Review

Gut microbiome: pertinence in fibromyalgia

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ABSTRACT

The human gut microbiome constitutes a diverse and dynamic community of microorganisms that inhabit the digestive tract. In recent years, there is growing appreciation for the role of the gut microbiome in host health and disease. Gut bacteria are involved in the pathogenesis of numerous medical conditions in a variety of medical fields including gastroenterology, metabolic, rheumatologic, neurologic and psychiatric disorders. Recently, evidence is mounting that gut bacteria could also play a role in chronic pain and specifically fibromyalgia (FM). The composition of the gut bacterial community is altered in individuals with FM, with an altered abundance of a small subset of bacterial species. Some of these species, either with increased or decreased abundance in patients, have established metabolic activity which could have pertinence in the expression of FM symptoms. The putative mechanisms which could allow these bacterial species to affect pain, fatigue, mood and other symptoms include the entry of short-chain-fatty-acids, bile acids, neurotransmitters and bacterial antigens into the host circulation. While these are merely the first steps in understanding the role of the gut microbiome in chronic pain and specifically FM, one might envision exciting future perspectives for better mechanistic understanding of FM, for the development of objective diagnostic aids and potentially for new therapeutic modalities.

Introduction

Almost every patient with fibromyalgia (FM) will at some time ask the question "is there anything I can do about my diet to help my condition?" To date, physicians have mostly recommended that patients adhere to a good balanced diet, with attention to total calorie control and with reduced fats and sugars, but without evidence that these strategies will specifically impact FM. Now, with the emergence of the science of the microbiome in both health and disease, this question has renewed pertinence for the care of patients with FM. Since the first reports of a syndrome characterised by chronic widespread pain without any defining clinical or laboratory abnormality, FM has defied the usual modern trajectory of clinical diagnosis and remains an ethereal condition (1). There are even those that contest the very existence of FM (2). Neurophysiological study in the past few decades has pointed towards a primary neurological mechanism as the underpinnings of the pathogenesis of FM, with changes characterised as a sensitisation phenomenon in both the peripheral as well as the central nervous system (CNS). Researchers have looked to understand mechanisms that might explain the development of sensitisation, with the new preferred term of nociplastic pain, defined as pain arising from altered nociception in the absence of specific tissue changes causing activation of nociceptors or abnormality of the nervous system (3). This is where the gut microbiome has potential clinical implications in the understanding and care of patients with FM. Some patients consistently report either exacerbation or relief of symptoms related to some food products, but without any consistent evidence to support these reports. So enter the concept of the gut-brain axis, a hypothesis with increasing scientific validity, that gut microbiome, either via leakage of whole microbes, components of microbes or the microbe-associated metabolites enter the body and have effects on nervous system functions (4-7). There is a bidirectional interplay between the gut and the nervous system. Neurological effects mediated via the autonomic nervous system as well

as the hypothalamic pituitary adrenal axis are directed to intestinal functional effector cells, which in turn are under the influence of the gut microbiota. The gut microbiome affects many aspects of neurological functioning, including emotional and cognitive effects, but with increasing evidence for effect in pain conditions. This therefore raises the question of whether the microbiome may have implications in FM.

Gut microbiome

The gut microbiome consists of a huge number of microorganisms existing in an ever-changing state in the human gut. These include bacteria, archaea, fungi, viruses, protozoa and helminths, all forming a rich and complex ecosystem. The composition of the gut microbiome is dynamic and is influenced by both host and environmental factors. From first colonisation within a few hours of birth, the microbiome changes as diet progressively changes towards an adult diet. While the composition of the microbiome is shaped by host and environmental features, the microbiome in turn has effects on physiologic, metabolic and immune functions of the host (8). The mechanisms which allow for these bi-directional effects include, but are not limited to, infiltration of bacterial antigens eliciting an immune response, secretion of biologically active bacterial metabolites that have effect on multiple host organs, metabolism and degradation of ingested nutrients and medications to name only a few (8). These, as well as other mechanisms are thought to facilitate the critical role of the gut microbiome on the host's health and diseases.

