Immune-Microbiota Crosstalk Underlying Inflammatory Bowel Disease

Congmin Xu[1], Quoc D. Mac[1], Qiong Jia[2], and Peng Qiu[1(✉)]

[1] The Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Georgia, USA
peng.qiu@bme.gatech.edu

[2] The Department of Pediatrics, Peking University Third Hospital, Beijing, China

Abstract. Long-time evolution has shaped a harmonious host-microbiota symbiosis consisting of intestinal microbiota in conjunction with the host immune system. Inflammatory bowel disease (IBD) is a result of the dysbiotic microbial composition together with aberrant mucosal immune responses, while the underlying mechanism is far from clear. In this report, we creatively proposed that when correlating with the host metabolism, functional microbial communities matter more than individual bacteria. Based on this assumption, we performed a systematic analysis to characterize the co-metabolism of host and gut microbiota established on a set of newly diagnosed Crohn's disease (CD) samples and healthy controls. From the host side, we applied gene set enrichment analysis on host mucosal proteome data to identify those host pathways associated with CD. At the same time, we applied community detection analysis on the metagenomic data of mucosal microbiota to identify those microbial communities, which were assembled for a functional purpose. Then, the correlation analysis between host pathways and microbial communities was conducted. We discovered two microbial communities negatively correlated with IBD enriched host pathways. The dominant genera for these two microbial communities are known as health-benefits and could serve as a reference for designing complex beneficial microorganisms for IBD treatment. The correlated host pathways are all relevant to MHC antigen presentation pathways, which hints toward a possible mechanism of immune-microbiota cross talk underlying IBD.

Keywords: IBD · Microbial symbiont · MHC · Co-metabolism

1 Introduction

The inflammatory bowel disease (IBD) known as Crohn's disease (CD) and ulcerative colitis are a result of accumulating alterations in intestinal microbiota and disorders of the immune system. However, the mechanisms leading to the chronic mucosal inflammation that characterize IBD are ambiguous. There has been a dramatic increase of metagenomic and metabolomic studies of IBD

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in the past decades [1] aiming to characterize IBD from host metabolic activities and the accompanied microbial dysbiosis. Studies aiming to understand the host pathways involved in IBD initiation have revealed that IBD are strongly associated with the immune system, including antigen processing and presentation pathways linked with major histocompatibility complex (MHC)[2] Antigen presentation by intestinal epithelial cells (IEC) is crucial for intestinal homeostasis. Disturbances of MHC I- and II-related presentation pathways in IEC are involved in an altered activation of CD4+ and CD8+ T cells in IBD [3]. From the microbial side, current literature has clearly demonstrated a perturbation of the gut microbiota in IBD patients [4]. Gevers et al. linked alterations in mucosal-associated microbiota with CD status using metagenomic analysis [5]. A meta-analysis reported 467 out of 536 patients with CD (87%) experienced resolution of diarrhea after fecal microbiota transplant treatment [6], which proved the significance of microbial dysbiosis in CD patients.

The microbes inside the human gut often have correlated functions, and can be aggregated into different functional communities that are able to dynamically respond to or modulate the host metabolic activities [7]. When correlating with the host metabolites, the functional communities of microbes matter more than the relative abundance of individual microbes [8]. We proposed to apply community detection algorithm on the microbial composition of human gut to identify microbial communities and then cross-link these communities with gene pathways enriched by IBD-associated genes. With this approach, genera often reported as beneficial, such as *Bacteroides*, *Blautia*, *Faecalibacterium* and *Propionibacterium*, are revealed as negatively interacting with the those host immunological pathways enriched in IBD patients, especially those relevant to MHC presentation.

2 Materials and Methods

2.1 Sample and Data Description

We retrieved data for 21 subjects with both 16S rRNA sequencing data of mucosa-luminal interface (MLI) microbiota and proteome data of colon or ileum from a previous study [9], including 11 Crohn's disease patients and 10 healthy controls (Table 1). These Crohn's disease samples represent new-onset teenagers, so there are no treatment influence and few co-morbidities compared with samples from adults. More details about the sampling and sequencing technologies could be found in the reference [9].

