Role of the Microbiome in Disease: Implications for Treatment of Irritable Bowel Syndrome

Brian E. Lacy, PhD, MD

CONTINUING MEDICAL EDUCATION

LEARNING OBJECTIVES:

1. Describe the findings of the Human Microbiome Project and Gut Microbiome Initiative
2. Describe the evidence linking alterations in the human microbiome with disease, including irritable bowel syndrome
3. Describe results of treatments that act upon the gut microbiota in patients with irritable bowel syndrome

TARGET AUDIENCE

Family physicians and clinicians who wish to gain increased knowledge of the gut microbiome and its implications in the primary care management of IBS.

DISCLOSURES:

The IAFP adheres to the conflict of interest policy of the ACCME and the AMA. It is the policy of the IAFP to ensure balance, independence, objectivity, and scientific rigor in all its educational activities. All individuals in a position to control the content in our programs are expected to disclose any relationships they may have with commercial companies whose products or services may be mentioned so that participants may evaluate the objectivity of the presentations. In addition, any discussion of off-label, experimental, or investigational use of drugs or devices will be disclosed by the faculty. Only those participants who have no conflict of interest or who agree to an identified resolution process prior to their participation were involved in the CME activity.

Dr. Lacy discloses that he is on the advisory boards for Ironwood Pharmaceuticals, Inc.; Prometheus Laboratories, Inc.; and Salix Pharmaceuticals, Inc.; and is a board member for the *American Journal of Gastroenterology*.

Stephen A. Brunton, MD, FAAFP, discloses that he is on the advisory boards and speakers' bureaus for AstraZeneca; Boehringer Ingelheim GmbH; Eli Lilly and Company; Janssen Pharmaceuticals, Inc.; and Novo Nordisk Inc. He is on the advisory boards for Abbott Diabetes Care Inc.; Allergan; Becton, Dickinson and Company; Cempra, Inc.; Intarcia Therapeutics, Inc.; and Mylan N.V.

Allan Wilke, MD, CME Reviewer and Greg Scott, PharmD, RPh, editorial support, disclosed no relevant financial relationship or interest with a proprietary entity producing, marketing, reselling or distributing health care goods or services.

Illinois Academy of Family Physicians/Family Practice Education Network staff and additional staff of PCEC disclosed no relevant financial relationship or interest with a proprietary entity producing, marketing, reselling or distributing health care goods or services.

This program does not include any discussion or demonstration of any pharmaceutical or medical devices that are not approved by the Food and Drug Administration (FDA) or that are considered "off-label."

CONFLICTS OF INTEREST

When individuals in a position to control content have reported financial relationships with one or more commercial interests, the Illinois Academy of Family Physicians works with them to resolve such conflicts to ensure that the content presented is free of commercial bias. The content of this activity was vetted by the following mechanisms and modified as required to meet this standard:

Content peer-review by an external CME reviewer

Content validation by internal clinical editorial staff

SPONSORSHIP

This educational activity is sponsored by the Illinois Academy of Family Physicians and Primary Care Education Consortium.

SUPPORTER

This activity is supported by an educational grant from Valeant Pharmaceuticals.

ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of The Illinois Academy of Family Physicians/Family Practice Education Network and Primary Care Education Consortium. The Illinois Academy of Family Physicians (IAFP) / Family Practice Education Network (FPEN) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

AMA PRA CATEGORY 1

The Illinois Academy of Family Physicians/Family Practice Education Network designates this activity, "Role of the Microbiome in Disease: Implications for Treatment of Irritable Bowel Syndrome," for a maximum of 1.0 AMA PRA Category 1 Credit™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Release Date: 1 April 2017

Expiration Date: 31 March 2018

METHOD OF PARTICIPATION

PHYSICIANS

To receive CME credit, please read the journal article and on completion, **go to www.pceconsortium.org/microbiome to complete the online evaluation and receive your certificate of completion.**

PHYSICIAN ASSISTANTS

AAPA accepts certificates of participation of educational activities certified for AMA PRA Category 1 Credit™ from organizations accredited by ACCME or a recognized state medical society.

