Gynecologic Oncology 138 (2015) 190–200

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Review

The vaginal and gastrointestinal microbiomes in gynecologic cancers: A review of applications in etiology, symptoms and treatment

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HIGHLIGHTS

- Dysbiotic gut and vaginal microbiota may be implicated in carcinogenesis, therapy-related side effects and treatment outcomes in gynecologic cancers.
- Changes in the microbiome following chemotherapy and radiation may impact patient quality of life and/or treatment outcomes.
- Further research is needed to determine optimal composition, function and efficacy of probiotics in reinstating mucosal homeostasis and barrier function.

ARTICLE INFO

Article history:
Received 29 January 2015
Accepted 25 April 2015
Available online 5 May 2015

Keywords:
Dysbiosis
Commensal microbiota
Radiation and chemotherapy
Probiotics
Epithelial barrier function
Quality of life
Bacterial therapeutics

ABSTRACT

The human microbiome is the collection of microorganisms in the body that exist in a mutualistic relationship with the host. Recent studies indicate that perturbations in the microbiome may be implicated in a number of diseases, including cancer. More specifically, changes in the gut and vaginal microbiomes may be associated with a variety of gynecologic cancers, including cervical cancer, uterine cancer, and ovarian cancer. Current research and gaps in knowledge regarding the association between the gut and vaginal microbiomes and the development, progression, and treatment of gynecologic cancers are reviewed here. In addition, the potential use of probiotics to manage symptoms of these gynecologic cancers is discussed. A better understanding of how the microbiome composition is altered at these sites and its interaction with the host may aid in prevention, optimization of current therapies, development of new therapeutic agents and/or dosing regimens, and possibly limit the side effects associated with cancer treatment.

Contents

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http://dx.doi.org/10.1016/j.ygyno.2015.04.036
0090-8258/

1. Introduction

The human microbiome is the aggregate of microorganisms that reside in the body's mucosal surfaces [1]. These microbes help perform essential functions that include nutrient absorption, establishing/regulating the immune system, and protecting against pathogenic insults. These microbiota also occupy critical niches to prevent pathogen invasion. While these microbial communities populate all mucosal surfaces, the composition of each community varies from site to site within the body depending upon a myriad of host-derived factors (Fig. 1). These factors include available nutrients (e.g. diet), hormone levels, host genetics, race and age [1–3]. This core human microbiome can also be altered by cancer and cancer treatment, which may result in the variable human microbiome as depicted in Fig. 1.

Our knowledge of the human microbiome and its implications in health and disease has grown exponentially in recent years, largely due to advancements in sequencing technology and the establishment of the several international consortia to characterize and understand the role of the human microbiome in health and multiple types of diseases. The most common sequencing methods to characterize the microbiome are pyrosequencing and 16S ribosomal RNA (rRNA) sequencing [4]. The latter method relies on the identification f hypervariable regions in the 16S rRNA gene, which are unique from species to species. This method is advantageous because ribosomes and rRNA are present in all cells, and rRNA sequences are highly conserved. This method can also facilitate the identification of new or lesser-known bacterial species [5].

These sequencing methods have been used to characterize diverse microbial ecosystems, including the human microbiome. A healthy gastrointestinal (GI) microbiome is typically populated by five major bacterial phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria*. These bacteria make up approximately 90% of the total microbiota in the gut (Fig. 2), although the relative abundance of GI microbiota may vary throughout the host's lifespan [6]. In contrast, a healthy vaginal microbiome is typically populated by members of the *Firmicutes* phylum (Fig. 2),dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii* [1]. Similar to GI microbiota, the relative abundance of each of these bacteria may change over time [1].

The sequencing and characterization of GI microbiota have greatly expanded our understanding of how the human microbiome impacts the overall health of the host. For example, the GI microbiome supports functions that include immune system development, digestion, fat metabolism, epithelial homeostasis, enteric nerve regulation, and

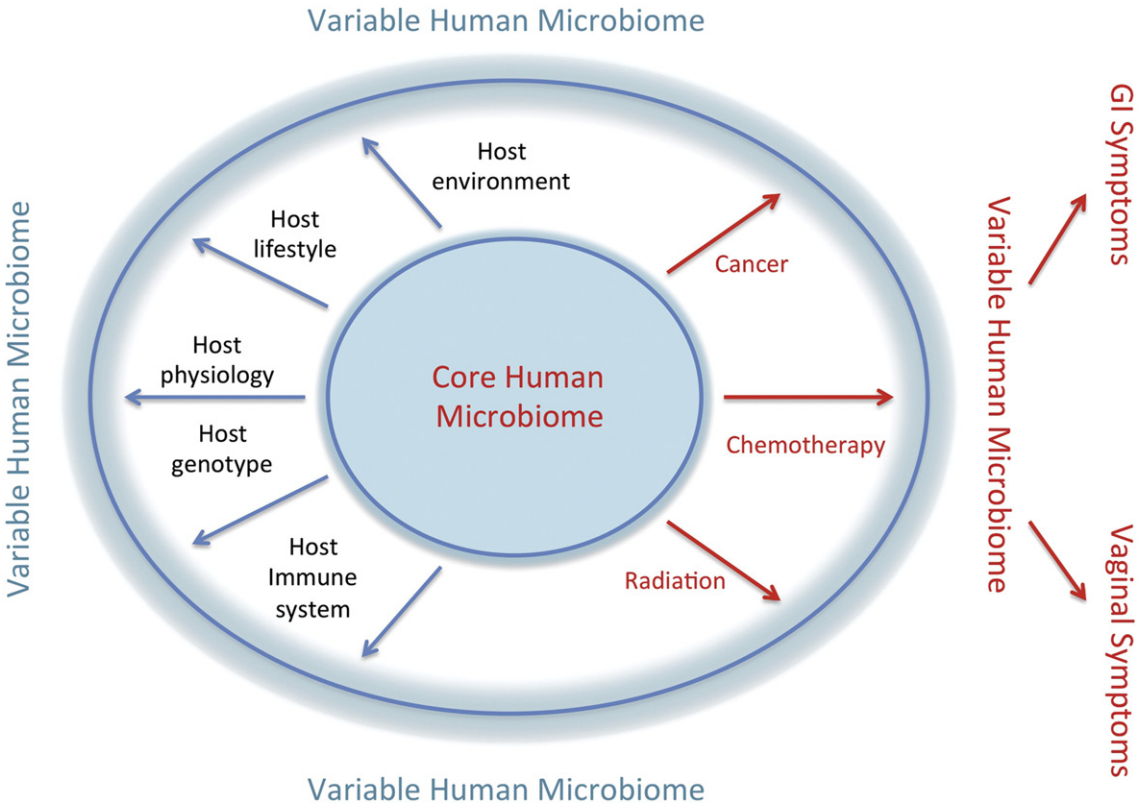

Fig. 1. The core human microbiome is variable, depending on a myriad of factors. These factors (blue) include host environment, lifestyle, physiology, genotype, and immune system. Other conditions (red) that can impact the microbiome composition include cancer, chemotherapy, and radiation treatment, all of which can contribute to GI and vaginal symptoms in cancer patients.

