
EARLY LIFE
DIET
STRESS
ALLERGY

DYSBIOSIS
Post and Current infection

MOST COMMON FOOD ALLERGENS
TREE NUTS
SOY
FISH
PEANUTS
SHELLFISH
EGGS
WHEAT
DAIRY
Inflammatory bowel disease and irritable bowel syndrome: similarities and differences

Curr Opin Gastroenterol. 2014;30:352-8

Giovanni Barbara, Cesare Cremon, and Vincenzo Stanghellini

The Functional–Organic Dichotomy: Postinfectious Irritable Bowel Syndrome and Inflammatory Bowel Disease–Irritable Bowel Syndrome

CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2009;7:48–53

MADHUSUDAN GROVER,*† HANS HERFARTH,*∥ and DOUGLAS A. DROSSMAN*

Changing views on diverticular disease: impact of aging, obesity, diet, and microbiota

R. C. SPILLER

Neurogastroenterol Motil (2015) 27, 305–312
Pathophysiology and Therapeutic Strategies for Symptomatic Uncomplicated Diverticular Disease of the Colon

Eleonora Scaioli¹ · Antonio Colecchia¹ · Giovanni Marasco¹ · Ramona Schiumerini¹ · Davide Festi¹


**SUDD**

- F = M
- Age > 60 years
- Less frequent episodes of abdominal pain (months-years)
- Abdominal pain > 24 h
- Possible history of acute diverticulitis

**IBS**

- F > M
- Age 30-40 years
- More frequent episodes of abdominal pain (every 2-3 days)
- Abdominal pain < 24 h
- Roma III criteria

Abdominal pain
Discomfort
Bloating
Altered bowel habits
Model of overlap IBS/IBD
Distinguishing IBD, IBS, MC & DD overlap or truly different??

- Immunology - Biopsy – subtle and unusual inflammation
- Genetics – which genes are useful markers?
- Microbiome – can we measure alterations?
- How do these link to give rise to different outcomes of disease?
- In these diseases, cause unknown – associations (e.g. drugs in MC) – likely genetic predisposition, epigenetics & microbiome constitution leads to different inflammatory phenotypes expressed in mucosa
Different pathologies

• Obvious
• Subtle
• Clinicopathological correlation – let your pathologist know what is troubling the patient!
• ?IBD ?IBS ?MC, did you see DD?
• We need to know what to look for!
• Biopsy of different sites
Site of biopsy

- Coeliac disease
- IBD
- IBS
- MC
- DD

Please take enough biopsies to make a diagnosis
Histopathology phenotype

IBS

IBD

DD

MC

CrD

UC

DD - associated IBD-like colitis/segmental colitis associated with diverticulitis SCAD
If histopathology is not helpful...

- Other useful strategies?
- Non invasive tests
- Genetics and epigenetics
- Microbiome
- Biomarkers
**Genetics**

**New IBD genetics: common pathways with other diseases**

C W Lees,¹ J C Barrett,² M Parkes,³ J Satsangi¹

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**Figure 2** Inflammatory bowel disease susceptibility loci. The loci (depicted by lead gene name) attaining genome-wide significance ($P<5\times10^{-8}$) are shown for Crohn's disease (red), ulcerative colitis (blue) and IBD (black where $p<5\times10^{-8}$ in Crohn's disease (CD) and ulcerative colitis (UC); red where $p<5\times10^{-8}$ in Crohn's disease and $<5\times10^{-4}$ in ulcerative colitis; blue where $p<5\times10^{-8}$ in ulcerative colitis and $<5\times10^{-4}$ in Crohn's disease).

*Gut* 2011;60:1739—1753. doi:10.1136/gut.2009.199679
Numerous IBD susceptibility loci have been identified, but how genetics influence disease biology and how this information can translate to clinical practice needs further investigation.

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


34 819 patients

The genetic risk score representing all known risk alleles for inflammatory bowel disease showed strong association with disease subphenotype ($p=1.65 \times 10^{-78}$), even after exclusion of NOD2, MHC, and 3p21 ($p=9.23 \times 10^{-18}$).

Predictive models based on the genetic risk score strongly distinguished colonic from ileal Crohn’s disease. Location is a fundamental biological aspect of a patient’s disease, whereas behaviour (like surgery or treatment history) is a marker of disease progression.
The goal of integrating molecular and serological markers is very exciting but is premature at the present time.

TNFSF 15
Tumour necrosis factor superfamily 15

- T-cell receptor gene involved in T-cell maturation
- Polymorphism of this gene overexpressed in CD +/- UC
- As in CD, IBS - degree of heritability - family clusters
- A potential role for TNFSF15 in IBS patients - exaggerated response to infection and immune activating stimuli which results in IBS.

- SNP rs7848647 associated with TNFSF15 gene - associated with diverticulitis requiring surgery
- This SNP may be a marker of diverticular disease severity that might assist in surgical decision making.

Environmental Factors

- Fiber
- Aging
- Constipation

Genetic Factors

- Collagen
- Vascular Defect

Normal Colon → Diverticulosis → Diverticulitis

Gut Bacteria

Obstruction Ischemia Perforation

Surgical Diverticulitis

Immune Defect

Immune Compromise
Identification of genetic variants may define a specific disease phenotype to follow clinical progression to develop new targeted therapies.

