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A Microbiome Foundation for the Study of Crohn's Disease

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Abstract

Our 2014 study published in *Cell Host & Microbe*, “The Treatment-Naïve Microbiome in New-Onset Crohn’s Disease,” was designed to improve our understanding of the microbiome’s role in Crohn’s disease by studying a unique, well-suited cohort and sample set. This commentary provides a hindsight perspective of this original study as well as future outlook.

Many children diagnosed with Crohn’s disease (CD) experience a mild disease course throughout their life that is responsive to treatment; however, a small subset of children will go on to develop a more severe or complicated form that can significantly impair quality of life and may require surgery. Physicians are unable to predict how the disease will ultimately progress in new-onset patients, and therefore cannot identify who will benefit from more

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intensive treatment at the outset. The Pediatric RISK Stratification Study (RISK) is a large cohort study designed to address this challenge by identifying multiple factors in children that may be predictive of more severe disease, including genetic, microbiological and immunological factors. Our 2014 study published in *Cell Host & Microbe*, “The Treatment-Naïve Microbiome in New-Onset Crohn’s Disease,” was a subset of this larger study designed to improve our understanding of how the microbiome contributes to the inflammatory cascade of CD pathogenesis (Gevers et al., 2014).

At the onset of the study, we knew that inflammatory bowel disease (IBD) is a complex disorder involving both genetic and environmental components, and large-scale, genome-wide association studies had linked IBD to host-microbe pathways. Previous studies had examined the role of the microbiome in patients with established disease, but these studies had important limitations. The small cohorts were underpowered to link the microbiome to disease severity, the microbiome changed over time both with disease severity and with treatment making it difficult to associate microbial changes with disease, and most importantly, many of the biomarker findings could not be replicated in other cohorts.

Moreover, many of the new-onset studies that existed at the time examined only the fecal microbiome. Although these studies were able to detect a disease signal, fecal bacterial ecosystems differ from those in the intestinal mucosa. Thus, studies using strictly fecal samples face limitations in identifying microbes more directly involved in disease initiation or progression.

A Unique Cohort

The RISK study design allowed us to address many of the limitations described above. Our large, multi-center cohort of pediatric new-onset CD patients (3 – 17 years of age; n = 668) provided us with the opportunity to analyze the microbiome at the very earliest stage of disease and prior to treatment intervention. To date, this study remains the largest single cohort microbiome study related to new-onset IBD (n = 447) and represents the largest characterization of mucosal-associated microbiota in non-IBD subjects (n = 221). We were able to include patients representing the variety of disease phenotypes with respect to location, severity and behavior. We concurrently sampled multiple sites in each patient prior to treatment initiation, including mucosal tissue biopsies (terminal ileum and rectum) and serum samples. A subset of patients also provided a fecal sample.

The use of a large cohort increased the resolution and statistical power for studying the role of the microbiome in disease. For example, several taxa were only reliably associated with disease when using several hundreds of samples, and capturing microbial shifts in their full complexity required a large study design. Furthermore, we combined two additional cohorts with the RISK cohort, resulting in a total of 1,742 samples from pediatric or adult patients with either new-onset or established disease. This multi-cohort study allowed us to position the unique RISK cohort in the context of a comprehensively defined diversity landscape of IBD, and to identify potential biomarkers with increased robustness.

A Few Limitations

Despite our strong study design, we did face study limitations. Although 1,100 patients were recruited for the larger study, sample collection for microbiome analysis was not mandated, but optional. Thus, we were only able to capture microbiome data from a smaller subset of patients. Also, diet is a factor that can affect the microbiome, but this was not captured as part of the patient history. Finally, we did not collect follow-up biopsy samples from these patients following their treatment to see how the microbiome changes with intervention. Although a small subset of patients (approximately 125) volunteered to provide follow-up biopsy samples, these samples have yet to be analyzed.

Data from an independent small cohort study of 19 treatment-naïve pediatric IBD patients (and 10 healthy controls) suggests that the fecal dysbiosis may not change with disease progression as hypothesized in our work. Interestingly, pretreatment microbiome signatures may be able to predict remission and response to treatment with 76.5% accuracy (Shaw et al., 2016), which holds tremendous translational potential.