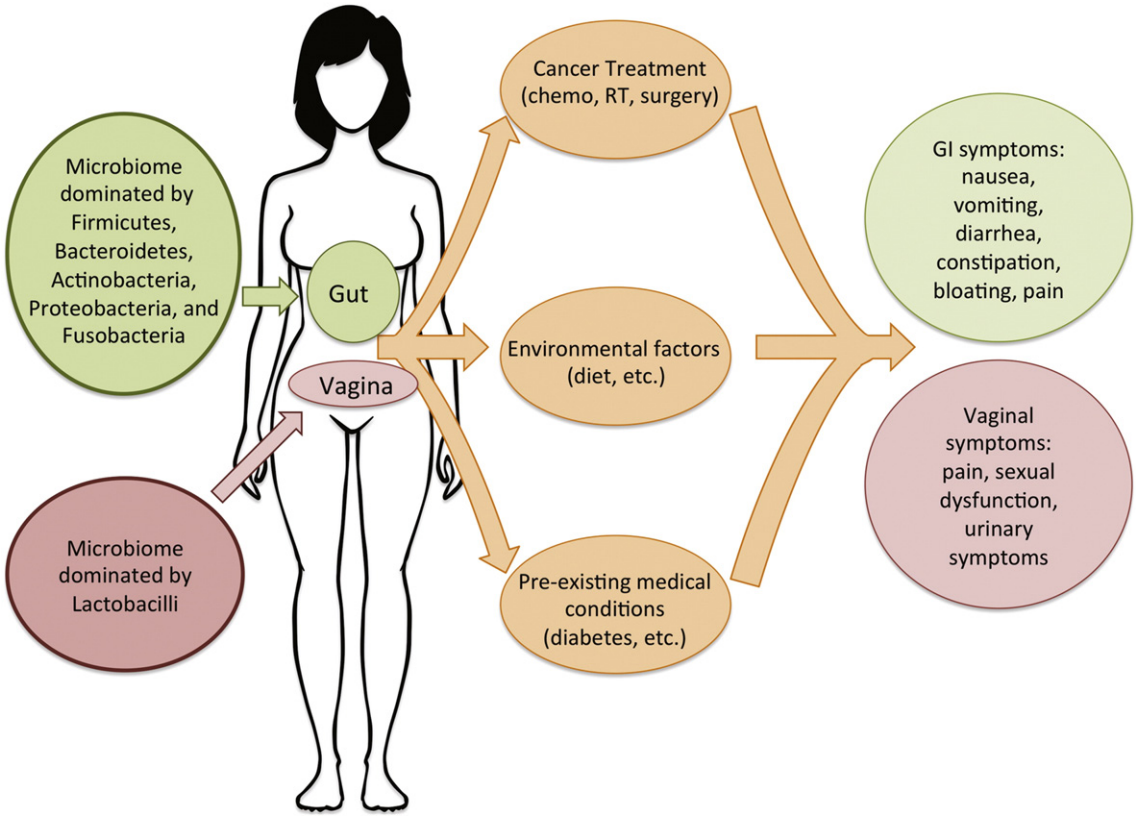

Fig. 2. Factors that impact the gut and vaginal microbiomes that can result in toxicity during cancer and cancer-related treatment. The gut of a healthy female is dominated by *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria*. Cancer treatment, environmental factors, and pre-existing medical conditions can alter the GI microbiota and cause GI symptoms like nausea, vomiting, diarrhea, constipation, bloating, and abdominal pain. The vaginal microbiome of a healthy female is dominated by *Firmicutes*, specifically *Lactobacilli* spp. Perturbations of the vaginal microbiota can contribute to vaginal symptoms including pain, sexual dysfunction, and urinary symptoms.

angiogenesis. As such, disruption of the microbiome, termed dysbiosis, can impact these important processes and result in disease, including cancer [7]. In a healthy female, microbiota in the gut and vagina are separated from the host by a multi-level barrier system that includes, among other factors, a mucus layer, secretion of soluble immune mediators, and intact epithelium with tight junctions (Fig. 3). This barrier system is also supported by immune cells. For example, B cells produce IgA that helps to neutralize pathogenic bacteria. When this multifaceted barrier system fails, pathogenic bacteria can translocate across the gut and vaginal epithelia, causing low-grade chronic inflammation that leads to disease, including cancer [8]. Conversely, cancers of the GI and reproductive tracts cause inflammation (Fig. 3) that results in dysbiosis, resulting in a positive feedback loop that may result in promoting disease [8].

A better understanding of how dysbiosis contributes to disease will allow for the development of novel bacterial therapies that can manipulate the microbiome to reinstate homeostasis. One such example is the use of fecal microbiota transplantation (FMT) in the treatment of *Clostridium difficile* infection (CDI). CDI often follows antibiotic use and is characterized by a decrease in the amount of resident, beneficial bacteria in the gut, which allows *C. difficile* to proliferate and cause dysbiosis of the GI microbiota [9]. FMT reintroduces the beneficial bacteria from a healthy donor into the gut of the infected patient and this aids in recolonization [10]. This technique has been successful for treating these infections and supports the concept that microbiota can be manipulated to restore homeostasis in states of dysbiosis.

This review highlights the existing knowledge from the literature and gaps in our current understanding of the gut and vaginal microbiomes related to specific gynecologic cancers and therapy-related side effects. This review focuses on the three most common gynecologic malignancies and is organized accordingly: cervical, uterine, and ovarian. Within each of these primary sections, there are subsections dedicated to carcinogenesis and therapy-induced symptoms as they relate to microbiota perturbations. We close this review with a section dedicated to symptom management and the potential use of probiotics. The purpose of this review is to highlight the importance of future research focused on studying the gut and vaginal microbiota (VMB) compositions and impacts on the host during carcinogenesis, progression and treatment of gynecologic cancers. Targeting the microbiota could possibly influence patient-reported quality of life (QOL) by reducing cancer- and treatment-related complications in these women.

2. Methods

A comprehensive literature search was conducted using PubMed to search for articles reported in English and published within the last 10 years. The search terms used are detailed below in their respective sections. Relevant abstracts and full-text articles were considered for inclusion in this review. Review articles were also examined to identify additional relevant studies. Studies using human subjects were prioritized, but studies using animal models were included when human data was unavailable.

3. Results

3.1. Cervical cancer

3.1.1. Carcinogenesis

3.1.1.1. The microbiome and HPV infection. Human papillomavirus (HPV) is a well-established causative agent of cervical cancer. More specifically, persistent infection with high-risk HPV (especially HPV

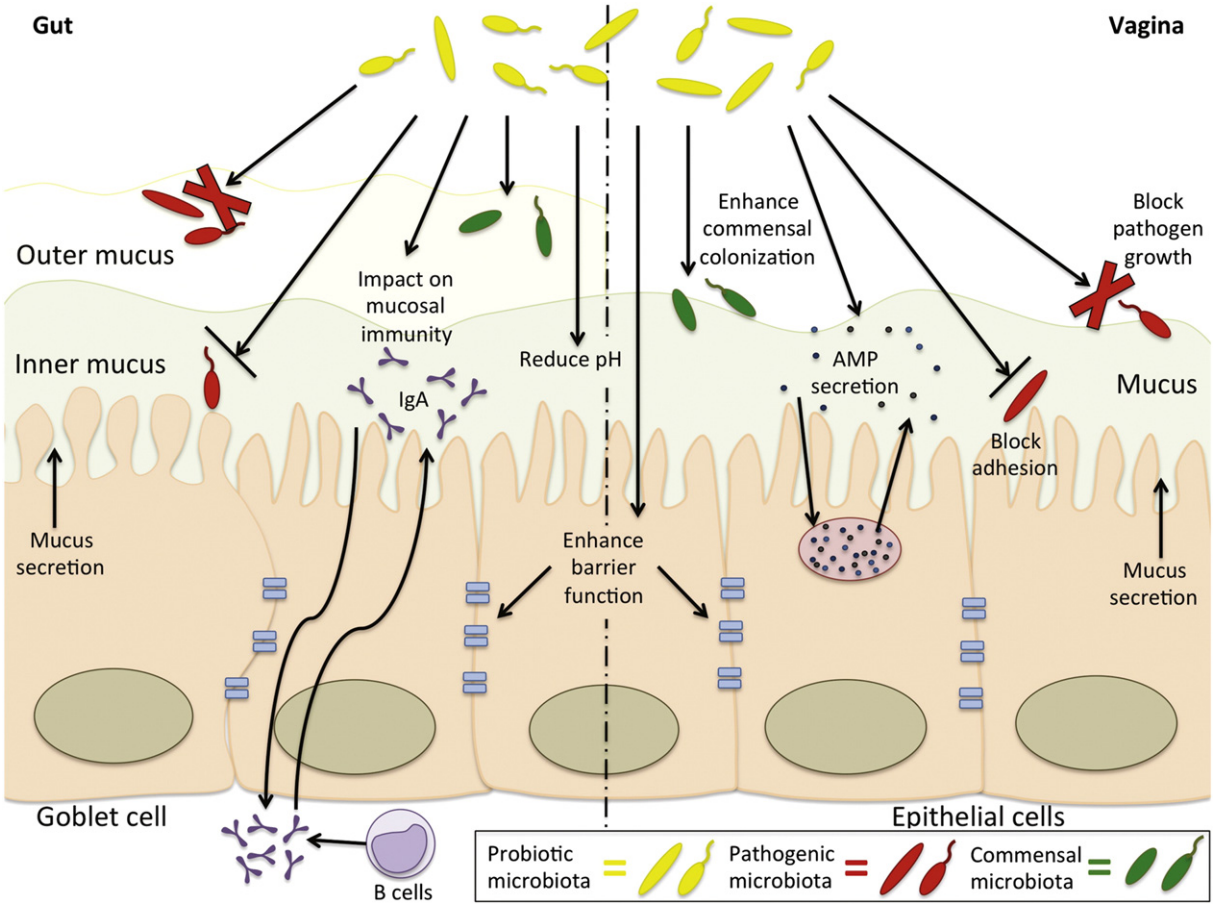

Fig. 3. There is a complex relationship between probiotic, pathogenic, and commensal microbiota in the gut and vagina. At both mucosal sites, probiotic bacteria (yellow) have beneficial functions including lowering the pH locally, bolstering mucosal immunity by interacting with IgA and altering antimicrobial peptide (AMP) secretion, blocking pathogen (red) growth in the mucus layer, and blocking pathogen adhesion to epithelial cells. In addition, probiotic bacteria may serve a beneficial role by enhancing commensal (green) colonization. In both the gut and vagina, these beneficial functions help to enhance epithelial barrier function. When the microbiota are disrupted, however, pathogenic bacteria are able to proliferate in the mucus and adhere to the gut and vaginal epithelial cells. In this state of dysbiosis, epithelial barrier function is disrupted and pathogenic bacteria are able to translocate through the epithelium resulting in inflammation and infection. Microbial translocation can also result in chronic, low-grade inflammation in the gut and vagina, which may cause GI and vaginal symptoms and may even drive carcinogenesis.