Table 1. Sample information

Groups	Number	Age	Male	Female
Healthy controls	10	14.25 ± 2.70	6	4
Crohn's diseases	11	13.3 ± 2.92	6	5

2.2 Annotation of the 16S rDNA Sequencing Data of the Mucosa-Luminal Interface Microbiota

The 16S rDNA sequencing data of the MLI microbiota were processed in a standard pipeline [10]. Raw reads were downloaded from NCBI with accession code SRP056939 [9]. Read quality control was conducted by applying FastQC. Those high-quality reads passing quality controls were converted into fasta format and imported into QIIME using QIIME import command. Duplications were removed for speeding up the annotation process. Dereplicated contigs were clustered into operational taxonomic units (OTUs) using a closed-reference OTU picking workflow against the Greengenes 16S rRNA gene database (version gg-13-8) based on an average percentage of identity 0.97, after which a set of representative sequences and an OTU relative abundance (proportion) matrix were obtained. A taxonomic annotation was assigned to the representative sequence of each OTU using classify-sklearn of QIIME. By summing up the abundance of OTUs assigned to the same genus, a taxonomic abundance matrix can be obtained on genus level.

2.3 Microbial Community Detection

The microbes inside human gut aggregate into different communities for functional purposes. When analyzing crosstalks with the host metabolic pathways, considering microbes in the same community as a whole is likely to shed new light on the interaction mechanism between microbiota and host pathways. In order to identify microbial communities, we first calculated a pairwise similarity matrix for all OTUs. The similarity was quantified using the correlation between each pair of OTUs regarding their relative abundance across all samples. In order to make sure the microbes in the same community correlate with each other in the same direction and also exclude spurious correlations induced by the unit-sum constraint, only positive correlations were kept while negative correlations were set as zeros. Furthermore, weak and insignificant correlations (i.e., correlation coefficient $|R| < 0.2$ or p-value $P > 0.05$) were discarded and set as zeros. Once the similarity matrix was generated, Louvain community detection algorithm [11] was applied on it to identify OTU clusters. 12 OTU clusters were identified, and each cluster of OTUs was considered as one microbial community.

We then defined the level of activity for each microbial community. Since only positive correlations were considered during the community detection, the cross-sample alteration of OTUs in the same microbial community are in the same trend. The easiest way to quantify the level of community activity is summing up the relative abundance of all OTUs in each OTU cluster/community.

2.4 Proteome Data of the Host Tissue (Human Colon or Ileum)

For the same set of 21 subjects with 16S rDNA sequencing data of MLI, the biopsies of their colon or ileum were profiled by mass spectrometry to characterize their proteome. We retrieved the relative abundance matrix of 3,861 proteins/genes from a public data source released by Mottawea et al. [9].

2.5 Gene Set Enrichment Analysis

IBD enriched gene pathways were identified by applying Gene Set Enrichment Analysis (GSEA) [12] using gene sets in the KEGG database [13] as the reference database. Genes were sorted according to their fold change, and the fold change (FC) for gene i was defined as

$$FC_i = \frac{\frac{1}{N_C}\sum_{j=1}^{j=N_C} C_{ij} - \frac{1}{N_H}\sum_{j=1}^{j=N_H} H_{ij}}{\frac{1}{(N_C+N_H)}(\sum_{j=1}^{j=N_C} C_{ij} + \frac{1}{N_H}\sum_{j=1}^{j=N_H} H_{ij})} \quad (1)$$

where, N_C is the total number of samples in the Crohn's group and N_H is the total number of samples in the group of healthy control. C_{ij} and H_{ij} are the relative abundance of gene i in the j^{th} Crohn's sample and j^{th} healthy sample, respectively. When performing GSEA, the number of permutations was set as 1000, the minimal gene set size was set as 20, and the cutoff for p-value was set as 0.05 .