However, the evidence that genetic factors contribute in small part to disease pathogenesis confirms the important role of microbial and environmental factors.
• Successful translation of genetic advances to clinical practice requires understanding of intermediate phenotypes/biomarkers
  – to measure disease activity
  – pathophysiologic mechanisms
  – therapeutic response
• Epigenomics could provide new insight into the pathogenesis of IBD.
• The role of miRNA in IBD represents a new pathway for discovery of disease mechanisms, diagnostics, and therapeutics.
Microbiome - PI-IBS

Walkerton Health Study May 2000

- Municipal water supply contaminated with *Escherichia coli* 0157:H7, *Campylobacter jejuni*
- Acute GE >2,300 residents.
- 36.2% -> PI-IBS, 2-3yrs ff exposure
- 21.4% @ 4yrs, 14.3% @ 6yrs, 8.4% @ 8yrs
- *CDH1, IL6 & TLR9* gene variants independent risk factors for PI-IBS
- Vigorous innate immune response with inefficient downregulation following infection
- **Epithelial barrier function, innate response to bacteria...**


Villani et al. GASTROENTEROLOGY 2010;138:1502-1513
Microbiome – Current Infection

Colonic Spirochetosis is Associated with Colonic Eosinophilia and Irritable Bowel Syndrome in a General Population in Sweden

- The prevalence of CS in a general population is 2%
- There was a threefold increased risk of IBS in CS (OR 3.59, 95% CI 1.27-10.11, p= 0.015)
- Colonic eosinophilia with lymphoid follicles may signify the presence of CS.

Walker MM, Talley NJ et al
Hum Pathol. 2015 Feb;46(2):277-83
<table>
<thead>
<tr>
<th>Microbial composition</th>
<th>Decrease in α diversity</th>
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<tr>
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<td>Decrease in <em>Bacteroides</em> and <em>Firmicutes</em></td>
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<td>Increase in Gammaproteobacteria</td>
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<td>Presence of <em>E.coli</em>, specifically adherent-invasive <em>E.coli</em></td>
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<td>Presence of <em>Fusobacterium</em></td>
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<td>Decrease in Clostridia, Ruminococaceae, <em>Bifidobacterium</em>, <em>Lactobacillus</em></td>
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<td>Microbial function</td>
<td>Decrease in <em>F. prausnitzii</em></td>
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<td>Decrease in SCFAs, butyrate</td>
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<td>Decrease in butanoate and propanoate metabolism</td>
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<td>Decrease in amino acid biosynthesis</td>
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<td>Increase in sulfate transport</td>
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<td>Increased oxidative stress</td>
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<td>Increase in type II secretion system, secretion of toxins</td>
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Microbiome and Biomarkers

• How the microbiome orchestrates gut architecture is evolving

• Microbial-derived metabolites can initiate epigenetic changes that influence gut homeostasis

• Some of these can be measured

• Soubières et al World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 41-50
• Tursi A. Biomarkers in diverticular diseases of the colon. Dig Dis. 2012;30:12-8
Koslic A et al

Subjects

Samples

Sample handling

Assays

Microbe: 16S rRNA gene profiling
Microbe: Metagenome sequencing
Microbe: Metatranscriptome seq
Microbe: Metabolite profiling
Microbe: Single-cell assays
Microbe: DNA & RNA virome
Microbe: Proteomic profiling
Host: Fecal calprotectin test
Microbe: 16S rRNA gene profiling
Separate microbes and Meta’omic seq
Host: Transcriptome sequencing
Host: Epithelial cell profiling
Host: Bisulfite sequencing (RRBS)
Host: SNP profiling
Host: Serological profiling

Host
Microbial

Covariates to control for:
- Host genome
- Disease activity
- Treatment details
- Diet info
- Age, gender, race
- Sample type, collection, extraction

Stool samples (self collected)

Biopsies

Blood samples
Biomarkers

- Metabolomics, proteomics
- Stool
  - Faecal calprotectin
  - Faecal lactoferrin - activity
- Blood
  - ESR, CRP, ASCA/pANCA
- There is no biomarker reliable enough to make a confident diagnosis of IBD without going on, in the case of a positive test, to perform confirmatory colonoscopy.
- Soubières A et al World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 41-50
Chronic gut syndromes - Diagnosis

- Clinical presentation and history
- Symptoms - pain, diarrhoea / disordered bowel habit
- Endoscopy and biopsy
- Mucosal immune response – subtle pathologies
- Genetics – TNFSF 15, IBD/IBS/DD
- Epigenetics, Microbiome – reduced diversity in IBD, CS
- Biomarkers, serology and stool – measures of activity
Multifactorial model of development of diverticulosis and symptomatic diverticular disease

Genetics
- Genetic predisposition
- Ageing
- Smoking

Epigenetics
- Weakening of colonic wall

Microbiome
- Impairment of epithelial barrier
- Faecolith impaction
- Microbiota

Inflammation
- Post inflammatory hypersensitivity

Development of diverticulosis

Epigenetics
- Neural degeneration
- Altered receptor sensitivity
- Circular/longitudinal muscle thickening

Diet

OUTCOME
- Diverticulitis
- Mucosal inflammation
- Chronic gut syndromes
- Environment & Microbiome

Spiller RC
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