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EDITORIAL

Analyzing the intestinal microbiome in inflammatory bowel disease: From RNA to multiomics

The evolution of methods for the analysis of the microbiome in the gastrointestinal tract is one of the major developments in medical science over the past two decades. Although the initial interest was in gastrointestinal disorders, the field has expanded exponentially to include endocrine disorders such as diabetes, metabolic disorders such as non-alcoholic fatty liver disease, and a variety of other disorders where the composition of the microbiome may influence immune responses, mental well-being, and psychiatric syndromes. For the gastroenterologist, the focus has largely been on the inflammatory bowel disorders (IBD), ulcerative colitis (UC) and Crohn's disease (CD). The hope was that microbiome analysis would identify an excess of pro-inflammatory bacteria or a deficiency of anti-inflammatory bacteria that could be corrected by diet or dietary supplements. Although important progress has been made, it is still unclear whether changes in the microbiome influence susceptibility to IBD or are secondary to the disease process. In addition, currently, there is no consensus on the efficacy or otherwise of dietary supplements.

The purpose of this editorial is to highlight the increasing sophistication of methods for the analysis of the intestinal microbiome by summarizing one article in this journal and three articles published elsewhere. It now seems unlikely that the pathogenesis of IBD involves a simple excess or deficiency of one or a small number of bacteria. A more likely explanation is a complex interplay between the microbiome, the mucosal immune system, and other factors in the intestinal environment.

In this issue of the journal, de Alencar *et al*.[1] examine the prevalence of four groups of bacteria in the feces of 54 CD patients and 51 healthy control subjects. They found that CD patients had elevated concentrations of the *Bacteroidete* phylum when compared to the control group and a reduction in *Bacilli* class and *Bifidobacteriaceae*. This finding demonstrates a significant decrease in the diversity of the microbiota in CD patients, especially with regard to *Bifidobacteriaceae*, one of the most abundant genera in the healthy gut.[2] Given the role that *Bifidobacteriaceae* plays in homeostasis of the gastrointestinal tract, it is perhaps not surprising to find a decrease in this family or that dysregulations of this family have previously been associated with several other conditions.[2] However, the increase in *Bacteroidete* in CD is discrepant from previous studies that have reported a depletion of this phylum in CD patients.[3]

De Alencar *et al*. utilized reverse transcriptase polymerase chain reaction (rt-PCR) of the 16s ribosomal RNA in their study, a longstanding and accessible technique for studying the microbiome of IBD. Using this technique, it is not possible to resolve bacterial populations at a species or subspecies level. In addition, by prespecifying the phyla of interest, it is not possible to accurately assess changes in abundance relative to the many other species that were not targeted. This bias may partially explain the, seemingly, contradictory results of *Bacteroidete* in CD. As *Bacteroidete* is a very large phylum, comprising 7000 different bacterial species,[4] it is not unexpected that conflicting results would arise when looking for associations at the phylum level. This is not to discount the importance of the results but to recognize that they should be used to guide further investigation.

In another study, Vich Vila *et al*., (2018)[5] used high-resolution shotgun metagenomic sequencing to assess the microbiomes of healthy volunteers, IBD patients, and patients with irritable bowel syndrome. Using these techniques, they were able to identify associations at a higher resolution, down to species and strain levels. First, they used these tools to develop a microbiome identification pattern for each condition, finding differences in 219 taxa that were associated with CD and 102 taxa that were associated with UC. Of these differentially regulated taxa, 87 were common to both conditions. Some of the species that were unique to ulcerative colitis were the complex sugar-metabolizing bacteria *Bacteoides uniformis*[6]and *Bifidobacterium bifidum*.[7] In contrast, patients with CD were found to have a decrease in bacteria such as *Faecalibacterium prausnitzii*, a butyrate-producing bacteria with anti-inflammatory properties, and *Bifidobacterium longum*, bacteria known to protect against infection by harmful bacteria. CD patients also had an increase in the *Enterobacteriacea* species, *Escheria* and *Shigella*, both known to cause damage to the gut epithelium.[5]

In addition to examining the abundance of bacterial species in IBD, the authors went on to investigate how disease states impact strain level diversity, finding 21 species in CD and 15 species in UC that had alterations in their strain level diversity.[5] Of note was a decrease in the strain diversity of *Faecalibacterium prausnitzii*, which, as mentioned above, also had a decrease in abundance. Conversely, *Roseburia intestinalis* showed a decrease in strain diversity but did not show a decrease in abundance. Furthermore, Vich Vila *et al*. used the metagenomic data to characterize the functional changes that had occurred in the gut due to the differential regulation of the microbiome in IBD with the use of the HUMAnN2[8](https://huttenhower.sph.harvard.edu/humann) data analysis pathway. This protocol found differences in microbial functions involved with the synthesis of amino acids, neurotransmitters, and vitamins, as well as negative impacts on the metabolic pathways used in the degradation of complex carbohydrates and the fermentation of short-chain fatty acids. The authors also noted an increase in the abundance of virulence factors in IBD patients compared to control. Based on homology to the Virulence Factor Database, 262 known microbial virulence factors saw an increase