INTRODUCTION

As many as 1 in 5 Americans have symptoms of irritable bowel syndrome (IBS), but only about 30% seek medical attention.[1,2] Even so, IBS accounts for approximately 12% of visits to primary care physicians and 28% of referrals to gastroenterologists.[3] With emerging evidence to support some practices, many people with IBS turn to complementary health practices, including dietary manipulation and the use of alternative medicine such as probiotics and prebiotics, to help relieve their symptoms.[1,4,5] Therefore, patients with IBS who seek medical care for their IBS symptoms may have questions about diet and alternative treatments or may be self-managing.

Dietary and some other treatments for IBS are supported by a growing body of evidence, much of which comes from programs such as the Human Microbiome Project and Human Gut Microbiome Initiative, which were intended to identify and characterize microorganisms found in association with both healthy and diseased humans. These programs used state-of-the-art technology to characterize the human microbiome from multiple body sites.[6] This evidence indicates that the gut microbiome plays an important role in IBS and some other gastrointestinal (GI) disorders. The human microbiome is the collective genome (ie, genetic material) of all the microorganisms living in association with the human body, the vast majority of which reside in the distal gut.[7,8] The gut microbiota refers to the complex ecosystem of more than a thousand microbial species inhabiting the intestine, most of which are bacteria, and accounts for 60% of the fecal biomass.[6,9,10] While research is still in its infancy, these programs suggest that microorganisms carry out a range of biological functions critical to the health of the individual.[11]

Emerging evidence also suggests that changes in the composition of the gut microbiota (dysbiosis) correlate with numerous diseases, including type 1 and type 2 diabetes, obesity, asthma, and several cancers, as well as anxiety and depression.[7,12-15] Perhaps least surprising is the increasing evidence implicating gut microbiota alterations in gastrointestinal diseases such as inflammatory bowel disease and IBS.[16]

Brian E. Lacy, PhD, MD, Professor of Medicine, Chief, Section of Gastroenterology & Hepatology, Dartmouth-Hitchcock Medical Center, Lebanon, NH

DISCLOSURES

Dr. Lacy discloses that he is on the advisory boards for Ironwood Pharmaceuticals, Inc.; Prometheus Laboratories, Inc.; and Salix Pharmaceuticals, Inc.; and is a board member for the *American Journal of Gastroenterology*.

SUPPORT

This CME article is jointly sponsored by the Illinois Academy of Family Physicians/Family Practice Education Network and Primary Care Education Consortium and supported by an educational grant from Valeant Pharmaceuticals.

IRRITABLE BOWEL SYNDROME

Microbial complications of IBS

The most convincing evidence that suggests gut microbiota are involved in the pathogenesis of IBS is the finding that IBS can develop in predisposed individuals following a bout of infectious gastroenteritis.[17] The odds of developing IBS are increased more than sixfold after an acute GI infection, and the onset of new IBS symptoms after a bout of infectious gastroenteritis is reported by 6% to 18% of IBS patients.[17]

Additional evidence supporting a role for the gut microbiota in IBS include differences in the colonic microbiota between IBS and non-IBS populations, symptomatic response of IBS to antibiotic and probiotic administration, and recent anecdotal reports of responses to fecal microbial transplantation.[11,18-21] Numerous studies have reported differences in the mucosal and/or fecal microbiota of patients with IBS compared with healthy controls, such as reduced diversity of the microbial population, altered proportion of specific bacterial groups, different degree of variability in the microbiota composition, a higher degree of temporal instability, and more abundant mucosal bacteria.[22] Some patients experience small intestinal bacterial overgrowth (SIBO), a condition in which bacteria colonize the small intestine, creating localized inflammation, altering intestinal absorption, and potentially using nutrients needed by the body, which in turn causes malnourishment.

While our understanding of the pathophysiologic role of the gut microbiota in IBS is still developing, several possible mechanisms have been proposed. The current working hypothesis is that altered composition and metabolic activity of the gut microbiota activate mucosal innate immune responses and inflammation.[9,17] These processes, in turn, increase mucosal permeability, promote epithelial barrier dysfunction, activate nociceptive sensory pathways, and dysregulate the enteric nervous system.

