Gut Microbial Signatures Underline Complicated Crohn’s Disease but Vary Between Cohorts; An In Silico Approach

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Inflammatory Bowel Diseases, Volume 25, Issue 2, February 2019, Pages 217–225
Introduction

IBDs exhibit significant heterogeneity

CD: Montreal subclassification defines different subphenotypes

• age at diagnosis (A1 <17, A2 >17–<40, A3 >40 years of age)
• location (L1 ileal, L2 colonic, L3 ileocolonic, L4 isolated upper disease modifier)
• behavior (B1 nonstricturing, nonpenetrating, B2 stricturing, B3 penetrating, p perianal disease modifier)

Crucial need for identification of factors than can predict complicating behavior

Satsangi J et al, 2006
Introduction

**Gut microbiome**: the collective genomes of the microbes located in the gut

Extensive research linking **dysbiosis in the gut microbiome** to the **pathophysiology of host** functions and phenotypes:

→ atherosclerosis and cardiovascular disorders\(^1\), diabetes, metabolic and antiphospholipid syndromes\(^2\), cancer\(^3\), chronic kidney disease\(^4\), neurological\(^5\) and neuropsychiatric\(^6\) disorders

**Existence of dysbiotic microflora**, which implies a functional role of the microbiome in the **pathogenesis of IBD**\(^7\).

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Hypothesis and Aim of the Study

**Hypothesis:** There are different taxonomic and metabolic microbial signatures among the B1, B2, and B3 subphenotypes of CD; the microbiome is associated with disease behavior.

**To test our hypothesis:** We utilized and compared results from 2 publicly available data sets (data set 1\(^1\) and data set 2\(^2\))

**Aim:** To study the microbiome with regards to disease behavior in 2 data sets generated years apart, under different methods, in patients of different ethnicities and locales, different lifestyles and dietary habits, and undergoing different, if any, treatments.

Results

Data selection

Analysis
FIGURE 1. \( \alpha \)-diversity

- Overall decreased in Crohn’s disease compared with healthy controls
- More pronounced dysbiosis in the stricturing B2 and penetrating B3 subphenotypes

\( \alpha \)-diversity definition: an estimate of within-sample diversity, how diverse the microbiome of each sample is.
The B2 and the B3 cohorts were co-localized in the same area for both data sets.

B1 and HC were dispersed over an area different than that of B2 and B3.

**β-diversity definition:** the explicit comparison of microbial communities of different samples.
• Complicated disease has similar enterotypes
• Differs from B1 and healthy controls
Supplementary Figures 4 and 5. Relative abundances of microbial families

Relative Abundance of Microbial Families (ANOVA, CSS+log2)

Dataset 1
- Faecalibacteriaceae
  p-value: 2.3e-10
- Lachnospiraceae
  p-value: 7.8e-12
- Peptococcaceae
  p-value: 5.0e-07
- Rikenellaceae
  p-value: 5.0e-11

Dataset 2
- Bacteroidales
  p-value: 5.0e-10
- Clostridiales
  p-value: 1.0e-13
- Enterobacteriaceae
  p-value: 1.0e-17
- Christensenellaceae
  p-value: 1.0e-13

Relative Abundance of Microbial Families (ANOVA, log2)

Dataset 1
- Lachnospiraceae
  p-value: 1.0e-06
- Enterobacteriaceae
  p-value: 1.0e-07
- Peptococcaceae
  p-value: 1.0e-16

Dataset 2
- Unord. Lachnospirales
  p-value: 1.0e-11
- Netbacteriales
  p-value: 1.0e-16
- Enterobacteriales
  p-value: 1.0e-12
- Desulfovibrionales
  p-value: 1.0e-13
- Campylobacterales
  p-value: 1.0e-07
Supplementary Figure 3. Relative abundances of microbial taxa

Microbial Family Abundance

Taxa of Dataset 1 identified by the rank test of Dataset 2
- Pasteurellaceae (p=0.039)
- Desulfobacteraceae (p=0.041)
- Lactobacillaceae (p=1.5e-12)
- Rikenellaceae (p=1.4e-16)

Taxa of Dataset 2 identified by the rank test of Dataset 1
- Barnesiellaceae (p=1.2e-07)
- Lactobaccilaceae (p=1.2e-15)
- Peptococcaceae (p=0.1)
- Actinomycetaceae (p=4.5e-06)
- Odoribacteraceae (p=8e-08)
- Enterobacteriaceae (p=3.2e-06)
- Fusobacteriaceae (p=5e-09)
- Christensenellaceae (p=2.9e-08)

B2 and B3 subphenotypes have similar enterotypes
FIGURE 4. Spearman’s correlation coefficient of genera to CD subphenotypes and HC

- **B2** and **B3** subphenotypes had similar microbial signatures
- **Distinct** from those of **B1** and healthy controls

Specific taxa had **a strong positive correlation** with the B2 and B3 subphenotypes cluster and subsequently **a strong negative correlation** with the B1 and HC cluster and vice versa.

Color pallet gradient: positive (red) to negative associations (blue).
Several taxa show similar associations to CD subphenotypes, and thus further support the distinct microbial signature of B2 and B3 vs B1 and healthy controls:

Could potentially serve as universal biomarkers of CD subphenotypes
Similar metabolic patterns for the B2 and B3 subphenotypes as opposed to the B1 subphenotype.

A) **aLAM**: alpha-linolenic acid metabolism, **BIEC**: bacterial invasion of epithelial cells, **PEcI**: pathogenic *Escherichia coli* infection, **Sh**: Shigellosis, **PKII**: biosynthesis of type II polyketide products

B) **PTS**: Phosphotransferase system, **DD**: Dioxin degradation, **XD**: Xylene degradation, **GLM**: Glycerolipid metabolism, **CCD**: Chloroalkane and chloroalkene degradation, **Germ**: Germination
Conclusions

• Using a bioinformatics approach and publicly available clinical data: we highlighted that behavioral subphenotypes of CD (B1, B2, and B3) are characterized by unique microbial signatures.

• B1 subphenotype’s microbiome is on a different plane from the microbiomes of the complicated B2 and B3 subphenotypes.

• In this work, we suggest that markers of dysbiosis, such as reduced biodiversity, distinct enterotypes, and predicted metabolotypes, correlate with complicated behavior in CD.
Thank you!