type 16 or 18) is directly involved in the pathogenesis of approximately 70% of cervical cancer cases [11]. A PubMed search for the term "vaginal microbiome and HPV" returned 11 results published within the last 10 years, 7 of which were in English and involved human subjects. Four of those 7 papers were primary research articles and are discussed here. The first paper followed 32 women who collected vaginal swabs twice weekly for 16 weeks [12]. These samples were analyzed using pyrosequencing to determine the VMB composition and HPV status of each patient. Results of this study showed that women with *L. gasseri*-dominated microbiota had the fastest HPV remission rate. Additionally, women whose VMB were low in *L. gasseri* and high in *Atopobium* had the slowest rate of HPV remission. Overall, the composition of the VMB was significantly associated with changes in HPV status ($p < 0.001$) [12]. In the second paper, the vaginal microbiota of 70 women — 32 HPV-negative and 38 HPV-positive — were sequenced using PCR [13]. Results of the sequencing showed that there was significantly greater diversity in the microbiota of HPV-positive women ($p < 0.001$) and that *L. gasseri* and *Gardnerella vaginalis* were found in significantly higher frequencies in HPV-positive women ($p = 0.005$ and $p = 0.031$, respectively) [13]. The third paper examined the association between vaginal pH, which is determined by the microbiota composition, and HPV infection. In this study, the vaginal pH and HPV status were determined for 9165 women. Results showed that an elevated vaginal pH, associated with decreased abundance of *Lactobacilli*, was associated with a 30% greater risk of infection with LSIL and with multiple HPV types [14]. The fourth paper was a twin study that followed 68 HPV-infected or uninfected females [15]. The researchers used 454-pyrosequencing to determine the microbiota composition of each woman and found that HPV-positive women had significantly higher microbial diversity and a lower proportion of *Lactobacilli* species, specifically *L. iners* ($p = 0.03$) [15]. Given that the VMB composition has been shown to play a role in HPV infection and the rate of HPV clearance, the vaginal microbiome structure may be associated with the development of cervical cancer secondary to a persistent HPV infection.

3.1.2. Therapy-induced symptoms

3.1.2.1. The microbiome and radiation-induced enteritis. Radiation therapy, used either alone or in conjunction with surgery and/or chemotherapy, is the standard of care for most locally advanced cervical cancers. Unfortunately, radiation therapy can secondarily affect the bowel. Up to 80% of patients experience bowel symptoms — including abdominal pain, urgency, diarrhea, fecal incontinence, and bloating throughout their radiation treatment [16]. While these symptoms often resolve spontaneously within 8 weeks following cessation of treatment, as many as 50% of patients may have chronic radiation enteritis (CRE), defined as symptoms persisting for at least 3 months post-treatment [16]. Using the search term "radiation enteritis and cervical cancer," we identified a key study that followed 117 women who underwent radiotherapy for endometrial or cervical cancer to determine how treatment impacted their QOL [17]. Of the 117 women in this study, 47% indicated that they experienced CRE and that it negatively impacted their QOL.

Younger women and women with cervical cancer were most likely to experience CRE. There was no association found between patient-reported severity of CRE and dose of radiation or stage of cancer, therefore suggesting that alteration of the local GI microbiota could be playing a role in this radiation-mediated toxicity [17].

The search term "microbiome and radiation therapy" yielded a key study that demonstrated that the GI microbiome is altered by radiotherapy specifically in gynecologic cancer patients [18]. Fecal samples from patients undergoing pelvic radiotherapy were compared using 454-pyrosequencing to samples from healthy women, and it was found that the GI microbiota between the two groups differed significantly. The relative abundance of *Actinobacteria* was 30 times higher in cancer patients prior to treatment compared to healthy individuals ($p = 0.001$), whereas the relative abundance of *Fusobacteria* was 7.4 times lower ($p = 0.001$). The relative abundance of each of these bacteria is important because *Actinobacteria* are dominant in the gut and help prevent symptoms like bloating and abdominal pain [19], whereas *Fusobacteria* include invasive, proinflammatory bacteria associated with diseases like inflammatory bowel disease [20]. The study also monitored changes in microbiota at several time points: before treatment (T0), after first radiotherapy fraction (T1), after fifth radiotherapy fraction (T2), and at the end of radiotherapy treatment (T3). The relative abundance of *Fusobacteria* was 6 times higher at T2 compared to T0 ($p = 0.05$), and the abundance of unclassified bacteria increased by 10% between T0 and T3 ($p = 0.04$). While the sample size in this study was small these data suggest that there is a link between the composition of the GI microbiota, health status, and pelvic radiotherapy [18]. It is important to note that health status changes through gynecologic cancer can impact both local and distal mucosal site (e.g. gut) microbiota compositions as shown in this study.

Collectively, there has been very little research that has directly examined GI microbiome composition during radiotherapy and how changes in composition correlate to patient-reported QOL. Given the compelling research demonstrating that CRE has a negative impact on patient QOL and that the GI microbiome is significantly altered by radiotherapy, it is important that future studies investigate how changes in the microbiome correlate with patient-reported QOL.

3.1.2.2. The microbiome and radiation-induced cystitis. Cystitis, inflammation of the bladder wall, is another common side effect of radiation therapy for cervical cancer due to unintentional irradiation of the bladder during treatment. There has been little research on perturbations of the GI microbiome specifically related to radiation-induced cystitis; our literature search for the term "microbiome and radiation-induced cystitis" did not yield any results. However, literature does suggest that there is significant cross-talk between the GI tract and the bladder [21,22]. A clinical study of irritable bowel syndrome (IBS) patients found that rectal lidocaine provided relief not only from bowel symptoms, but also from abdominal pain [21]. While the exact mechanism for this phenomenon is unknown, animal studies have shown that the spinal nerves that respond to colon stimulation also respond to bladder stimulation and vice versa [22]. While these studies must be replicated in humans, this neuronal cross-talk suggests that the bowel may be able to modulate bladder function and pain. With regard to the GI microbiome, perturbations of the microbiota have been implicated in a number of bowel inflammatory and/or pain conditions, including Crohn's patients, whom exhibit diminished bacterial diversity among *Firmicutes* in their GI tracts [23]. There is also growing evidence of the link between human–microbial interface along the human GI tract and the inclusion of the enteric nervous system, which through dense projections carried within the vagus nerve to the brain, can potently modify behaviors or neurological signals through changes in neuro-peptide release [24]. Given that alterations in the GI microbiome are associated with inflammation and pain of the bowel, and given that the bowel and bladder have significant cross-talk through neuronal networks, it is possible that perturbations in the GI microbiome may also contribute to the bladder pain characteristic of interstitial cystitis [25]. Therefore, GI microbiome perturbations during radiotherapy may be partially responsible for the bladder-specific pain of radiation-induced cystitis. However, additional research in this area is necessary to support this hypothesis.

3.1.2.3. The microbiome and other symptoms associated with cervical cancer treatment. There are additional symptoms commonly associated with cervical cancer treatment, including pelvic pain and sexual dysfunction. However, there were no relevant results found using the search terms "microbiome and pelvic pain" and "microbiome and sexual dysfunction." Notably, pelvic pain is a common symptom of cervical cancer and may persist throughout treatment, possibly due to unintentional irradiation of healthy pelvic structures [26]. Sexual dysfunction can also be a side effect of treatment for cervical cancer and may be due to pain following treatment, among other factors [27]. Since conventional photon radiotherapy for cervical cancer irradiates parts of the healthy tissue of the reproductive tract, including the vagina, it is possible that this treatment perturbs the VMB. This perturbation may disrupt epithelial barrier function, permitting translocation of pathogenic bacteria and causing an inflammatory response [8]. This dysbiosis and local inflammation may contribute to pelvic pain and sexual dysfunction in cervical cancer patients. Given the relative lack of current literature related to these topics, it is important that additional research be conducted to investigate these areas.