Treatment approaches focused on altering the gut microbiome

While our knowledge about the gut microbiome and its role in IBS pathophysiology continue to develop, the gut microbiota has been a therapeutic target for years, if not decades.[17]

Dietary modification

Diet has been shown to significantly influence the composition and metabolic activity of the gut microbiota. In fact, dietary modification can substantially alter the gut microbiome in as

little as 3 days.[9,23-25] Additionally, 60% to 70% of patients with IBS report a worsening of symptoms after meals, and 50% to 70% report intolerance to various foods.[3]

The most compelling evidence for a beneficial impact of diet on IBS exists for a diet that restricts a group of short-chain carbohydrates known collectively as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). FODMAPs are found in such foods as wheat, legumes, milk, some fruits, and sorbitol.[4,26] Rapid fermentation of these incompletely absorbed carbohydrates leads to gas production and increased luminal water content, resulting in luminal distention that may account for IBS symptoms.[4] Implementation of a low FODMAP diet for IBS reduces overall gastrointestinal symptoms and individual symptoms such as abdominal pain, bloating, constipation, diarrhea, abdominal distention, and flatulence.[26,27] In a randomized, single-blind, crossover study of 30 patients with IBS and 8 healthy controls who received 21 days of either a low FODMAP or typical Australian diet, 70% of patients with IBS experienced improvement in overall GI symptoms.[28] Dietary intervention guided by specialized dietitians appears to be vital for the success of the diet, which is fairly complex.[26] The ideal length of time for a patient to adhere to a low FODMAP diet has not been adequately studied; however, strict adherence to a low-FODMAP diet is not recommended long-term due to potential risks of inadequate nutrient intake.[26]

Very limited data suggest that gluten may exacerbate IBS symptoms in patients with IBS but not celiac disease whose symptoms are already controlled on a gluten-free diet. This observation suggests that a gluten-free diet may help some patients with IBS.[29] However, a more recent study by the same investigators demonstrated that implementation of a gluten-free diet in patients with IBS already on a low FODMAP diet did not provide added benefit.[30]

Fiber has long been considered a mainstay of therapy for relief of IBS symptoms. The beneficial effects of fiber are thought to reflect colonic fermentation with production of short-chain fatty acids or its action as a prebiotic.[3] A recent systematic review and meta-analysis of 14 trials found moderate-quality evidence that soluble fiber—but not bran fiber—is effective at improving global IBS symptoms and should remain a first-line therapy for IBS, given its affordability and safety.[31]

Probiotics and prebiotics

Prebiotics (eg, fructooligosaccharides and inulin) are ingredients in food that remain undigested and which may stimulate either the growth or the activity of bacteria that are also beneficial to human health.[19] In contrast, probiotics are live microorganisms that, when ingested in adequate amounts, confer a health benefit to the host.[4] Synbiotics combine prebiotics and probiotics, with a potentially synergistic action.[19] There is a paucity of evidence for the efficacy of prebiotics or synbiotics in IBS.[22]

Probiotics, principally those containing *Lactobacillus sp.* and *Bifidobacterium sp.*, have been studied extensively as a way to beneficially modulate the GI microbiota in the treatment of IBS.[17,19,32,33] *Lactobacillus sp.* and *Bifidobacterium sp.* modulate several mechanisms that might be implicated in the pathogenesis of IBS, including effects on intestinal microbiota composition, GI dysmotility, visceral hypersensitivity, altered gut epithelium and immune function, and luminal metabolism.[22] Interpreting results from probiotic studies in IBS is challenging due to inclusion of patients with different IBS subtypes and the use of multiple probiotic strains and doses across studies, which may obscure the beneficial effects of individual strains within that species.[19,32]

In a meta-analysis of 35 studies of probiotics vs placebo for IBS, probiotics improved overall symptoms, with a relative risk of 0.79 (95% confidence interval, 0.70-0.89) for IBS symptoms persisting. Probiotics reduced abdominal pain, bloating, and flatulence. The number needed to treat (NNT) was 7. Some combinations of probiotics were superior to individual species or strains, although no specific combination was superior to another.[19] Adverse events were more common with probiotics (16.5%) compared with placebo (13.8%), with a number needed to harm (NNH) of 35.[19]

Antibiotics

The alteration of the gut microbiota, and particularly the possible role of an SIBO in at least some patients with IBS, has prompted the evaluation of antibiotics as a treatment for IBS.[22] Neomycin, a nonabsorbable antibiotic, was the first investigated for IBS. Neomycin produced a 50% improvement in global IBS symptoms compared with placebo, but also induced rapid bacterial resistance.[22]