2.6 The Activeness of Each Gene Pathway

The activeness of a metabolic pathway can be quantified by the expression levels of genes in the pathway. The simplest idea for calculating the activeness of a pathway is to compute the average of gene expression levels in this pathway. However, genes in the same gene pathway may be positively correlated but may also be negatively correlated. Therefore, when computing the simple average within a gene pathway, the negatively correlated genes will cancel out each other. As an alternative approach, principle component analysis (PCA) was adopted here. PCA performs dimension reduction by linearly combining the genes/features to derive principle component scores that maximally preserve the variance. In a gene pathway where a large portion of genes are correlated, the first principle component score is typically dominated by a weighted combination of the correlated genes, where the signs of the weights are able to avoid the cancelling effect due to negative correlations among genes in the pathway. Therefore, operationally, given the gene list for a gene pathway, a sub-matrix containing the relative abundance of these genes was retrieved. The first principle component of the sub-matrix was used to represent the overall activeness of this pathway.

3 Results

As shown in Fig. 1, based on the proteome data of host colon and ileum, our approach aims to identify gene pathways significantly enriched by those genes associated with the IBD condition. In parallel, our approach takes the taxonomic composition of intestinal microbiota, and identifies microbial communities. After that, the correlations between gene pathways and OTU communities are examined to discover OTU communities that are closely linked with IBD-enriched pathways.

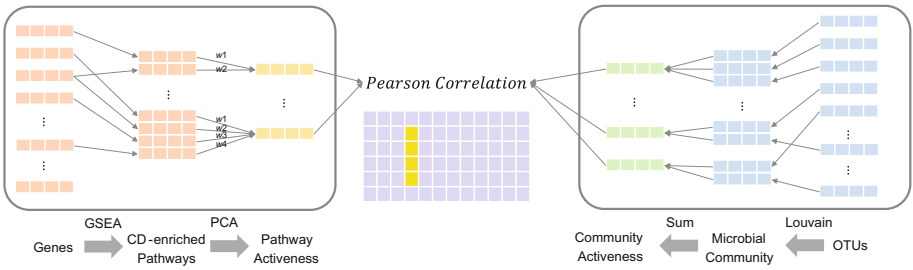

Fig. 1. Schematic diagram of the analysis pipeline. The left side shows the procedure of identifying host pathways and calculating their activeness. Genes were sorted according to their fold change of expression levels in CD *vs* healthy controls. Then GSEA identified those KEGG pathways significantly enriched/depleted in CD patients. Then the activeness of these KEGG pathways were calculated using PCA analysis as described in the Material and Method section. The right side of Fig. 1 illustrated how microbial OTUs were aggregated into different communities. The activeness of each microbial community was calculated by simply summing up the relative abundance of every OTU in that community. Finally, for each combination of host pathway and microbial community, a Pearson Correlation was calculated based on their activeness. Significant correlation implied a strong interaction between host metabolic pathways and activities of those bacteria in the corresponding microbial community.