3.2. Uterine cancer

3.2.1. Carcinogenesis

3.2.1.1. The microbiome and pelvic inflammatory disease. Pelvic inflammatory disease (PID) results when pathogenic bacteria ascend through the cervix into the upper genital tract and cause inflammation of the uterus, fallopian tubes, and/or ovaries [28]. Recent studies have indicated that dysbiosis of the VMB, specifically as seen with bacterial vaginosis (BV), is associated with an increased risk of PID [28,29]. BV is a disease state characterized by a decrease in the abundance of *Lactobacilli* and an increase in the abundance of anaerobic organisms including *Gardnerella*, *Prevotella*, *Atopobium*, *Mobiluncus*, *Ureaplasma*, and *Mycoplasma* [29]. A PubMed search for "bacterial vaginosis and pelvic inflammatory disease" yielded 80 results published within the past 10 years. Of these 80 papers, 61 were in English and involved human subjects; 28 were review articles and were thus excluded from this review. One of the most comprehensive and relevant studies was a multi-center study of 1140 women. In this study, vaginal swabs were used to investigate the association between the dysbiosis seen in BV and the development of PID [29]. Results of the study showed that women with a decrease in *Lactobacilli* species and a relative abundance of *G. vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and anaerobic Gram-negative rods were significantly more likely to develop PID [29]. Therefore, perturbations in the VMB are risk factors for PID. The chronic inflammatory state in the upper genital tract caused by PID may result in uterine endothelial dysfunction. As discussed previously, endothelial dysfunction and chronic inflammation drive carcinogenesis [8]. Therefore, one hypothesis may be that vaginal dysbiosis leads to PID and thus could be associated with the development of endometrial cancer, but additional research is necessary to support this theory.

3.2.1.2. The microbiome and obesity. Our literature search for "microbiome and obesity and uterine cancer" did not yield any relevant results. However, obesity is a well-known risk factor for uterine cancer [30] and has recently been associated with alterations in the GI microbiota [31]. One hypothesis for the association between GI microbiota and obesity is that the composition of the microbiota influences nutrient absorption,

which influences adiposity and body composition [32]. A twin study consisting of 154 individuals was conducted recently to characterize the GI microbiome in lean and obese humans [31]. Results showed a lower relative abundance of *Bacteroidetes* ($p = 0.003$) and a higher abundance of *Actinobacteria* ($p = 0.002$) in obese individuals compared to lean individuals. There was no significant difference in the relative abundance of *Firmicutes* ($p = 0.09$). Obese individuals also had decreased diversity of the GI microbiota in general [31]. These results indicate that alterations in the GI microbiota are associated with obesity. Given that obesity is an established risk factor for uterine cancer, it is possible that perturbations in the GI or vaginal microbiota, or even upper reproductive tract microbiota (as discussed above) are involved in development of this cancer.

3.2.1.3. The microbiome and sex hormones. The composition of the VMB varies significantly as women undergo hormonal changes throughout their lifetime. A recent study of 87 women characterized the composition of the VMB in premenopausal, perimenopausal, and postmenopausal women [33]. The researchers found that the VMB of premenopausal women were dominated by *L. crispatus* and *L. iners*. In contrast, perimenopausal women had higher levels of *L. gasseri*, and postmenopausal women had higher levels of *Streptococcus* and *Prevotella* and an overall decrease in *Lactobacilli* spp. [33]. These results suggest that the VMB composition fluctuates as women undergo hormonal changes throughout their lifetime.

The vaginal microbiome is not the only microbial community associated with hormonal changes in women. Human GI microbiota are closely associated with sex hormones via the estrobolome, defined as the collection of bacterial genes whose products metabolize estrogens [34]. More specifically, enzymes produced by gut bacteria can conjugate and deconjugate estrogen, thus impacting circulating and excreted estrogen levels [34]. This association is important in gynecologic oncology because excess estrogen is a well-established risk factor for the development of uterine cancer [35–37]. Perturbations of GI microbiota have been shown to impact metabolism and excretion of estrogen. Because the composition of the GI microbiome is crucial for the proper metabolism and excretion of estrogen, it is possible that perturbations of the microbiota may result in an increased risk of estrogen-driven cancers, including uterine cancer.

3.2.2. Therapy-induced symptoms

3.2.2.1. The microbiome and vaginal atrophy. Vaginal atrophy is a significant concern for women undergoing treatment for uterine cancer, because atrophy can result from cellular damage due to radiation therapy [38]. A PubMed search for "microbiome and vaginal atrophy" returned 6 results, one of which was a review and thus was not included in this paper. Of the 5 remaining studies, one of the papers specifically examined the association between the VMB and vulvovaginal atrophy. The authors found that women with vulvovaginal atrophy had significantly less *Lactobacillus* than women without the condition [33]. Given that radiation therapy damages the vaginal epithelium [38], pathogenic bacteria may be able to cross the epithelium and cause chronic inflammation that contributes to vaginal atrophy. Vaginal atrophy contributes to sexual dysfunction, which is known to affect QOL post-treatment [39].

Another study identified in this literature search further supported the finding that women with vulvovaginal atrophy have significantly less *Lactobacillus* in their vaginal microbiome than women without the condition [40]. In this 2011 study, the VMB of 32 post-menopausal women were analyzed. From that group of 32 women, 6 healthy women and 4 women with vaginal dryness were selected to identify whether there were any differences in VMB associated with atrophy [40]. Results showed an inverse relationship between the amount of *Lactobacillus* and degree of dryness, and an increase in bacterial diversity among women experiencing moderate to severe dryness [40]. While the sample size of this study was small, the results suggest that vaginal atrophy and dryness are associated with changes in the VMB. These data support the continued investigation of host–VMB interactions in the context of the vaginal epithelium and vulvovaginal atrophy.

3.3. Ovarian cancer

The GI microbiome is of particular interest in ovarian cancer patients since ovarian cancer often presents initially with GI symptoms, including abdominal pain, bloating, indigestion, constipation, and early satiety [41–43]. Patient-reported abdominal discomfort scores are highest at presentation, and they improve slightly throughout treatment. However, compared to other gynecologic cancers, GI symptoms remain heightened throughout ovarian cancer treatment in general [44,45].

3.3.1. Carcinogenesis

3.3.1.1. The microbiome of the upper reproductive tract. The etiology of ovarian cancer is still largely unclear, although a chronic inflammatory state has been implicated in the carcinogenesis [46]. Some hypothesize that BV promotes the ascension of dysbiotic VMB to the upper reproductive tract or that BV could indicate colonization of the upper tract [47–49]. In addition, BV-associated bacteria is a risk factor for pelvic infection and studies have shown that BV-associated bacteria are found in the upper reproductive tract (uterus and fallopian tubes) suggesting a direct link [50–52]. Inflammation in the upper tract could establish a chronic inflammatory state that may influence the development of ovarian cancer, especially in light of recent studies indicating that this process may arise in the fallopian tube [53]. Additional studies are required to determine if there is a link between the microbiome, inflammation and carcinogenesis at these previously considered "sterile" sites of the upper female reproductive tract.

3.3.2. Therapy-induced symptoms

3.3.2.1. The microbiome and changes with chemotherapy. It is important to understand how the GI microbiota change during chemotherapy, because the composition of the GI microbiome may impact the effectiveness of chemotherapy used to treat ovarian cancer. Our literature search for "microbiome and chemotherapy and ovarian cancer" did not yield any relevant results with human subjects. However, this search identified several key animal studies. Alkylating agents like cyclophosphamide are chemotherapy agents used to treat advanced, recurrent and/or persistent ovarian cancer [54]. A recent mouse study examined how cyclophosphamide alters the composition of the microbiota in the small intestine and how these microbiota facilitate an anticancer immune response [55]. In this study, cyclophosphamide treatment resulted in disruption of the epithelial barrier of the small intestine and a decrease in the relative abundance of *Lactobacilli* and *Enterococci*, suggesting that this drug facilitates the translocation of certain bacterial species across the gut epithelium [55]. These bacteria were detected in mesenteric lymph nodes and the spleen within 48 h of treatment [55]. By homing to lymphoid organs, bacteria from the gut helped stimulate an immune response characterized by a significant increase in helper and memory T cells, which may have facilitated the antitumor effects of cyclophosphamide. Additionally, germ-free mice lacking commensal GI microbiota showed a reduction in T cell activation, and their tumors were significantly less responsive to cyclophosphamide [55]. This study demonstrates the importance of GI microbiota in the effectiveness of chemotherapy and suggests that perturbations in GI microbiota may have an impact on chemotherapy effectiveness.

