

end points for drug trials in IBS-C, such as those recommended by the US Food and Drug Administration, and this issue should be addressed in any future trials. These end points can help to facilitate comparisons of efficacy against other existing treatments in trials that also use them. In addition, the inclusion of patients with IBS-C means that, in terms of Rome criteria diagnosis at least, the study population is heterogeneous. If FC and IBS-C are truly separate conditions, we might expect the effects of mizagliflozin to differ between these 2 patient groups, making a case for separate trials. There is evidence to suggest that FC and IBS-C overlap and are, in fact, different points on the same clinical spectrum (Am J Gastroenterol 2015;110:580–587), and that patients can switch between the 2 disorders (Am J Gastroenterol 2010;105:2228–2234). If this is true, then including patients with either FC or IBS-C in the same drug trial may be clinically justifiable and informative, because it more truly reflects real-world practice. However, there is also evidence that the pathophysiological mechanisms responsible for causing constipation differ between patients with FC and those with IBS-C. Visceral hypersensitivity may be more prevalent in IBS-C than in FC, whereas delayed transit has been observed to a greater extent in FC compared with IBS-C (Gastroenterol Hepatol 2016;12:171–178). These differences in the underlying pathophysiology of FC and IBS-C could limit the ability of drug trials to demonstrate treatment effects when patients with FC and IBS-C are grouped together.

Mizagliflozin failed to demonstrate an impact on abdominal discomfort and bloating; however, in a survey of 759 Japanese patients with IBS-C, abdominal discomfort and bloating were among the three most commonly reported GI symptoms (Biopsychosoc Med 2018 Sep 4;12:12). Moreover, in a survey of patients with FC, these symptoms were frequently reported to be bothersome (Aliment Pharmacol Ther 2007;25:599–608). Therefore, it is doubtful that mizagliflozin will offer constipated patients the symptomatic relief they desire if, in addition to increasing the number of bowel movements, it does not also lead to improvements in abdominal discomfort and bloating. The drug also failed to show any significant improvement in quality-of-life measures for either dose compared, with placebo at 4 weeks. This finding may be due to the short trial duration. Again, these secondary end points will also require further evaluation in larger numbers of patients with a longer duration of follow-up.

In conclusion, the results of this study suggest that the SGLT1 inhibitor mizagliflozin may represent a new class of drug for the treatment of FC and IBS-C. It therefore merits further study in larger trials, with a longer duration of follow-up, and that use US Food and Drug Administration-recommended end points, where possible. This trial also highlights questions about the classification and subgrouping of functional GI disorders in general, and in particular whether FC and IBS-C are distinct disorders or not. Further research is needed to try and resolve this issue, which has important implications for how study populations are selected for drug trials in FC. Finally, we should be mindful that, although RCTs can help to determine the efficacy of a

new drug relative to placebo, trying to understand how any new drug ranks alongside existing treatments in terms of efficacy and safety can be difficult.

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## Insights into How Probiotics Colonize the Healthy Human Gut



Zmora N, Zilberman-Schapira G, Suez J, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 2018;174:1388–1405.

Probiotic use is widespread throughout the world. Although mouse studies have provided significant insight into their effects in health and in disease models, data from humans remain limited. In a Herculean effort by scientists and human study volunteers alike, Zmora et al from the Weizmann Institute of Science provide new insight into human host-microbial interactions during probiotic consumption (Cell 2018;174:1388–1405). Healthy human volunteers each underwent invasive colonoscopy and endoscopy procedures at baseline and during consumption of probiotics or placebo to allow for a person-specific multi-omics assessment of global probiotics effects on the human gastrointestinal (GI) tract. Mouse experiments were conducted in parallel. In summary, the investigators report person-specific differences in probiotic persistence that can be explained in part by the indigenous microbiome compositions of the human hosts, and by transcriptional features of biopsies of their stomachs and ilea. Importantly, the investigators demonstrate that stool, often used as a proxy for sampling the gut microbiota, is limited in its representation of both species presence and abundance within the GI tract.