Alterations in the gut microbiome have been observed in countless disorders, involving almost all body organs. In some cases, these alterations remain within the realm of a mere correlation, while in others a causal role for gut bacteria has been demonstrated (8-11). Altered gut microbiome has been shown to play a role in gastrointestinal diseases ranging from inflammatory bowel diseases to irritable bowel syndrome and cirrhosis (7, 12, 13); in rheumatic disorders such as rheumatoid arthritis and ankylosing spondylitis (14-17); in metabolic disorders such as diabetes and obesity (10, 18); and similarly, in oncologic, cardiovascular, dermatologic and dental disorders (11, 14, 19, 20). One of the most intriguing aspects of the gut microbiome interaction with the host is the effect on the CNS. This so called 'gut-brain-axis' appears to be an intricate bidirectional communication axis between the gut and the brain, allowing members of the gut bacterial community to affect the function of CNS circuits (4, 6, 7).

Evaluation of the composition and function of the gut microbiome

Since many of the bacterial taxa inhabiting the human gut are difficult to culture (21), the composition of the gut bacterial community is typically based on the identification of their DNA. This can be achieved either by targeted amplification of specific bacterial gene sequences, or by metagenomic sequencing of all the DNA found in the sample (whole genome sequencing). Targeted amplification typically targets the 16S ribosomal RNA (rRNA) gene, which encodes for the RNA component of the prokaryotic ribosome, and is preserved across bacterial taxa. Sequence differences (polymorphisms) in the hypervariable regions of the 16S rRNA gene, allow for the taxonomic identification of bacteria present in a given sample. The taxonomic resolution of 16S rRNA analysis is sometimes limited by ambiguity, secondary to sharing of 16S versions across species. Whole genome sequencing allows for higher taxonomic resolution as well as quantitative functional annotation of genes and metabolic pathways, including entire bacterial genomes as well as DNA pertaining to other microbiome organisms, the host and ingested plants and animals (8, 22).

Functional evaluation of the microbiome can be achieved by targeting bacterial RNA (transcriptomics), proteins (proteomics) or metabolites (metabolomics). In recent years, the taxonomic resolution of microbiome studies has steadily increased, thanks to the decrease in sequencing costs, more detailed bacterial genomic databases and improved bio-informatic algorithms. This allows the discussion to be shifted away from general population-based descriptive features such as diversity, towards higher- resolution species- and strain-level taxonomic identification.

Role of the gut microbiome in animal models of pain, other pain conditions in humans

The apparent effect of the gut microbiome on the function of the CNS, and particularly its putative role in affective disorders and in overall CNS excitability raise the question of its involvement in pain processing. Chronic pain is known to involve brain circuits with an established role in affective and cognitive functions. Indeed several pioneering studies have explored microbiome correlates of chronic pain both in animal models and in humans.

Microbiome role in animal models of pain

In a mouse model of visceral pain, Luczynski *et al*. demonstrated that germ-free mice exhibit higher visceral sensitivity, altered spinal cord gene expression and increased volume of the anterior cingulate gyrus (23). In contrast, supplementation with certain bacterial species was shown to be protective against experimental visceral pain in rodents (24). Gut bacteria were also shown to influence neuropathic pain in a mouse model of chemotherapy induced neuropathy, with attenuation of pain in germ free or antibiotic treated mice. In this model, neuropathic pain was mediated by an inflammatory reaction to the translocation of lipopolysaccharide (LPS), a molecule derived from bacterial membrane, to the dorsal root ganglia (DRG) (25). Influence of the microbiome on mood has been suggested by differences in gut microbiome composition between rats who developed anhedonia following spared- nerve-injury and those who did not. Fecal microbiome transplantation from anhedonic rats augmented anhedonia in recipient rats while transplantation from resilient rats was protective (26). In a mouse model of opioid tolerance, pre-administration of antibiotics impeded the development of opioid tolerance in mice (27).