Another recent study used a mouse model to determine whether disruption of the GI microbiota affects the efficacy of CpG-oligonucleotide immunotherapy and platinum chemotherapy, two common anticancer treatments [56]. Compared to wild type mice, mice treated with antibiotics to disrupt the GI microbiota had a diminished response to

immunotherapy [56]. This therapy inhibits tumor growth by stimulating cytokine production, particularly tumor necrosis factor (TNF) from tumor-associated myeloid cells; the cytokines induce a hemorrhagic necrosis of the tumor [56]. In antibiotic-treated mice, cytokine production was significantly diminished and there was minimal tumor necrosis compared to untreated mice [56]. In addition, TNF production was correlated with microbiota composition. It was determined that the presence of *Lactobacillus* species, which have anti-inflammatory effects and reduce TNF production, attenuated tumor response to immunotherapy, while the presence of *Ruminococcus* and *Alistipes* species reconstituted TNF production [56]. A similar response was seen in mice treated with platinum chemotherapy instead of immunotherapy; antibiotic-treated mice had significantly reduced tumor regression and survival [56]. The antitumor effects of platinum chemotherapy are mediated by reactive oxygen species (ROS) and inflammatory responses independent of TNF [56]. In antibiotic-treated mice, expression of pro-inflammatory genes was downregulated and ROS production decreased, accounting for the diminished response to chemotherapy [56]. The results of this study suggest that commensal bacteria in the gut affect the inflammatory responses required for immunotherapy and platinum chemotherapy to be effective, thus indicating that manipulation of particular communities of GI microbiota may enhance cancer treatment and could be a potential target to optimize therapeutic outcomes (Fig. 2).

3.3.2.2. The microbiome and chemotherapy-induced symptoms. Chemotherapy-induced nausea and vomiting (CINV) and chemotherapy-induced fatigue are among the most common side effects of treatment for ovarian cancer. There does not appear to be any current literature directly investigating the association between these symptoms and perturbations in the GI microbiota. However, given how much the microbiome is altered during chemotherapy, it is possible that these changes are associated with nausea/vomiting and fatigue. Therefore, further research is needed in these areas.

3.3.2.3. The microbiome and cachexia. Cachexia is another concern in ovarian cancer patients; malnutrition and cachexia markedly reduce the effectiveness of treatment and increase morbidity and mortality [57]. A PubMed search for "microbiome and cachexia" yielded 5 results, two of which were relevant for this review. One of these articles described a possible mechanism by which cancer-related cachexia and the GI microbiome are related [58]. This report suggested that cachexia in cancer patients is due to increased intestinal permeability secondary to microbial pathogens and/or intestinal inflammation [58]. As discussed above, chemotherapy can result in increased intestinal permeability, resulting in microbial translocation of gut microbes out of the intestine, which then triggers a systemic immune response [58]. This immune response may lead to chronic inflammation that may contribute to metabolic dysfunction, malnutrition, and cachexia. Maintenance of gut barrier function may help counteract symptoms of cancer cachexia, thus illustrating the importance of a healthy GI microbiome in cancer patients [58].

The second relevant article from the literature search for "microbiome and cachexia" discussed how the GI microbiome and cachexia may also be associated via the "GI microbiota–muscle axis," which has both positive and negative effects on muscle mass [59]. A positive effect is that GI microbiota contribute to amino acid bioavailability, which is critical for the production and maintenance of adequate muscle mass to combat cachexia [59]. However, a negative effect of the GI microbiota–muscle axis is that a variety of bacteria in the gut can activate toll-like receptors (TLRs) that activate NF-kB, which in turn can cause muscle wasting [59]. Specific TLRs are triggered by certain microbial products in the gut [60]. NF-kB, a downstream target of TLRs, promotes muscle wasting and atrophy by stimulating the production of proinflammatory cytokines that mediate the cachectic process [61]. Therefore, GI microbiota are closely involved in the TLR/NF-kB pathway that results in muscle atrophy and cachexia [59].

An additional study aimed to characterize the GI microbiota in a mouse model of leukemia with signs of fat loss, muscle atrophy, anorexia, and inflammation. This report showed that the relative abundance of *Lactobacillus* in the gut, which has immunomodulatory properties, was decreased in the mice with cachexia compared with healthy mice [62]. The number of total bacteria and relative abundance of *Bacteroidetes* species did not change significantly [62]. Most telling, however, was the fact that following supplementation with *Lactobacillus reuteri* and *L. gasseri*, levels of inflammatory cytokines that contribute to muscle atrophy and cachexia were reduced, and the weight of the tibialis muscle increased by 8% ($p = 0.05$) [62]. These results suggest that there are specific changes in the composition of the GI microbiota that accompany cachexia in cancer patients, and treatment of this dysbiosis may be beneficial for cachectic patients. However, while these data are promising, it is still necessary to validate the results using human patients by comparing the relative abundance of *Lactobacillus* in the guts of healthy patients to those of cachectic ovarian cancer patients.

3.4. Symptom management

3.4.1. Probiotics

As discussed throughout this review, perturbation of the microbiome may be implicated in many GI and vaginal symptoms associated with gynecologic cancer and treatment. Probiotics, defined as "a product or preparation containing viable and defined microorganisms in a number thought to be sufficient to alter by implantation or colonization the host's microbiota and thereby exert beneficial effects,"[63] are currently under investigation as a method of reinstating microbiome homeostasis (Fig. 3). We searched PubMed for the following terms: "cervical cancer and radiation and probiotics," "uterine cancer and radiation and probiotics," "endometrial cancer and radiation and probiotics," "ovarian cancer and radiation and probiotics," "pelvic cancer and radiation and probiotics," "cervical cancer and chemotherapy and probiotics," "uterine cancer and chemotherapy and probiotics," "endometrial cancer and chemotherapy and probiotics," "ovarian cancer and chemotherapy and probiotics," and "pelvic cancer and chemotherapy and probiotics." The results of these searches were combined, and we identified a total of four relevant primary research articles published in English within the last 10 years (Table 1). All four of these papers examined whether probiotics are effective at reducing radiation-induced diarrhea in gynecologic cancer patients [63–66]. The first paper was a randomized, double blind, placebo controlled trial of 63 advanced cervical cancer patients that investigated whether a probiotic containing live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* could reduce the incidence of diarrhea as the patients underwent radiotherapy with concurrent cisplatin. Grade 2–3 diarrhea was initially reported in 45% of the placebo group and 9% of the study group. Upon follow-up, antidiarrheal medication use was significantly decreased in the placebo group ($p = 0.03$), while the patients in the study group had a significantly improved stool consistency ($p < 0.001$) [64]. The second study was a double blind, placebo controlled trial that followed 482 patients with sigmoid, rectal, or cervical cancer and gave them either a probiotic or a placebo beginning the first day of radiation therapy [63]. The patients given the probiotic had significantly less diarrhea than those given the placebo ($p < 0.001$), and the severity of the diarrhea of patients given the probiotic was significantly lower ($p < 0.001$) [63]. Furthermore, daily bowel movements among the placebo group were 14.7 +/− 6, and only 5.1 +/− 3 in the probiotic group ($p < 0.05$) [63]. The third paper was a randomized, double blind, placebo controlled trial that demonstrated the beneficial effects of probiotics in reducing GI symptoms in patients with either cervical carcinoma or endometrial adenocarcinoma. Forty-one patients were assigned to the placebo group and 44 were assigned to the probiotic group, which involved drinking a probiotic drink daily [66]. The probiotic drink did not significantly reduce the incidence of radiation-induced diarrhea or

Table 1
Clinical trials investigating the impact of probiotics on preventing or alleviating symptoms following gynecologic cancer treatment. PubMed was used to search for the following terms: "cervical cancer and radiation and probiotics," "uterine cancer and radiation and probiotics," "endometrial cancer and radiation and probiotics," "ovarian cancer and radiation and probiotics," "pelvic cancer and radiation and probiotics," "cervical cancer and chemotherapy and probiotics," "uterine cancer and chemotherapy and probiotics," "endometrial cancer and chemotherapy and probiotics," "ovarian cancer and chemotherapy and probiotics," and "pelvic cancer and chemotherapy and probiotics." The results from all of these searches were combined, and we identified a total of 4 relevant primary research articles published in English within the last 10 years. The design and results of these studies are presented below. R = randomized, DB = double blind, PC = placebo controlled.

