



A microbial signature for Crohn's disease

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Crohn's disease (CD) results in the chronic inflammation of the intestinal mucosa and is characterized by periods of acute exacerbation and remission. It is one of the two main forms of inflammatory bowel disease (IBD). Although the etiology of IBD is unclear, the two main contributing factors in genetically predisposed individuals include environmental factors and pathogenic infections. After discovering NOD2/CARD as a susceptibility-associated gene, the role of innate immunity has become clear in the pathogenesis of CD (1,2). Genetic, immunologic, and microbiologic studies have shown that the etiology of IBD involves a reduced tolerance to components of the intestinal commensal microbiota. To prevent IBD, There should be perfect homeostasis mediated by the integrity of the intestinal barrier and functional immune tolerance to intestinal microbiota and luminal antigens.

Dysbiosis (alterations in the microbiome) has been reported in IBD over the last 10 years (3-5). In particular, CD patients have reported that they have decreased complexity in their commensal bacterial profiles and higher numbers of mucosa-associated bacteria, which is different from the microbiomes of controls. Dysbiosis appears to be more prevalent - in CD than in ulcerative colitis (UC), which involving more groups of microbes (5). Besides, the reduced expression of peptides among antimicrobial proteins called defensins has been shown in patients with CD (6). Thus, commensal and pathogenic bacteria can infiltrate the intestinal epithelium, resulting in inflammation. Therefore, microbiota as a key modulator of the immune system may play an important role together with the mucosa in the pathogenesis of CD (7).

In a recently published cohort study, Pascal et al. attempted to identify microbial biomarkers of CD and validate their outcome with the outcomes of several other studies: a Belgian CD cohort, a Spanish irritable

bowel syndrome (IBS) cohort, a UK healthy twin cohort, and a German anorexic cohort (8). It was published online on February 2017 in GUT. To study differences in the microbiome composition between patients with IBD and healthy subjects and between the inactive and active form of the disease, 40 healthy controls, 34 patients with CD, and 33 patients with UC were enrolled for a follow-up study in the Spanish cohort. They also included healthy relatives of 36 patients with CD and 35 patients with UC patients in the study. At inclusion and during the follow-up (every 3 months), they collected diagnostic criteria location and behavior of CD, extension of UC and clinical data including tobacco use and medical treatment. Blood samples were collected for determining the erythrocyte sedimentation rate, C-reactive protein - level, and complete blood count. A total of 415 fecal samples for microbiome analysis were collected from 178 participants at various time points. Patients with CD and those with UC who showed recurrence during the study period also provided a stool sample at the time of recurrence. The following patients were excluded: pregnant and breast-feeding patients, patients with severe concomitant disease, and patients who were treated with antibiotics during the previous 4 weeks. Healthy controls provided a single fecal sample, whereas the healthy relatives of patients provided two fecal samples within a 3-month interval. During the 1-year follow-up, 13 patients with CD (38%) and 18 patients with UC (54%) developed recurrence. In the Belgian prospective cohort, 54 patients with CD undergoing ileocecal resection were included. Patients were enrolled before operation, and a total of 187 fecal samples were collected at four time points before and during the postoperative follow-up period (baseline and 1, 3, and 6 months after surgery). -Authors also used the UK and German cohort results; thus, the researchers analyzed 2045 fecal samples from patients with and those without IBD.

Genomic DNA was extracted from fecal samples following the recommendations of the International Human Microbiome Standards (9). Authors reported higher instability of the microbiome of patients with CD than their relatives. Conversely, UC patients presented a more stable microbiome compared with controls. Furthermore, over the 1-year follow-up, the comparison of samples collected at baseline and the remaining time points demonstrated that the microbiome of patients with CD was significantly more unstable than that of patients with UC (mixed ANOVA $p < 0.001$). They reported the microbiome of patients with CD and that of patients with UC was significantly different from that of controls by multivariate analysis ($p = 0.001$). They suggested that dysbiosis is greater in patients with CD than in those with UC because six genera were enriched in patients with CD compared with 12 in healthy controls, while there were only two enriched genera in patients with UC compared one in healthy controls at baseline. They could not find any biomarker either for CD or UC predictive of recurrence. Their results indicate that a loss of beneficial microorganisms is more common in patients with CD than a gain of more pathogenic ones. They reported the lower relative abundance of *Faecalibacterium* as a butyrate-producing beneficial microorganism in patients with CD and also showed that this genus is not missing in patients with UC, thus making it a useful marker to discriminate patients with CD from those with UC.

They tested the link between smoking and disease activity. They did not find any association between being a smoker or ex-smoker and disease severity. Moreover, they studied the association of the relative abundance of groups of bacteria and smoking habits and found that certain bacteria had higher abundance in patients with CD and those with UC according to smoking habits. The examination of the link between the relative abundance of the group of bacteria and disease location yielded the result that *Enterococcus faecalis* and an unknown species belonging to Erysipelotrichaceae were more abundant in the stool sample of patients with ileal CD than in the ileocolon. They also reported that proctitis is associated with a higher relative abundance of an unknown Clostridiales, *Clostridium*, and an unknown Peptostreptococcaceae and Mogibacteriaceae in the stool. They did not find any relationship between medication use and microbiome composition.

They examined the groups of microbes that presented most significant differences between patients with CD and those with UC and between patients with CD and healthy controls using the Kruskal-Wallis test to discriminate CD and non-CD. Eight bacterial genera showed the potential to discriminate between patients with CD and those with UC and healthy controls: *Faecalibacterium*, Peptostreptococcaceae, Anaerostipes, Christensenellaceae, *Fusobacterium*, *Escherichia*, *Collinsella*, and *Methanobrevibacter*. The researchers found that *Faecalibacterium*, *Anaerostipes*, *Methanobrevibacter*, and an unknown genus of Christensenellaceae were abundant in healthy controls and patients with UC and almost absent in

patients with CD, while *Fusobacterium* and *Escherichia* were abundant in CD patients and almost absent in healthy controls and patients with UC. This finding may be the microbial signature of CD and had an overall sensitivity of 80% and a specificity of 94% for detecting patients with CD over healthy controls. Its specificity was 91% for distinguishing CD from UC, 94% for distinguishing CD from anorexia, and 89% for distinguishing CD from IBS.

Pascal et al. (8) attempted to identify microbiomarkers that would facilitate the discrimination between CD and UC in their study. These microbiomarkers may decrease the systematic use of endoscopy for diagnosing these diseases or help confirm its diagnosis. The findings of this study may help future treatments based on the use of microorganisms by fecal microbiota transplantation through the appropriate selection of missing microorganisms in CD. Though those microbiomarkers should be validated in all types of patients with IBD, it would be very useful even if they will only help distinguish CD.

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