The 16S rRNA gene-sequencing or whole metagenome shotgun-sequencing of the microbiome was performed on endoscopically obtained samples from multiple locations throughout the upper GI and lower GI (LGI) tracts, as well as stool, of 25 healthy participants. Taxonomic composition analysis revealed a similarity gradient, with more taxonomic similarity between the LGI and stool than the upper GI and stool. However, multiple taxa including *Bacteroides thetaiotaomicron*, *Blautia obeum*, and *Dorea longicatena* differed significantly in abundance between the LGI and stool. Further, mapping of shotgun reads to KEGG orthologous pathways identified 100 pathways to be differentially represented in stool metagenome as compared to the LGI lumen and mucosa.

Fifteen healthy human volunteers also ingested either an 11-strain probiotic cocktail (commercially available in

Israel) or placebo twice daily for 4 weeks, with mucosal and luminal samples as well as biopsies collected from the upper GI and LGI at baseline and 3 weeks. Seven of 11 strains were shown by quantitative polymerase chain reaction to be significantly enriched in the feces of probiotic-treated participants during consumption, but this enrichment decreased to baseline after cessation of treatment. Although there was almost no increase in luminal levels of the probiotic strains, 9 of 11 strains were significantly enriched in mucosal samples on the last day of treatment. Importantly, although fecal shedding of probiotics was universally detected in the treated group, only a subset of this group had increased levels in the mucosal samples, implying that fecal shedding may not represent colonization by a strain, but rather transient passage.

Microbiome phylogenetic profiles and KEGG orthologous pathways at baseline of colonization-permissive participants clustered separately from colonization-resistant participants (visualized on principal coordinate analysis plots), indicating that person-specific microbiome features may explain the variation in colonization resistance. In particular, permissive individuals had lower baseline levels of strains included in the probiotic formulation that later bloomed in their mucosa. Germ-free mice humanized with stool from permissive or resistant participants recapitulated the observed difference in colonization resistance when gavaged with the probiotic formulation. In addition, transcriptomic profiling of GI biopsies at baseline revealed an up-regulation of genes involved in adaptive and innate immunity in the stomachs of resistant individuals, whereas immune-related genes were up-regulated in the ilea of permissive individuals.

During the course of treatment, differences in transcriptional profiles between resistant and permissive individuals diminished. Nevertheless, the ceca of permissive individuals were enriched for pathways related to dendritic cells and antigen presentation, whereas those of resistant individuals were enriched for innate immune activation and antibacterial defense against gram-positive bacteria.

**Comment.** A fundamental challenge of microbiome studies has been explaining the compositional variability of “healthy” gut microbiomes. Human microbiomes are highly individual specific at the species and strain levels (Nature 2012;493:45–50), and although efforts to identify a core set of taxa that invariably populate the human gut have been unsuccessful, the abundance of microbial metabolic pathways has been shown to be more consistent (Genome Med 2016;8:51). This study adds a wealth of evidence that variable responses to probiotic treatment observed in the population are due in part to the indigenous microbiomes of the participants and the functions encoded therein. As illustrated in Figure 7D of the article, there seems to be a role for metabolic exclusion in colonization resistance. Whereas an enrichment in certain metabolic functions is present in colonization-permissive individuals during probiotic treatment, the colonization-resistant group had relatively high levels of these functions before probiotic treatment. Further, the investigators report an inverse

relationship between baseline levels of probiotic species and their fold change during treatment, supporting the prior observation that earlier colonizers of a species can inhibit colonization by a naïve population of that species due to metabolic exclusion or slight mutational advantages (Infection Immunity 2009;77:2876–2886).