3.1 Pathways Enriched in IBD Patients

Several metabolic pathways were identified as significantly enriched or down-regulated in IBD patients compared to the healthy controls. Using the KEGG pathway database as reference, we performed GSEA and identified 17 KEGG pathways as significantly enriched (as shown in Fig. 2). Among these 17 KEGG pathways, five pathways are involved in virus infection, i.e., Epstein-Barr virus infection, Herpes simplex virus 1 infection, Measles, Hepatitis B and Influenza A; two pathways are related to bacterial infection, i.e., Tuberculosis and Staphylococcus aureus infection; one pathway is associated with Toxoplasmosis, which is also an infectious disease. These infectious diseases are all linked with disordered immune responses [14]. The other nine pathways are also relevant to immune response. NOD-like receptor (NLR) signaling pathway mediates the production of pro-inflammatory cytokines. NLR together with inflammatory factors enhance the body's inflammatory response and antimicrobial infection [15]. Pathway Complement and coagulation cascades, and pathway Antigen processing and presentation are well known as part of immune system. Pathway Phagosome is linked to abnormal immune response. Transcriptional misregulation in cancer is a NF-kappa B related pathway. Osteoclast differentiation is mainly regulated by signaling pathways activated by immune receptors. Systemic lupus erythematosus is an autoimmune disease. IL-17 signaling pathway is mainly involved in mucosal host defense mechanisms. The IL-17 family signals via their correspondent receptors and activates downstream pathways that include NF-kappaB, MAPKs and C/EBPs to induce the expression of antimicrobial peptides, cytokines and chemokines.

Eight of the 17 identified KEGG pathways were indicated as infectious diseases, and these eight pathways cover a broad range of biological processes. To identify the key effectors of the metabolic alterations in these pathways, we used the Hallmark gene sets in MsigDB [16] as the reference database to perform another set of GSEA analyses. Six pathways were significantly enriched by genes associated with IBD, i.e., Complement, Interferon gamma response, Allograft rejection, Coagulation, Interferon alpha response, and TNFA signaling via NFKB. These pathways points to the up-regulation of adaptive immune responses during IBD, which is consistent with what we found in the KEGG pathway analyses, and confirms our previous conjecture that these eight KEGG pathways were related to infectious diseases, indicating alterations of the immune system.

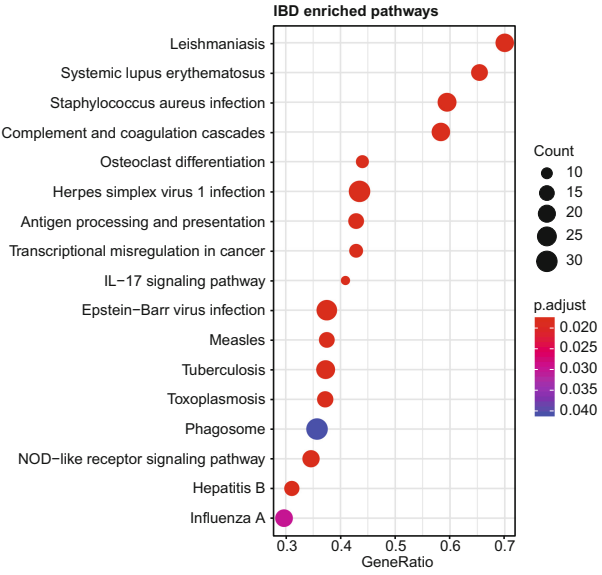

Fig. 2. Pathways enriched in CD patients. These 17 pathways were identified through GSEA with KEGG database as reference. The size of each dot represents the number of genes in each gene set and the adjusted P values of testing enrichment significance were illustrated using different colors as shown in the color bar.

3.2 OTU Communities Within Human Gut

Stintzi and his colleagues reported significant OTUs as those negatively correlated with the severity of host suffering IBD. In contrast, our analysis takes a different perspective. We proposed to examine microbial communities, which are aggregated by multiple OTUs. Before being manifested in disease severity, alterations in the human gut microbiota first interact with the host metabolism. Instead of individually interacting with the host metabolism, different microbes

share common set of metabolic activities aggregated into functional microbial communities. After taxonomic binning of all high-quality raw reads of whole-genome-sequenced human gut microbiota, we searched for microbial communities based on pairwise correlations between OTUs. The correlations were quantified using Spearman correlation coefficients, and the OTU communities were identified using Louvain community detection algorithm. Overall, 12 OTU communities were discovered. Different OTU communities were dominated by different genera, and the genera in the same community are supposed to participate in the same sets of metabolic pathways.