References	No. of patients (probiotics/control)	Type of study	Bacterial strain investigated/formulation/frequency of treatment	Clinical setting	% of patients with treatment-induced diarrhea	Severity of treatment-induced diarrhea	Anti-diarrheal medication use following probiotic treatment	Stool consistency
Chitapanarux et al. [64]	63 (32/31)	R, DB, PC	*L. acidophilus* plus *Bifidobacterium bifidum*/capsule/twice a day before meals (morning and evening)	Women undergoing radiation with concurrent cisplatin for cervical cancer	–	–	Use decreased in placebo group ($p = 0.03$)	Improved in probiotic group ($p < 0.001$)
Delia et al. [63]	482 (243/239)	DB, PC	VSL#3 (combination of *L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *L. salivarius* subsp. *thermophilus*)/sachet/three times a day	Patients undergoing adjuvant radiation after surgery for sigmoid, rectal, or cervical cancer	51.8% of placebo group vs. 31.6% of probiotic group ($p < 0.001$)	Less severe in probiotic group ($p < 0.001$)	Mean time to medication use was longer in probiotic group ($p < 0.001$)	–
Giralt et al. [66]	85 (44/41)	R, DB, PC	*L. casei* DN-114 001/probiotic drink/three times a day	Women undergoing radiation for cervical carcinoma or endometrial adenocarcinoma	–	–	–	Improved in probiotic group ($p = 0.04$)
Demers et al. [65]	229 (140/89) Standard dose probiotic: n = 81 High dose probiotic: n = 59	R, DB, PC	*L. acidophilus* LAC-361 plus *Bifidobacterium longum* BB-536/capsule/standard dose of 1.3 billion CFU twice a day OR high dose of 10 billion CFU three times a day	Patients undergoing radiation, with or without chemotherapy, for gynecologic, rectal, or prostate cancer	–	Less severe in group receiving standard dose of probiotic ($p = 0.04$)	–	–

use of anti-diarrheal medication ($p = 0.568$), but patients in the probiotic group had significantly better stool consistency ($p = 0.04$) [66]. The fourth and final paper was a randomized, double blind, placebo controlled trial that followed 229 patients with pelvic cancer (either gynecologic, rectal, or prostate) undergoing radiation with or without chemotherapy [65]. Of the 140 patients assigned to the probiotic group, 81 patients took a standard dose (1.3 billion CFU twice a day) and 59 took a high dose (10 billion CFU three times a day). After 60 days, 35% of patients in the standard dose group had no symptoms of moderate to severe diarrhea, compared to only 17% of patients in the placebo group ($p = 0.04$) [65]. Collectively, the results of these studies indicate that probiotics may mitigate radiation-induced diarrhea in gynecologic cancer patients and warrants further study (Table 1).

Another major area of probiotic research is the potential use and beneficial effects on the VMB. As illustrated in Fig. 3, probiotics including *Lactobacilli* may improve vaginal health through a variety of mechanisms [67]. A variety of *Lactobacilli* strains have been studied for reinstating vaginal health in humans. Each strain and combination functions differently. *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 strains have been shown to modulate host defense, interfere with pathogen growth, induce anti-adhesion and antivirulence factors and disrupt biofilm formation [68–71]. Oral administration of the GR-1/RC-14 combination in humans was shown to decrease urinary tract infection (UTI) recurrence and to increase the probability of a *Lactobacilli* dominant VMB [68–71]. Intravaginal delivery of *L. crispatus* CTV05 has shown to decrease levels of bacterial vaginosis-associated bacteria when this strain persists more than 28 days and significantly reduced UTI recurrence [72]. A recent, well-designed meta-analysis also focused on the role of probiotics in the treatment of BV [73]. This meta-analysis identified 12 high-quality studies in the field and specifically singled out 4 papers as being the highest quality of these 12 [74–77],due to appropriate randomization, double-blind structure, and description of withdrawals and dropouts. Two of these 4 papers reinforced the efficacy of oral GR-1/RC-14 probiotics in treating BV [74,75]. The other 2 papers described the efficacy of different bacterial strains as probiotics, including *Lactobacillus brevis* CD2, *Lactobacillus salivarius* FV2, and *Lactobacillus plantarum* [76,77]. Taken together, these limited studies support the continued investigation of probiotics and bacterial therapeutics in the treatment of BV and vaginal dysbiosis. However, more research is needed to determine the optimal strain(s), dosage, formulation, timing and route of administration to reinstate vaginal health.

Probiotics have also been investigated for their beneficial role in the clearance of HPV, a virus associated with the development of cervical cancer. Our literature search on PubMed for "probiotics and HPV" yielded 6 results published within the last 10 years. Of these 6 articles, one was a review article and thus is not discussed in this paper. Two of the papers examined whether recombinant and non-recombinant lactic acid bacteria could be used for vaccination against HPV; since these studies investigated the role of probiotics as vectors, not as a therapeutic treatment, they were not included in this section. The remaining 3 papers regarding probiotic treatment and HPV clearance are discussed here. The first in vitro study examined the impact of probiotic treatment on the expression of HPV16 E6 and E7 in SiHa cells. Results indicated that after 48 h, both E6 and E7 mRNA transcript levels decreased following treatment with *Bifidobacterium adolescentis* in cervical cancer cells ($p < 0.01$ and $p < 0.05$, respectively); however, E6 and E7 protein expressions were not significantly changed [78]. The significant decline in mRNA transcript levels indicates that *B. adolescentis* may exhibit

some level of antiviral activity against HPV E6 and E7 [78]. The second study investigated the cytotoxic effects of *Lactobacillus* on normal cervical cells compared to HeLa (cervical cancer) cells [79]. Normal cervical and HeLa cells were exposed to culture supernatants, cytoplasmic extracts, cell wall extracts, and live cells of *L. gasseri* and *L. crispatus*. Cell growth inhibition was measured using an MTT assay. Upon exposure to the supernatant of *Lactobacilli*, HeLa cell growth was significantly inhibited compared to normal cells ($p < 0.05$), indicating that *Lactobacilli* exert cytotoxic effects on cervical cancer cells, but not on healthy cells [79]. However, it is important to note that the effects of bacteria on cultured cells may not accurately approximate the effects of probiotics on cells in vivo, therefore more research is needed in this area. In the third study, 54 women with HPV+ low-grade squamous intraepithelial lesions were monitored for 6 months; the intervention group consumed a daily probiotic drink containing *Lactobacillus casei*, while the control group received no treatment [80]. After 6 months, the probiotic users had double the chance of clearance of cytological abnormalities (60% vs. 31% in women who received no treatment, $p = 0.05$). Additionally, HPV was cleared in 29% of probiotic users and only 19% of control patients, although not significant ($p = 0.41$) [80]. These results suggest that probiotics may play a role in promoting the clearance of HPV, although the mechanism is unknown. Collectively, these studies highlight the need to further study the pleiotropic mechanisms of beneficial bacteria and their application to promoting vaginal health. Additional research is needed to investigate the mechanisms of specific bacterial strains delivered orally or locally and how dosage and formulation may impact VMB composition, recolonization and restoration of vaginal health and how it could be applied to the prevention and/or treatment of gynecologic cancer.

3.4.2. Necessity of a personalized approach to symptom management

Despite the strong evidence indicating the efficacy of probiotics in reducing GI symptoms in gynecologic cancer patients, it is necessary to take a personalized approach when considering this treatment. One important consideration is whether a patient is immunocompromised, as the *Lactobacillus* found in probiotics may be an opportunistic pathogen and a rare cause of sepsis in immunocompromised patients [81,82]. The primary concern is that immunocompromised cancer patients may have GI endothelial barrier dysfunction that allows the *Lactobacillus* from the probiotic to translocate across the epithelium and cause sepsis [83]. However, it is important to note that cases of *Lactobacillus* sepsis are very rare and probiotics are often used in cancer patients with no adverse effects [83,84].

Other considerations for the use of probiotics in GI symptom management should include factors like the age, race, diet, and hormonal status of the patient, because all of these factors have been shown to affect the composition of the GI microbiome [1–3]. Therefore, these factors may also impact the efficacy of probiotics. There does not appear to be any research directly investigating the impact of these factors on the efficacy of probiotics, but these variables may be another aspect to consider when recommending probiotic treatment for gynecologic cancer patients.

3.5. Future perspectives

As demonstrated in this review, there are large gaps in knowledge regarding the microbiome and gynecologic cancers. Continued investigation of the core and variable microbiome in the context of gynecologic cancer is warranted (Figs. 1 and 2). In addition, further investigations into the host–microbiota and metabolome are needed to improve treatment and develop new interventions for women's health.