The rifamycin-derivative rifaximin is an oral, nonsystemic, broad-spectrum antibiotic associated with a low bacterial resistance profile and a favorable side-effect profile.[20] Rifaximin appears to have anti-inflammatory, host-response, and gut microbiota modulatory activities.[34] Rifaximin has shown efficacy in several small-scale studies of IBS as well as 2 large-scale, identically designed, phase 3, double-blind, placebo-controlled, multicenter trials (Targeted non-systemic Antibiotic Rifaximin Gut selective Evaluation Treatment [TARGET] 1 and TARGET 2) (**TABLE**).[20,35]

In TARGET 1 and TARGET 2, patients affected by IBS without constipation (N=1258) received either rifaximin 550 mg or placebo 3 times daily for 2 weeks, then were followed for an additional 10 weeks.[20] Significantly more

TABLE TARGET-1, -2, and -3 trials for rifaximin in the management of irritable bowel syndrome[20,35]

Study design	Patients	Treatment	Primary efficacy outcomes	Secondary efficacy outcomes	Safety
R, DB, PBO-C; TARGET 1 and TARGET 2 combined	IBS (Rome II criteria) with abdominal pain and discomfort	Rifaximin 550 mg tid (n = 624) vs PBO (n = 634)[a] for 2 weeks	Adequate relief[b] of global IBS symptoms: rifaximin vs PBO: 40.7% vs 31.7%; *P* <.001	Adequate relief[b] of IBS-related bloating: rifaximin vs PBO, 40.2% vs 30.3%; *P* <.001	AEs comparable between groups Rifaximin vs PBO: Headache: 6.1% vs 6.6%; upper respiratory tract infection: 5.6% vs 6.2%; abdominal pain: 4.6% vs 5.5%
Open label, then R, DB, PBO-C; TARGET 3	IBS-D (Rome III criteria) with abdominal pain and bloating	Rifaximin 550 mg tid open-label for 2 weeks (n=1074) If relapsed during 18-week observation phase: rifaximin 550 mg tid (n=328) vs PBO (n=308)	Percentage of responders[c] after first repeat treatment: rifaximin vs PBO: 38.1% vs 31.5%; *P*=.03	Percentage of responders who did not have recurrence through end of 6-week repeat treatment observation phase and continued to respond without recurrence through end of second repeat treatment phase: rifaximin vs PBO: 13.2% vs 7.1%; *P* =.007	AEs comparable between groups Rifaximin vs PBO: Overall: 42.7% vs 45.5%; nausea: 3.7% vs 2.3%; upper respiratory tract infection: 3.7% vs 2.6%; urinary tract infection: 3.4% vs 4.9%

Abbreviations: AE, adverse event; bid, twice daily; DB, double-blind; IBS, irritable bowel syndrome; PBO, placebo; PBO-C, placebo-controlled; R, randomized; TARGET, Targeted non-systemic Antibiotic Rifaximin Gut selective Evaluation Treatment; tid, 3 times daily.

[a]Patients included in modified intention-to-treat analysis.

[b]Defined as relief of symptoms for ≥2 of first 4 weeks of treatment by self-report.

[c]Defined as a decrease in abdominal pain ≥30% from baseline AND a decrease in frequency of loose stools ≥50% from baseline for ≥2 weeks during a 4-week posttreatment period.

patients in the rifaximin group than in the placebo group had adequate relief of global IBS symptoms during the first 4 weeks after treatment (**TABLE**).[20,35] The percentage of patients with adequate relief decreased over time in both groups, but remained higher for patients treated with rifaximin compared with patients receiving placebo during all 3 months in both studies. The incidence of adverse events was similar in the rifaximin and placebo groups.

Most recently, the randomized, placebo-controlled TARGET 3 study indicated that repeat treatment with rifaximin 550 mg 3 times daily for up to three 2-week cycles in patients with diarrhea-predominant IBS (IBS-D) was significantly more efficacious than placebo (38.1% vs 31.5%, *P*=.03) in improving IBS symptoms. Treatment was well tolerated.[35]

Although not indicated for IBS-C (constipation predominant), rifaximin (400 mg 3 times daily for 7-10 days) has been evaluated in patients with IBS-C in 2 small, double-blind trials.[36] In one trial, rifaximin plus neomycin significantly improved severity of constipation and symptoms of bloating and straining for up to 4 weeks compared with neomycin plus placebo.[36] In the other trial, which utilized a crossover design, rifaximin significantly decreased bloating, abdominal pain, abdominal distension, and flatulence compared with placebo.[37]

Overall, these data suggest that rifaximin, with its favorable safety profile and demonstrated efficacy, is a therapeutic option for patients with IBS-D.