3.3 Multiple Health Beneficial Genera Are Negatively Correlated with Inflammation-Relevant MHC Pathways

As described in the Material and Methods section, the correlation between microbial OTU communities and host metabolic pathways could be computed based on the activeness of each OTU community and host metabolic pathway. Two out of the 12 OTU communities (OTU community number 2 and number 7) were identified to be negatively correlated with seven Crohn-enriched host metabolic pathways (Pearson correlation with correlation coefficient $|R| > 0.4$ and the correlating significance test p-value $P < 0.05$) (Fig. 3).

By counting the occurrences of different genera in these two OTU communities, the dominant genera were found to be beneficial ones. Nine most dominant genera (assigned to > 5 OTUs) these two OTU communities affiliated to include *Bacteroides*, *Blautia*, *Clostridium*, *Dorea*, *Faecalibacterium*, *Propionibacterium*, *Prevotella*, *Ruminococcus* and *Parabacteroides*. Out of these nine dominant genera, five genera *Blautia*, *Roseburia*, *Ruminococcus*, *Clostridium* and *Faecalibacterium* were reported as negatively correlated with IBD severity in the paper where we obtained the raw data [9], which supported our findings here. Comprehensive literature review of these nine dominant genera advanced our understanding about the metabolic roles of these genera and provided evidence of the health beneficial roles of these genera. *Bacteroides* has been shown to have the ability to influence the host immune system and inhibit the activities of other competing pathogens [17]. *Blautia* is associated with the remission of IBD and one of the most important features characterizing disease activity levels in pediatric IBD patients [18]. *Clostridium* spp. takes colonization resistance in the mucosa and plays an important role in host immune response, and is one of those strong inducers of colonic T regulatory cell (Treg) accumulation [19]. *Dorea* genus has also been reported to play an important role in host immune system activity [20], suggested by an elevated abundance in patients with an autoimmune condition. As a butyrate-producing genus, *Faecalibacterum* are decreased in Crohn's diseases compared to healthy controls [21]. Species in genus *Propionibacterium* have been shown to display promising immunomodulatory properties and anti-inflammatory effects via interacting with surface proteins [22,22,23]. *Prevotella* was associated with T helper type 17 (Th17) immune response, which can be beneficial to the host during infection [24]. *Ruminococcus* species *R. albus*, *R. callidus*, and *R. bromii* are less abundant in patients with IBD compared to the

