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Altered Fecal Bacteria of IBS Patients Characterized

The fecal microbiota is significantly altered in irritable bowel syndrome, and the microbial composition also differs among patients with diarrhea-predominant, constipation-predominant, and mixed types of the syndrome, according to an article appearing in the July 2007 issue of Gastroenterology.

Previous studies have linked irritable bowel syndrome (IBS) with changes in gastrointestinal fermentation patterns and with quantitative alterations in microbiota, wrote Dr. Anna Kassinen of the University of Helsinki and her colleagues.

To characterize these differences more specifically, the researchers analyzed the microbial genomes in fecal samples collected from 24 patients with IBS and 23 gender- and age-matched controls. The IBS patients were classified by their disease type, with 8 patients in the constipation-predominant (IBS-C) group, 10 in the diarrhea-predominant (IBS-D) group, and 6 in the mixed-type (IBS-M) group.

The genome preparations were pooled, centrifuged, and fractionated within 5% intervals based on their guanine and cytosine content. When differentiated by their percentage of guanine and cytosine, considerable variations were detected among the four pooled samples (IBS-D, IBS-C, IBS-M, and healthy controls) in three distinct fraction groups, which were further analyzed via high throughput cloning and sequencing of the 16S rRNA gene to determine whether the differences were significant. Some of the phylogenetic differences were confirmed by quantitative polymerase chain reaction assays on individual samples from each test subject, the authors wrote.

Out of 3,753 high-quality sequences identified, 53 operational taxonomic units (OTUs) corresponding to 98 sequences were characterized as novel relative to other publicly available sequences, with the majority of the novel OTUs included in phyla *Firmicutes* and *Actinobacteria*. A comparison of the sequence data with the Bayesian analysis of population structure confirmed "significant divergences [among] control samples and various IBS subtypes," they wrote.

To assess the validity of the observed differences at the level of the individual samples, real-time quantitative PCR (qPCR) analysis was used. "In the phylum *Firmicutes*, the real-time PCR assays for *Coprococcus eutactus*- and *Clostridium cocleatum*-related sequences revealed differences between the IBS patients and healthy control subjects, while the other assays for members of *Firmicutes* remained statistically nonsignificant," the authors wrote.

"In the phylum *Actinobacteria*, real-time qPCR analysis revealed significant differences in the counts of *C. aerofaciens*." While the latter bacterium was found in 96% of all of the control samples, its prevalence was considerably lower among IBS patients.

The high-resolution methods for analyses of gastrointestinal microbiota used in this study "provide a substantially more comprehensive view of the diversity and alterations of this complex ecosystem," the authors wrote. In addition, the "striking observation" that the gastrointestinal microbiota is significantly altered in IBS "emphasizes that additional research on the host gastrointestinal microbiota interactions in IBS is needed," they concluded. ■

By Diana Mahoney, Elsevier Global Medical News

Dr. Emeran A. Mayer, Associate Editor of Gastroenterology, comments:

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The study by Kassinen represents a milestone in the unfolding saga of research into the mechanisms underlying symptoms in IBS. Even though the results are based on a small sample of healthy control subjects and IBS patients, classified and subdivided by symptom criteria (the so-called "Rome criteria" which by themselves have been changing about every 3-4 years), they clearly point the way of how to study the role of the microflora in the emerging concept of a bidirectional brain-gut-microflora axis.

Preliminary and inconclusive, often nonreproducible evidence for a possible role of the microflora in IBS symptoms has come from various directions over the past decade: the sometimes beneficial, sometimes adverse effects of antibiotics on IBS symptoms and the possible therapeutic effect of certain probiotics. The IBS-like symptoms arising in a small number of individuals after a gastroenteric infection, and the possibility that bacteria may be the source for altered profiles of serine proteases in the stool of IBS patients all point toward some role of the microflora in IBS symptoms. In contrast to the great majority of indirect evidence for alterations in the microflora of IBS patients obtained from such studies, the Kassinen paper for the first time successfully applies cutting-edge methodology to the identification of differences in several bacterial genera between healthy control subjects and patients with the symptom complex of IBS.

With the advent of such high throughput technologies, several specific questions need to be addressed: Are the findings reproducible in a larger sample? Are the differences truly related to IBS symptom severity or can they be detected in some asymptomatic healthy control subjects? Are they related to the chronic use of certain treatments (including alternative and complementary remedies) or are they related to common psychiatric comorbidity? Are there really differences between bowel habit-based subtypes (not generally agreed upon by the field), or are these differences really due to differences in GI motility patterns and associated transit times which can be found in some IBS patients?

Once these initial caveats have been addressed, some fascinating questions arise: Are the differences in microflora composition related to the nature and quality of early life experiences, in particular the early interaction between the mother and the infant? Such a relationship could provide an epigenetic mechanism underlying the significant role of IBS diagnosis in the mothers of IBS patients. What role do host factors (altered central regulation of enterochromaffine cells and the release of their content into the gut lumen) play in these observed changes? Are the observed differences similar to those already reported in response to psychological stressors in animals, and do mood and affect alter the microflora?

The findings by Kassinen provide another piece in the jigsaw puzzle which will have to be added to the growing list of pieces already assembled to understand the brain-gut axis and its dysregulation in IBS. They can clearly be viewed as the opening of a new chapter in IBS research.

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