Animal models will be critical in studying the role of the microbiome in cancer development and progression. As discussed previously in this review, animal models have been used to assess different aspects of the relationship between dysbiosis, cancer, and GI/vaginal symptoms. These models may also be useful for evaluating the possible association between probiotics and sepsis in immunocompromised patients.

Human cell culture models and human clinical trials will also be of critical importance for future research. Human cell culture models are being utilized to investigate host–microbiome interactions [85] and gain a better understanding of the relationship between epithelial barrier dysfunction, bacterial translocation, and carcinogenesis [8]. Furthermore, it is of critical importance that we identify how these bacteria are interacting with host cells through evaluation of host cell–microbe interactions. Host factors that may impact the microbiome composition, including ethnic and/or socioeconomic diversity, will also be important for addressing health disparities related to cancer. Most clinical trials to date have focused on the efficacy of probiotics for reducing GI symptoms in gynecologic cancer patients [63,64,66,78,80,86,87], but there is little research on vaginal symptoms or the microbiota. Additional clinical trials will be useful for determining the efficacy of these agents for reducing GI and vaginal symptoms and whether they should be recommended for symptom management in gynecologic cancer patients.

4. Conclusion

Foundational studies on the human microbiome are increasing, largely due to technological advances with human in vitro models, sequencing technology and large scale international research consortia, such as the Human Microbiome Project. New knowledge in this field has the potential to revolutionize our approach to health and disease. Much of the current research has focused on the GI microbiome and how it impacts the health of the host in general. For example, the relationship between the GI microbiome and colorectal cancer is well documented. However, there is an important gap in the studies investigating the association between the gut and vaginal microbiomes and gynecologic cancers. Studies to date are encouraging, but there must be more research conducted on the topic of the human microbiome and gynecologic cancer. Dysbiosis or community structure may directly impact development, progression, and persistence of cancer. Additional research in this field is also necessary to help determine whether the relationship between microbiota perturbations and gynecologic cancers is correlational or causational, as research in this area is still in its infancy. Employing clinical, translational, and basic science strategies and partnerships are required to better understand the role of the human microbiome in gynecologic cancer and develop new preventatives or treatment strategies. Enhanced understanding of the microbiota composition and host–microbiota interactions will ultimately aid in improving therapeutics, development of new agents and/or dosing regimens and possibly limit toxicities associated with treatment.

Conflict of interest disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Acknowledgments

The authors would like to thank Mr. Sean Winkle for graphical assistance and Ms. Daniele Sumner for her editorial and administrative support. In addition, we apologize to all of those whose papers could not be cited due to scope and space limitations. This work was supported in part by the Flinn Foundation (grant no. 1974) (DMC and MMH-K).

References

[1] Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 2011; 108(Suppl. 1):4680–7.

[2] Jaaback K, Johnson N, Lawrie TA. Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer. Cochrane Database Syst Rev 2011; 11:CD005340.
[3] Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol 2012;66:371–89.
[4] Balzer S, Malde K, Lanzen A, Sharma A, Jonassen I. Characteristics of 454 pyrosequencing data—enabling realistic simulation with flowsim. Bioinformatics 2010; 26(18):i420–5.
[5] Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 2007;45(9):2761–4.
[6] Kosiewicz MM, Zirnheld AL, Alard P. Gut microbiota, immunity, and disease: a complex relationship. Front Microbiol 2011;2:180.
[7] Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome–host metabolic signal disruption in health and disease. Trends Microbiol 2011;19(7):349–59.
[8] Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013;13(11): 800–12.
[9] Chase DM, Kauderer J, Wenzel L, Ramondetta L, Cella D, Long 3rd HJ, et al. Factors associated with grade 3 or 4 treatment-related toxicity in women with advanced or recurrent cervical cancer: an exploratory analysis of NRG Oncology/Gynecologic Oncology Group Trials 179 and 204. Int J Gynecol Cancer 2015;25(2):303–8.
[10] Agito MD, Atreja A, Rizk MK. Fecal microbiota transplantation for recurrent *C. difficile* infection: ready for prime time? Cleve Clin J Med 2013;80(2):101–8.
[11] Ramakrishnan S, Partricia S, Mathan G. Overview of high-risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention. Biomed Pharmacother 2015; 70:103–10.
[12] Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM, Ravel J, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis 2014;210(11):1723–33.
[13] Gao W, Weng J, Gao Y, Chen X. Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. BMC Infect Dis 2013;13:271.
[14] Clarke MA, Rodriguez AC, Gage JC, Herrero R, Hildesheim A, Wacholder S, et al. A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection. BMC Infect Dis 2012;12:33.
[15] Lee JE, Lee S, Lee H, Song YM, Lee K, Han MJ, et al. Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PLoS ONE 2013; 8(5):e63514.
[16] Andreyev J. Gastrointestinal complications of pelvic radiotherapy: are they of any importance? Gut 2005;54(8):1051–4.
[17] Abayomi J, Kirwan J, Hackett A. The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. Eur J Oncol Nurs 2009;13(4):262–7.
[18] Nam YD, Kim HJ, Seo JG, Kang SW, Bae JW. Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. PLoS ONE 2013;8(12):e82659.
[19] Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care 2010;33(10):2277–84.
[20] Strauss J, Kaplan GG, Beck PL, Rioux K, Panaccione R, Devinney R, et al. Invasive potential of gut mucosa-derived *Fusobacterium nucleatum* positively correlates with IBD status of the host. Inflamm Bowel Dis 2011;17(9):1971–8.
[21] Verne GN, Robinson ME, Vase L, Price DD. Reversal of visceral and cutaneous hyperalgesia by local rectal anesthesia in irritable bowel syndrome (IBS) patients. Pain 2003;105(1–2):223–30.
[22] Ustinova EE, Gutkin DW, Pezzone MA. Sensitization of pelvic nerve afferents and mast cell infiltration in the urinary bladder following chronic colonic irritation is mediated by neuropeptides. Am J Physiol Renal Physiol 2007;292(1):F123–30.
[23] Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 2006;55(2):205–11.
[24] Mayer EA. Gut feelings: the emerging biology of gut–brain communication. Nat Rev Neurosci 2011;12(8):453–66.
[25] Klumpp DJ, Rudick CN. Summation model of pelvic pain in interstitial cystitis. Nat Clin Pract Urol 2008;5(9):494–500.
[26] Bergmark K, Avall-Lundqvist E, Dickman PW, Henningsohn L, Steineck G. Vaginal changes and sexuality in women with a history of cervical cancer. N Engl J Med 1999;340(18):1383–9.
[27] Greenwald HP, McCorkle R. Sexuality and sexual function in long-term survivors of cervical cancer. J Womens Health (Larchmt) 2008;17(6):955–63.
[28] Sharma H, Tal R, Clark NA, Segars JH. Microbiota and pelvic inflammatory disease. Semin Reprod Med 2014;32(1):43–9.
[29] Ness RB, Kip KE, Hillier SL, Soper DE, Stamm CA, Sweet RL, et al. A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease. Am J Epidemiol 2005;162(6):585–90.
[30] Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4(8):579–91.
[31] Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009;457(7228):480–4.
[32] Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, 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.
[33] Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, et al. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause 2014;21(5):450–8.
[34] Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe 2011;10(4): 324–35.
[35] Xu D, Lin TH, Yeh CR, Cheng MA, Chen LM, Chang C, et al. The wedelolactone derivative inhibits estrogen receptor-mediated breast, endometrial, and ovarian cancer cells growth. Biomed Res Int 2014;2014:713263.
[36] Antunes CM, Strolley PD, Rosenshein NB, Davies JL, Tonascia JA, Brown C, et al. Endometrial cancer and estrogen use. Report of a large case–control study. N Engl J Med 1979;300(1):9–13.
[37] Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 1995;85(2): 304–13.
[38] Dinkelspiel HE, Wright JD, Lewin SN, Herzog TJ. Contemporary clinical management of endometrial cancer. Obstet Gynecol Int 2013;2013:583891.
[39] Ye S, Yang J, Cao D, Lang J, Shen K. A systematic review of quality of life and sexual function of patients with cervical cancer after treatment. Int J Gynecol Cancer 2014; 24(7):1146–57.
[40] Hummelen R, Macklaim JM, Bisanz JE, Hammond JA, McMillan A, Vongsa R, et al. Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness. PLoS ONE 2011;6(11):e26602.
[41] Goff BA, Mandel LS, Drescher CW, Urban N, Gough S, Schurman KM, et al. Development of an ovarian cancer symptom index: possibilities for earlier detection. Cancer 2007;109(2):221–7.
[42] Goff BA, Mandel LS, Melancon CH, Muntz HG. Frequency of symptoms of ovarian cancer in women presenting to primary care clinics. JAMA 2004;291(22):2705–12.
[43] Goff BA, Mandel L, Muntz HG, Melancon CH. Ovarian carcinoma diagnosis. Cancer 2000;89(10):2068–75.
[44] Wenzel LB, Huang HQ, Armstrong DK, Walker JL, Cella D, Gynecologic Oncology G. Health-related quality of life during and after intraperitoneal versus intravenous chemotherapy for optimally debulked ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol 2007;25(4):437–43.
[45] Minig L, Velez JI, Trimble EL, Biffi R, Maggioni A, Jeffery DD. Changes in short-term health-related quality of life in women undergoing gynecologic oncologic laparotomy: an associated factor analysis. Support Care Cancer 2013;21(3):715–26.
[46] Poole EM, Lee IM, Ridker PM, Buring JE, Hankinson SE, Tworoger SS. A prospective study of circulating C-reactive protein, interleukin-6, and tumor necrosis factor alpha receptor 2 levels and risk of ovarian cancer. Am J Epidemiol 2013;178(8): 1256–64.
[47] Krohn MA, Hillier SL, Nugent RP, Cotch MF, Carey JC, Gibbs RS, et al. The genital flora of women with intraamniotic infection. Vaginal Infection and Prematurity Study Group. J Infect Dis 1995;171(6):1475–80.
[48] Silver HM, Sperling RS, St Clair PJ, Gibbs RS. Evidence relating bacterial vaginosis to intraamniotic infection. Am J Obstet Gynecol 1989;161(3):808–12.
[49] Hillier SL, Krohn MA, Cassen E, Easterling TR, Rabe LK, Eschenbach DA. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact fetal membranes. Clin Infect Dis 1995;20(Suppl. 2):S276–8.
[50] Hillier SL, Kiviat NB, Hawes SE, Hasselquist MB, Hanssen PW, Eschenbach DA, et al. Role of bacterial vaginosis-associated microorganisms in endometritis. Am J Obstet Gynecol 1996;175(2):435–41.
[51] Kiviat NB, Wolner-Hanssen P, Eschenbach DA, Wasserheit JN, Paavonen JA, Bell TA, et al. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. Am J Surg Pathol 1990;14(2):167–75.
[52] Mitchell CM, Haick A, Nkwopara E, Garcia R, Rendi M, Agnew K, et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. Am J Obstet Gynecol 2015;212(5):611.e1–9.
[53] Tone AA, Salvador S, Finlayson SJ, Tinker AV, Kwon JS, Lee CH, et al. The role of the fallopian tube in ovarian cancer. Clin Adv Hematol Oncol 2012;10(5):296–306.
[54] Malayev Y, Levene R, Gonzalez F. Palliative chemotherapy for malignant ascites secondary to ovarian cancer. Am J Hosp Palliat Care 2012;29(7):515–21.
[55] Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannani D, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013;342(6161):971–6.
[56] Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342(6161):967–70.
[57] Balogun N, Forbes A, Widschwendter M, Lanceley A. Noninvasive nutritional management of ovarian cancer patients: beyond intestinal obstruction. Int J Gynecol Cancer 2012;22(6):1089–95.
[58] Klein GL, Petschow BW, Shaw AL, Weaver E. Gut barrier dysfunction and microbial translocation in cancer cachexia: a new therapeutic target. Curr Opin Support Palliat Care 2013;7(4):361–7.
[59] Bindels LB, Delzenne NM. Muscle wasting: the gut microbiota as a new therapeutic target? Int J Biochem Cell Biol 2013;45(10):2186–90.
[60] Boyd JH, Divangahi M, Yahiaoui L, Gvozdic D, Qureshi S, Petrof BJ. Toll-like receptors differentially regulate CC and CXC chemokines in skeletal muscle via NF-kappaB and calcineurin. Infect Immun 2006;74(12):6829–38.
[61] Tisdale MJ. Biology of cachexia. J Natl Cancer Inst 1997;89(23):1763–73.
[62] Bindels LB, Beck R, Schakman O, Martin JC, De Backer F, Sohet FM, et al. Restoring specific lactobacilli levels decreases inflammation and muscle atrophy markers in an acute leukemia mouse model. PLoS ONE 2012;7(6):e37971.
[63] Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, et al. Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 2007;13(6):912–5.
[64] Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V. Randomized controlled trial of live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiat Oncol 2010;5:31.