Other prescription medications

Alosetron, a selective 5-HT_3 antagonist, and eluxadoline, a mixed opioid receptor agonist/antagonist, are also approved for IBS-D but have no effect on the gut microbiome.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) involves oral administration of encapsulated fecal material containing distal gut microbiota from a healthy person who serves as a donor.[14] The goal is to treat disease by restoring microbiota typically found in a healthy person. FMT has been effective for *Clostridium difficile* infection, generating speculation that the process may benefit other conditions associated with dysbiosis, including IBS.[14]

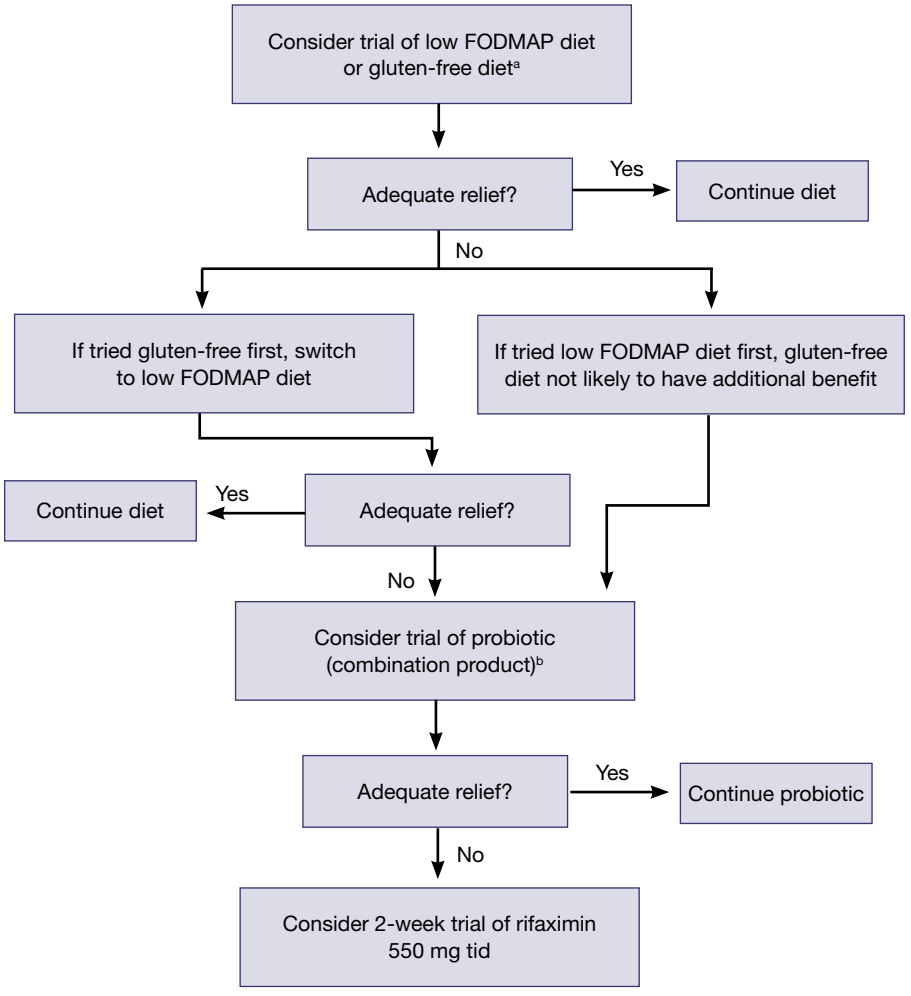
Data about the efficacy of FMT for IBS are scanty and far from conclusive at this time, consisting primarily of several case series reporting relief of symptoms in patients with

IBS who do not respond to conventional therapy.[21,38,39] Among concerns regarding FMT is the potential for long-term risks that may manifest as the development of chronic disease based on alterations in the gut microbiota.[14] For example, transplantation of human fecal microbiota from obese subjects to rodents has been shown to transmit an obesity phenotype.[40] FMT from lean subjects to obese subjects with metabolic syndrome, on the other hand, has proven beneficial, including an increase in insulin sensitivity.[41] Well-designed, large, randomized, controlled studies are required before FMT can be considered a therapeutic option in IBS.