Table 1 Summary of the findings of different methods of examining the IBD microbiome

Method	Principle	Conclusions in IBD
16s RNA sequencing	Sequences the 16s ribosomal RNA of bacteria, which permit identification and quantification of bacteria down to the genus level, depending on the sequence conservation in a given phylum.	↑ *Firmicutes* ↑ *Actinobacteria* ↓ *Bacilli* ↓ *Bifidobacteriaceae* ↕ *Bacteroidete* ↓ Microbiome diversity
Metagenomics	Using high-throughput methods, all DNA in a sample is sequenced. This identifies and quantifies bacteria at a species and even strain level and provides information about genes that reflect metabolic processes. In addition, this technique can be used to identify viruses, fungi, and protozoa. However, metagenomics is resource intensive.	↑ *Bacteroide* ↓ *Ruminococcus* ↓ *Roseburia hominis* ↑ *Streptococcus* ↑ *Clostridium bolteae, citroniae* ↓ *Clostridium aminophilum, asparagiforme, baratii, celatum, dakrense* ↓ *Bifidobacteriaceae* ↓ *Faecalibaterium prausntzii* ↓ *Faecalibaterium prausntzii* strain diversity ↓ *Roseburia* strain diversity ↑ *Enterobacteriacea. Escheria, Shigella*
Multiomics	Combines metagenomics with the analysis of other biological data such as proteomics, metabolomics, or transcriptomics. This multilateral approach can directly measure not just the bacteria that are present or absent but also the processes that are occurring, providing information about how bacteria react in response to disease. However, this is a complex and resource-intensive undertaking.	↓Taxonomic diversity ↓*Roseburia hominis* (↑transcription) ↓*Dorea formicigenerans* ↓*Ruminiciccus obeuml* ↓*Ruminococcus gnavus* (↓transcription) ↓ *Faecalibaterium prausntzii* ↑ *Escheria coli* (↑transcription) ↑ Sphingolipids ↑ Primary bile acids ↓ Secondary bile acids ↓ Short chain fatty acids ↑ Acylcarnitines

in abundance such as mu-toxin and methyl-accepting chemotaxis protein.

The Vich Vila paper is an excellent example of utilizing metagenomic data to its fullest. However, even more information about IBD and other microbiome disorders can be gleaned by a "multiomics" approach, integrating the study of different pools of biological data such as metagenomics and metabolomics.[9] Franzosa *et al*., 2019[10] used this approach in their study of the metabolic activity and associated microbiome of IBD using both untargeted liquid chromatography-mass spectrometry and shotgun metagenomic sequencing. Their metagenomic data were largely in agreement with previous studies, finding a general decrease in taxonomic diversity in IBD with strong reductions in the species *Roseburia hominis*, *Dorea formicigenerans*, and *Ruminococcus obeumI*. Furthermore, their metabolomic data identified 2700 metabolites that were differentially abundant in IBD compared to healthy patients. Of particular interest was the elevation of sphingolipids and bile acids, which have previously been identified as playing a role in the pathogenesis of IBD.[2,11]

Taking multiomics even further, Lloyd-Price *et al*.[9] combined metagenomics with metabolomics and transcriptomics. Examination of the transcriptome and the metabolome permits measurements of the genes that are active in a given setting, providing a picture of not only bacterial abundance but also what processes these bacteria are undertaking and what pathways have been impacted. The metagenomic data from Lloyd-Price were in alignment with previous studies in IBD showing decreases in obligate anaerobes such as *Faecalibacterium prausnitzii* and *Roseburia hominis*, while there was enrichment of facultative anaerobes such as *E. coli*. Transcriptionally, *F.prausnitzii* accounted for some of the strongest relationships with down-regulated enzymatic transcripts, while *E.coli* accounted for a large fraction of the upregulated enzymatic functions. In addition, they found several species that were both differentially abundant and that had an increase in transcriptional activity relative to their abundance, such as *Clostridium bolteae* and *Ruminococcus gnavus*, suggesting that these species may play a bigger role in IBD than their representation may indicate. Metabolically, short-chain fatty acids were reduced in IBD, consistent with the depletion of butyrate-producing bacteria such as *F.prausnitzii* and *R. hominis*. Similarly, there were reductions in the secondary bile acids, lithocholate and deoxycholate, that are formed when relatively rare bacteria, such as *R.gnavus*, mediate 7α-hydroxylation of primary bile acids. The findings of dysregulation of short-chain fatty acids and secondary bile acids are replicated in other studies.[10,11] The authors also identified previously undescribed

metabolic changes such as enrichment of acylcarnitines, a class of compounds associated with energy metabolism, which have also been found to be pro-inflammatory.

The results from the studies mentioned above have been summarized in Table 1. It seems increasingly likely that the pathogenesis of IBD will only be uncovered by the application of big data bioinformatics to datasets generated by metagenomics, metabolomics, and transcriptomics along with datasets that document other environmental factors in the gut lumen and the activity of mucosal immune pathways. Research on IBD still has some way to go, a situation it shares with many other chronic inflammatory disorders.

Declaration of conflict of interest

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