[65] Demers M, Dagnault A, Desjardins J. A randomized double-blind controlled trial: impact of probiotics on diarrhea in patients treated with pelvic radiation. Clin Nutr 2014;33(5):761–7.
[66] Giralt J, Regadera JP, Verges R, Romero J, de la Fuente I, Biete A, et al. Effects of probiotic *Lactobacillus casei* DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys 2008;71(4):1213–9.
[67] Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update 2015;21(3):353–77.
[68] Macklaim JM, Cohen CR, Donders G, Gloor GB, Hill JE, Parham GP, et al. Exploring a road map to counter misconceptions about the cervicovaginal microbiome and disease. Reprod Sci 2012;19(11):1154–62.
[69] Li J, Wang W, Xu SX, Magarvey NA, McCormick JK. *Lactobacillus reuteri*-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. Proc Natl Acad Sci U S A 2011;108(8):3360–5.
[70] McMillan A, Dell M, Zellar MP, Cribby S, Martz S, Hong E, et al. Disruption of urogenital biofilms by lactobacilli. Colloids Surf B: Biointerfaces 2011;86(1):58–64.
[71] Hummelen R, Changalucha J, Butamanya NL, Cook A, Habbema JD, Reid G. *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 to prevent or cure bacterial vaginosis among women with HIV. Int J Gynaecol Obstet 2010;111(3):245–8.
[72] Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, et al. Randomized, placebo-controlled phase 2 trial of a *Lactobacillus crispatus* probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis 2011;52(10):1212–7.
[73] Huang H, Song L, Zhao W. Effects of probiotics for the treatment of bacterial vaginosis in adult women: a meta-analysis of randomized clinical trials. Arch Gynecol Obstet 2014;289(6):1225–34.
[74] Anukam K, Osazuwa E, Ahonkhai I, Ngwu M, Osemene G, Bruce AW, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial. Microbes Infect 2006;8(6):1450–4.
[75] Martinez RC, Franceschini SA, Patta MC, Quintana SM, Gomes BC, De Martinis EC, et al. Improved cure of bacterial vaginosis with single dose of tinidazole (2 g), *Lactobacillus rhamnosus* GR-1, and *Lactobacillus reuteri* RC-14: a randomized, double-blind, placebo-controlled trial. Can J Microbiol 2009;55(2):133–8.
[76] Mastromarino P, Macchia S, Meggiorini L, Trinchieri V, Mosca L, Perluigi M, et al. Effectiveness of *Lactobacillus*-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. Clin Microbiol Infect 2009;15(1):67–74.
[77] Ya W, Reifer C, Miller LE. Efficacy of vaginal probiotic capsules for recurrent bacterial vaginosis: a double-blind, randomized, placebo-controlled study. Am J Obstet Gynecol 2010;203(2) (120 e1–6).
[78] Cha MK, Lee DK, An HM, Lee SW, Shin SH, Kwon JH, et al. Antiviral activity of *Bifidobacterium adolescentis* SPM1005-A on human papillomavirus type 16. BMC Med 2012;10:72.
[79] Motevaseli E, Shirzad M, Akrami SM, Mousavi AS, Mirsalehian A, Modarressi MH. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. J Med Microbiol 2013;62(Pt 7):1065–72.
[80] Verhoeven V, Renard N, Makar A, Van Royen P, Bogers JP, Lardon F, et al. Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. Eur J Cancer Prev 2013;22(1):46–51.
[81] Schlegel L, Lemerle S, Geslin P. *Lactobacillus* species as opportunistic pathogens in immunocompromised patients. Eur J Clin Microbiol Infect Dis 1998;17(12):887–8.
[82] Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. *Lactobacillus* sepsis associated with probiotic therapy. Pediatrics 2005;115(1):178–81.
[83] Liong MT. Safety of probiotics: translocation and infection. Nutr Rev 2008;66(4):192–202.
[84] Van den Nieuwboer M, Brummer RJ, Guarner F, Morelli L, Cabana M, Claasen E. The administration of probiotics and synbiotics in immune compromised adults: is it safe? Benefic Microbes 2015;6(1):3–17.
[85] Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. J Infect Dis 2014;209(12):1989–99.
[86] Collado Yurrita L, San Mauro Martin I, Ciudad-Cabanas MJ, Calle-Puron ME, Hernandez Cabria M. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. Nutr Hosp 2014;30(n02):244–52.
[87] Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, 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):1547–61.