IMPLICATIONS FOR CLINICAL PRACTICE

While our understanding of the role of the gut microbiota and dysbiosis in IBS continues to evolve, several treatment approaches that target the gut microbiota have already demonstrated efficacy in IBS. The current body of knowledge regarding these treatments suggests a logical sequence, or simple algorithm, to guide their use in clinical practice (**FIGURE**).

FIGURE Suggested algorithm for gut microbiota-targeted therapy for IBS



Abbreviations: FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; IBS, irritable bowel syndrome.

[a]Consider ruling out celiac disease in patients with persistent symptoms of gas, bloating, and diarrhea, and those with a family history.

[b]Consider at least a 4-week trial at adequate doses before judging response to treatment.

Diet manipulation should be considered first, including ruling out celiac disease in patients with persistent symptoms of gas, bloating, and diarrhea, as well as patients with a family history.[3] A gluten-free diet trial is a reasonable intervention, especially in patients with IBS-D, mixed irritable bowel syndrome, or predominant symptoms of gas and bloating. Alternatively, or in a patient not responding to a gluten-free diet, a 4-week trial of a low FODMAP diet under the guidance of a dietitian may be helpful. Longer trials need careful monitoring due to the potential for nutritional deficiencies.[3] Initiation of a gluten-free diet in a patient already on a low FODMAP diet is unlikely to provide additional benefit.

Probiotics may be considered in patients in whom dietary modification provides insufficient relief. While evidence does not suggest superiority of 1 microorganism over another, products containing combinations of microorganisms appear to be slightly more effective than single species/strain products. Trial duration should be at least 4 weeks before assessing treatment response.[17]

Rifaximin may be considered for patients with IBS refractory to dietary manipulation and probiotics. The drug is indicated only for the treatment of IBS-D, however.

In conclusion, IBS is one of the most common disorders treated by primary care physicians. Our rapidly accumulating knowledge about the pathophysiologic role of disturbances in the gut microbiota in IBS has prompted manipulation of the microbiota as a new therapeutic target for the disorder. A proposed algorithm suggests a logical approach for utilization of diet, probiotics, and antibiotics in clinical practice to manipulate the gut microbiota in the management of patients with IBS. ●