Potential Culprits

Before we initiated this study, we knew that microbial dysbiosis existed in patients with CD, but these data came from studies of older patients with a lot of confounding variables. By studying a pediatric, new-onset patient population, we discovered that significant mucosal dysbiosis exists even at the earliest stages of disease. When we analyzed the mucosa-associated microbiome, we found that inflammatory conditions were most strongly associated with an overall drop in species diversity and a change in the abundance of several taxa, some of which had been identified in previous studies. Disease status correlated strongly with an increased abundance of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae* and *Fusobacteriaceae*, and decreased abundance of *Erysipelotrichales*, *Bacteroidales* and *Clostridiales*. This disease signature that we identified has since been reproduced in other studies (Wright et al., 2015). Moreover, both the virome (Norman et al., 2015) and fungome (El Mouzan et al., 2016) are also beginning to be unraveled in IBD, which will ultimately contribute to a more comprehensive understanding of microbe-host interactions in these diseases.

Although we did not arrive at a single silver-bullet microbe that can point to a cure for CD, our work identified a clear list of potential culprits that may be involved in the initiation and/or progression of CD. The next step, already underway by numerous laboratories, is to determine if these microbes, either alone or in combination, actually cause CD or just thrive in an inflamed, diseased environment. Some approaches being used include the use IgA-coating to identify microbes most likely to be the root cause of disease (Palm et al., 2014) and the colonization of germ-free mouse models of disease with specific groupings of organisms identified from this study and others.

The scientific community is already starting to pursue a series of interventions that target the microbiome in CD patients. These efforts include, but are not limited to, direct interventions such as fecal microbial transplants (Vaughn et al., 2016) and enteral feeding (Walton et al.,

2016), as well as indirect approaches such as dietary interventions (Halmos et al., 2016). Broadly speaking, we have started to appreciate that if we can improve bacterial diversity, we can improve patient health.

A longer-term goal, however, is to go beyond crude interventions and to increase our understanding of the microbial products that cause or treat disease. These analyses benefit from high-throughput isolation of organisms, *in vitro* screening assays, and genetics targeting key members of the microbiota that characterize dysbiosis. Also, the compounds these microbes produce could be used to develop treatments that manipulate microbial populations. The challenge, however, is that many of the microbes identified cannot survive in the presence of oxygen and some exist in tight-knit interdependent communities with other species, which makes them difficult to isolate and study *ex vivo*.

Dysbiosis exists in other host species, but does not necessarily mimic what is observed in humans. For example, the same microbe that is harmful in the human network, notably *Fusobacterium*, has been found to be protective against CD in dogs; thus, the same microbe can play a beneficial role in one species and a harmful role in another (Vazquez-Baeza et al., 2016). This is an exciting observation in the context of switching from harmful to beneficial networks, and may change the way we think about using animal models to study CD.

A Powerful Diagnostic Tool

Our team aimed to quantitatively link the microbial imbalance to disease using the microbial dysbiosis index (MD-index), which is calculated as the log of [total abundance in organisms increased in CD] over [total abundance of organisms decreased in CD]. Specifically, the MD-index strongly correlated with clinical disease severity and negatively correlated with species richness, demonstrating that a severe disease state manifests as reduced species diversity in favor of more extreme dysbiosis.

The wide variation of the microbiome can be collapsed into the MD-index, which makes this a potentially powerful diagnostic tool. Although different types of dysbiosis exist across individuals with CD, they can easily be compared because the MD-index summarizes each with a score. We performed a meta-analysis across multiple cohorts and found that the MD-index held across different cohorts. Thus, the index is not only valid within the context of one individual study, but can be used to integrate results across different studies.

Furthermore, the index has since been extended and modified for use in other diseases, such as gingivitis and caries (Huang et al., 2014; Teng et al., 2015).

Although the MD-index can stratify healthy subjects versus CD patients, it currently cannot inform clinical care. We envision the MD-index to one day become part of the CD diagnostic armamentarium to stratify patients based on predicted treatment response.

Antibiotics Are Not The Solution

At the time of the publication of our study, overwhelming evidence already existed that antibiotics are detrimental to a healthy microbiome, and it was our hypothesis that the use of antibiotics impacts the overall community structure and increases the potential for dysbiosis.