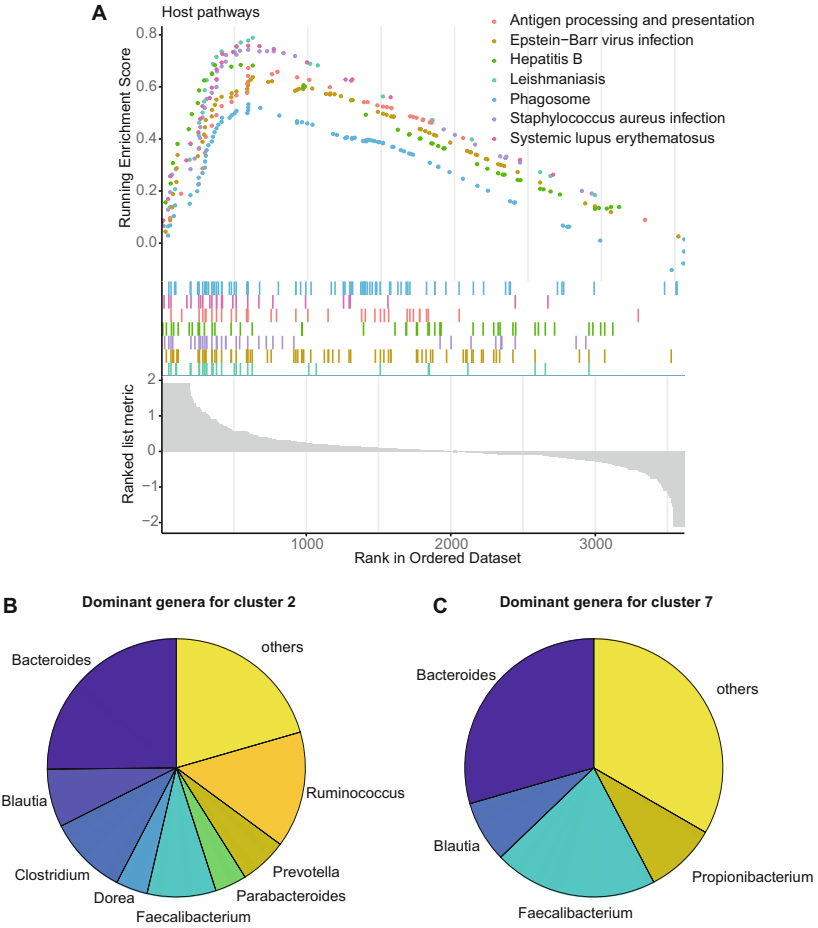

Fig. 3. Two microbial communities were closely correlated with seven immune related host pathways. A, Those seven host pathways enriched in CD samples and also correlated with the microbial communities. On the bottom of plot A, each vertical line represents the fold change of one gene regarding gene expression levels in CD vs. healthy controls. A positive value indicates this gene is more abundant in CD, otherwise more abundant in healthy controls. All genes were sorted in a descending order of fold changes. For each host pathway, an enrichment score is calculated based on the fold changes of those genes emerging in this pathway. A positive enrichment score indicates that pathway is up-regulated in CD, vice versa. B, Dominant genera for those two OTU communities closely correlated the host pathways. Only those genera with more than five OTUs affiliated with were illustrated. The area size on pie plot represents the number of OTUs assigned to that genus.

healthy controls [25]. Furthermore, *Prevotella*, *Parabacteroide*, *Bacteroides*, *Faecalibacterium* and *Clostridium* have been shown to have increased abundance in healthy controls compared to multiple sclerosis patients, which further proved

the immunomodulatory role of these genera [26,26–29]. Giri et al. also reported *Prevotella*, *Parabacteroides*, *Clostridium*, and *Adlercreutzia* as part of the anti-inflammatory symbionts [30].

Seven Crohn-enriched host metabolic pathways were correlated with the alteration of the microbial communities. These seven KEGG pathways are Leishmaniasis, Epstein-Barr virus infection, Staphylococcus aureus infection, Hepatitis B, Antigen processing and presentation, Systemic lupus erythematosus and Phagosome. Literature review of these seven pathways led to an interesting finding that they are all relevant to MHC processing and presentation pathways. Leishmaniasis was reported to be associated with the defective expression of MHC genes, which silences subsequent T cell activation mediated by macrophages, resulting in abnormal immune responses [14,14,31]. MHC class II was observed to be induced following Epstein-Barr virus infection [32,32,33]. Staphylococcus aureus expresses an MHC class II analog protein (Map), which influences the immune response of T cells [34]. MHC class I-related chain A (MICA) was induced after HBV infection compared with the uninfected control [35]. Antigen processing and presentation is closely relevant to EBV and MHC presentation. Systemic lupus erythematosus is closely linked with the MHC relevant pathways [36]. Bacterially derived antigens within the phagosome are closely linked with the MHC-I processing and presentation pathway [37].

4 Conclusions

In the literature, most studies on the host metabolism and the microbial community have been conducted separately. To the best of our knowledge, none of the previous studies correlated the host metabolism with microbes in a community manner. Here we explored a new analysis approach addressing the importance of microbial communities for the interplay between microbiota and host metabolic pathways. We identified two microbial communities of beneficial microbes that provide potential directions for developing beneficial microbes to treat IBD. Animal studies should be designed to test the influence of these beneficial microbes on host medical conditions, by transplanting the combination of these microbes to mucosal of IBD mouse.

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