REFERENCES

1. National Center for Complementary and Integrative Health. 6 Tips: IBS and complementary health practices. https://nccih.nih.gov/health/tips/IBS. Published 2015. Accessed January 27, 2017.
2. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol*. 2014;6:71-80.
3. Lacy BE. The science, evidence, and practice of dietary interventions in irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2015;13(11):1899-1906.
4. Ahmad OF, Akbar A. Dietary treatment of irritable bowel syndrome. *Br Med Bull*. 2015;113(1):83-90.
5. Dossett ML, Davis RB, Lembo AJ, Yeh GY. Complementary and alternative medicine use by US adults with gastrointestinal conditions: Results from the 2012 National Health Interview Survey. *Am J Gastroenterol*. 2014;109(11):1705-1711.
6. Jeffery IB, O'Toole PW. Diet-microbiota interactions and their implications for healthy living. *Nutrients*. 2013;5(1):234-252.
7. National Institutes of Health. NIH Human microbiome project. http://hmpdacc.org/overview/about.php. Published 2016. Accessed January 27, 2017.
8. Gordon JI, Ley RE, Wilson R, et al. Washington University. Extending our view of self: the Human Gut Microbiome Initiative (HGMI). https://www.genome.gov/pages/research/sequencing/seqproposals/hgmiseq.pdf. Published 2015. Accessed January 27, 2017.
9. Lee KN, Lee OY. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. *World J Gastroenterol*. 2014;20(27):8886-8897.
10. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *JAMA*. 2015;313(9):949-958.
11. Moraes-Filho JP, Quigley EM. The intestinal microbiota and the role of probiotics in irritable bowel syndrome: a review. *Arq Gastroenterol*. 2015;52(4):331-338.
12. Noval Rivas M, Crother TR, Arditi M. The microbiome in asthma. *Curr Opin Pediatr*. 2016;28(6):764-771.
13. Vogtmann E, Goedert JJ. Epidemiologic studies of the human microbiome and cancer. *Br J Cancer*. 2016;114(3):237-242.
14. Kelly CR, Kahn S, Kashyap P, et al. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. *Gastroenterology*. 2015;149(1):223-237.
15. Petra AI, Panagiotidou S, Hatziagelaki E, Stewart JM, Conti P, Theoharides TC. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. *Clin Ther*. 2015;37(5):984-995.
16. Forbes JD, Van DG, Bernstein CN. The gut microbiota in immune-mediated inflammatory diseases. *Front Microbiol*. 2016;7:1081.
17. Simren M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut*. 2013;62(1):159-176.
18. Dupont HL. Review article: evidence for the role of gut microbiota in irritable bowel syndrome and its potential influence on therapeutic targets. *Aliment Pharmacol Ther*. 2014;39(10):1033-1042.
19. Ford AC, Quigley EM, Lacy BE, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol*. 2014;109(10):1547-1561.
20. Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med*. 2011;364(1):22-32.
21. Pinn DM, Aroniadis OC, Brandt LJ. Is fecal microbiota transplantation the answer for irritable bowel syndrome? A single-center experience. *Am J Gastroenterol*. 2014;109(11):1831-1832.
22. Distrutti E, Monaldi L, Ricci P, Fiorucci S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World J Gastroenterol*. 2016;22(7):2219-2241.
23. Bennet SM, Ohman L, Simren M, Lee KN, Lee OY. Gut microbiota as potential orchestrators of irritable bowel syndrome. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. *Gut Liver*. 2015;9(3):318-331.
24. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-563.
25. Jumpertz R, Le DS, Turnbaugh PJ, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr*. 2011;94(1):58-65.
26. Nanayakkara WS, Skidmore PM, O'Brien L, Wilkinson TJ, Gearry RB. Efficacy of the low FODMAP diet for treating irritable bowel syndrome: the evidence to date. *Clin Exp Gastroenterol*. 2016;9:131-142.
27. Staudacher HM, Lomer MC, Anderson JL, et al. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutr*. 2012;142(8):1510-1518.
28. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology*. 2014;146(1):67-75.
29. Biesiekierski JR, Newnham ED, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. *Am J Gastroenterol*. 2011;106(3):508-514.
30. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology*. 2013;145(2):320-328.
31. Moayyedi P, Quigley EM, Lacy BE, et al. The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol*. 2014;109(9):1367-1374.
32. Mayer EA, Savidge T, Shulman RJ. Brain-gut microbiome interactions and functional bowel disorders. *Gastroenterology*. 2014;146(6):1500-1512.
33. Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World J Gastroenterol*. 2015;21(10):3072-3084.
34. Foxx-Orenstein AE. New and emerging therapies for the treatment of irritable bowel syndrome: an update for gastroenterologists. *Therap Adv Gastroenterol*. 2016;9(3):354-375.
35. Lembo A, Pimentel M, Rao SS, et al. Repeat treatment with rifaximin is safe and effective in patients with diarrhea-predominant irritable bowel syndrome. *Gastroenterology*. 2016;151(6):113-1121.
36. Pimentel M, Chang C, Chua KS, et al. Antibiotic treatment of constipation-predominant irritable bowel syndrome. *Dig Dis Sci*. 2014;59(6):1278-1285.
37. Di Stefano M, Tana P, Mengoli C, Miceli E, Pagani E, Corazza GR. Colonic hypersensitivity is a major determinant of the efficacy of bloating treatment in constipation-predominant irritable bowel syndrome. *Intern Emerg Med*. 2011;6(5):403-411.
38. Borody TJ, Paramsothy S, Agrawal G. Fecal microbiota transplantation: indications, methods, evidence, and future directions. *Curr Gastroenterol Rep*. 2013;15(8):337.
39. Holvoet T, Joossens M, Wang J, et al. Assessment of faecal microbial transfer in irritable bowel syndrome with severe bloating. *Gut*. 2016;doi:10.1136/gutjnl-2016-312513.
40. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*. 2013;341(6150):1241214.
41. Vrieze A, Van NE, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*. 2012;143(4):913-916.