However, antibiotics, particularly ciprofloxacin and metronidazole, are commonly prescribed for CD, but they are often unsuccessful.

A small subset of our patients (~10%) were on antibiotics during sample collection, which allowed us to compare microbiome data with and without antibiotic exposure. Our hypothesis was correct in that antibiotic exposure amplified the microbial dysbiosis associated with CD, and this finding has since been confirmed in multiple studies. The loss of protective microbes results in the proliferation of less beneficial taxa, thereby exacerbating the inflammation.

Genetic studies suggest that many of the genes associated with CD are responsible for immune system recognition of microbes, suggesting that these patients are unable to either nurture beneficial bacteria or manage the harmful ones (Jostins et al, 2012).

If antibiotics are not only ineffective, but exacerbate the disease, then alternative solutions are needed for treatment intervention.

Conclusion

It was the integration of multiple disciplines across multiple institutions that enabled us to provide a foundation for the study of CD. The resulting dataset was made publicly available and it continues to provide a tremendous benefit to the research community. To date, it remains one of the most requested datasets in microbiome research.

Three-year outcome data for the RISK cohort have been analyzed and will soon be made available upon publication. Although one of the limitations of this study is that follow-up biopsies were not collected (apart from a small subset of volunteers), the baseline microbiome described in our initial study can be correlated to these outcomes, and can be combined with other –omics datasets collected to identify potential biomarkers that can predict who will develop a severe form of the disease. We eagerly anticipate the results.

We have only a primitive understanding of how the gut microbiome influences the host immune system. With a complex disease such as IBD, even more important than the pathogens driving disease are the protective microbes that are important for establishing homeostasis between the gut and the microbiome. Ultimately, we need to gain a better understanding of the role of host-microbe pathways in maintaining homeostasis in order to provide the most value to patients therapeutically.

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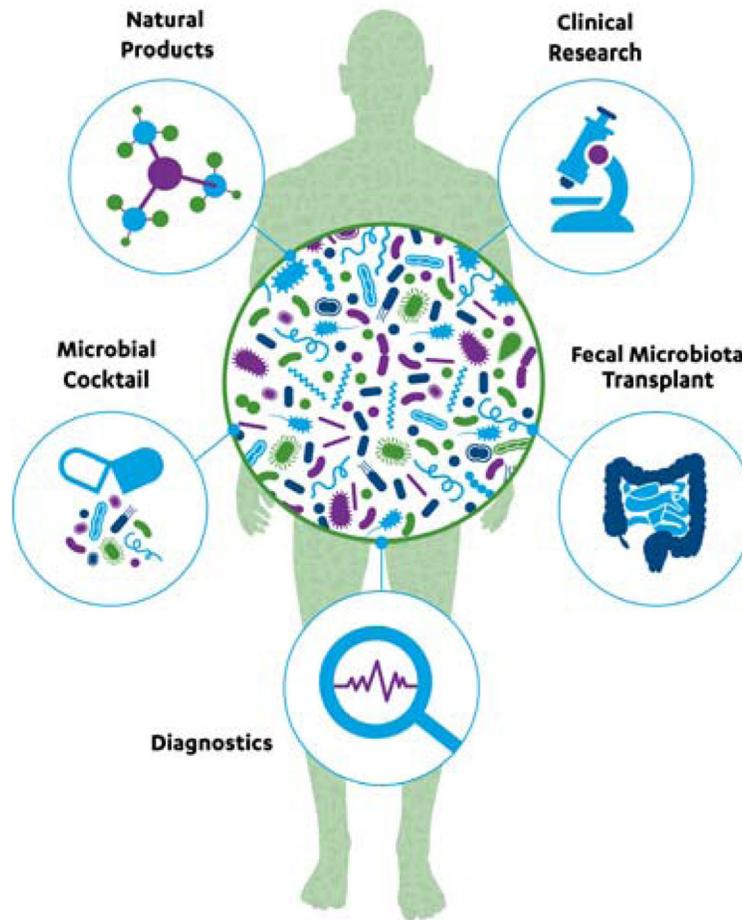


Fig. 1. Clinical research is a start to our understanding of the microbiome's role in Crohn's Disease needed to advance the development of both diagnostics and biotherapeutics.