The study is not clinically powered, but does provide motivation for a personalized approach toward probiotic therapy, especially in the light of the sister study published in the same issue which indicates that probiotic treatment may potentially be detrimental to individuals with vulnerable (low-diversity) microbiomes (Cell 2018;174:1406–1423). As sequencing costs decrease dramatically and improved algorithms for strain level identification from metagenomes are developed (Frontiers Microbiol 2016;7:712), it may become possible to develop diagnostic tools to aid personalized probiotic formulations based on person-specific predicted colonization efficacy. In contrast, increasing interest in engineered probiotic therapies, some of which have even begun clinical trials (Nat Biotechnol 2018;36:857–864), comes with an increasing interest to develop biocontainment tools to ensure transient passage through the GI tract and mitigate the risk of long-term colonization. Still, more work needs to be done to understand the molecular basis of these colonization resistance patterns, especially the host immune response and transcription profiles that define colonization permissiveness or resistance.

Relevant to this work is the question of the usefulness of 16S rRNA-sequencing in comparison to whole metagenome shotgun-sequencing when species- or strain-level identification is key, as in studies assessing the effect of a single species on microbiome composition and function and as the field progresses towards more mechanistic understandings of microbial interactions in the gut (Nat Rev Microbiol 2018;16:410–422). Although 16S rRNA sequencing has been, and continues to be, immensely useful in tracking overall compositional shifts of microbiomes (Nature 2012;486:207–214), it rarely reaches species-level resolution and may not be an accurate measure of low-abundance taxa that may nevertheless have important functional impacts on gut health. For example, the investigators highlight the important fact that probiotic formulations are not subject to regulation or strict assessment of composition before murine and human studies. In this study, 4 of 11 strains were identified by 16S rRNA sequencing at the species level, and 10 were identified by shotgun sequencing. This difference became relevant when the authors found no significant enrichment of *Lactobacillus* or *Bifidobacterium* genera but a 2.4-fold enrichment of a *Lactococcus* species in the stool of participants treated with probiotic. Three of 5 *Lactobacillus* species and 2 of 4 *Bifidobacterium* species were unable to be identified at the species level by 16S rRNA sequencing in the original probiotic formulation, and the resultant aggregation by genus may have obscured species-specific enrichment in the stool. In contrast, there was only 1 *Lactococcus* species in the original formulation which likely facilitated its identification by 16S. As such, the investigators appropriately developed a custom set of strain-specific primers with

demonstrated specificity and sensitivity by quantitative polymerase chain reaction, and relied on this method for accurate quantification of the probiotic strains throughout the study.

The study also illustrates the challenges that arise in applying results from murine microbiome studies to humans. Murine studies of the microbiome provide important insights into its impact on host health, because they allow for the rigorous control of environmental conditions, different immune states, testing of novel engineered probiotics therapies, and the ability to completely modulate microbiome states through use of gnotobiotic mice (Science 2013;341). Indeed, the investigators validated the role of the indigenous microbiome in colonization resistance by demonstrating a lack thereof in gnotobiotic mice compared with conventional mice, and used gnotobiotic mice pre-humanized with stool from colonization-permissive or -resistant participants to validate the conclusion that the indigenous microbiome is causal in person-specific colonization resistance. However, the colonization patterns the investigators describe in mice do not reflect those they describe in humans, which the investigators acknowledge is likely due in part to low intersubject variability in these inbred mice. Further limitations of murine microbiome studies include that the fitness of human probiotic strains likely differs between mouse and human microbiomes; mouse coprophagy often leads to within-cage microbiome sharing, such that multiple mice in a cage are effectively reduced to a sample size of 1; repeated daily gavages can be physically stressful to mice (and may explain the finding in this study that 10 of 14 taxa that bloomed in the LGI of gavaged mice were characteristic of the oral cavity and stomach); and it can be

technically challenging to extract sufficient bacterial DNA from the mucosa for confident relative abundance estimates from high-throughput sequencing data. Although murine microbiome studies are useful and often essential for establishing causal relationships between microbiome states and host health, an important advantage of this metagenomic study is its inclusion of not only human fecal samples, but also longitudinal endoscopic and colonoscopic luminal and mucosal samples, and tissue biopsies. Overall, this work underlines the importance of interpersonal variation as we develop therapies to modulate the gut microbiome.

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