Microbiome alterations in chronic pain in humans

Gut microbiome alterations have been observed in several chronic pain conditions in humans. Multiple studies have demonstrated alterations in several bacterial taxa in patients with irritable bowel syndrome (IBS), including a consistent significant decrease in *Bifidobacteria*, and *Lactobacilli* (genera) and *Faecalibacterium prausnitzii* (species) (9). Putative mechanisms include disrupted gut barrier, immune activation and the sensitisation of sensory neurons (7). Fecal microbiome transplantation have shown to be highly effective in the management of IBS- related symptoms in a dose dependent manner (28). Alterations in the composition of the gut microbiome were also observed in other conditions such as chronic pelvic pain, chronic fatigue syndrome and Gulf-war syndrome (29-31). Alterations in gut microbiome are now recognised to occur in patients with rheumatic diseases such as rheumatoid arthritis, ankylosing spondylitis and systemic lupus erythematosus (14, 15, 32, 33).

Gut microbiome in fibromyalgia

Early studies of the microbiome in FM used indirect methods to infer changes in this community. Using lactulose hydrogen breath test, small-bowel bacterial overgrowth was observed in 100% of FM patients, 84% of IBS patients and only 20% of healthy controls, with pain intensity in FM patients showing correlation with exhaled hydrogen levels (34). Elevated gut permeability was observed among FM and CRPS patients as compared to healthy controls when assessed by measurement of urine disaccharide excretion following a 3-sugar intestinal absorption test (35).

Two recent independent studies have used a combination of next generation DNA sequencing and metabolomic analysis to characterise the composition and function of the gut microbiome in FM. Clos-Garcia *et al*. investigated a cohort of 105 FM patients and 54 healthy controls (36). Using 16S rRNA sequencing they reported on significant differences in the overall composition of the gut microbiome between FM patients and healthy controls, characterised by 32 differentially abundant operational taxonomic units (OTUs), which were identified to the genera level. Of note, no correlations were observed between gender and medication consumption and the composition of the gut microbiome, which stands in contradiction to previous reports (37, 38).

A second study used both 16S rRNA amplification and whole-genome sequencing, allowing for high taxonomic resolution. In this study the overall composition of the gut microbiome of 77 FM patients and 79 controls was similar, but analysis at higher resolution identified significant differences in 72 OTUs, of which 19 were identified at the species level. Some of these differentially abundant species are well characterised for their metabolic activity, which could be biologically plausible in FM. Significant differences were observed in the relative abundance of certain short-chain fatty acids (SCFA) producing bacteria: *Faecalibacterium prausnitzii* and *Bacteroides uniformis* were found in lower relative abundance in FM patients, while higher relative abundance was observed for *Intestinimonas butyriciproducens*, *Flavonifractor plautii, Butyricoccus desmolans, Eisenbergiella tayi* and *Eisenbergiella massiliensis*. Species with a putative pro-inflammatory role such as *P. copri, Bacteroides Uniformis* and *Haemophilus parainfluenza* were depleted in FM patients in contrast to their increased abundance in inflammatory arthritis patients (17, 32, 39). Several members of the genera *Bacteroides* and *Clostridium*, both known for their established role in the metabolism of bile acids (40), were found differentially abundant in FM. Among them, *Clostridium scindens*, found in higher abundance in FM patients, is one of the only species capable of 7α-dehydroxylation, a metabolic process which converts primary- to secondary-bile acids (41). Microbiome derived secondary bile acids are thought to have nociceptor sensitising activity by direct activation of endogenic bile acid receptors (42, 43). Although gut microbiome is generally correlated with multiple covariates including demographic, anthropometric, comorbidities and medications features, FM specific microbiome alterations were independent of the effect of these covariates. Furthermore, the abundance of some differentially abundant species was correlated with symptoms severity.

Alterations in the gut microbiome of individuals with FM are not limited to the composition of the bacterial community, but involve significant alterations in the microbe metabolic function. Gut bacteria are the putative source of many of the small metabolites in the circulation, a large proportion of which are yet to be identified (44). Using targeted metabolomic analysis to evaluate small metabolites in the urine of 18 women with FM and compared to 41 healthy controls, Malatji *et al*. found alterations in some organic acids including hippuric, succinic and lactic acid (45, 46). In a further analysis of urine metabolites, 14 of 196 measured were increased in FM patients, including organic acids and saccharides involved in energy metabolism, digestion and metabolism of carbohydrates (45).

Metabolomic analysis of sera has also revealed changes in FM patients compared to controls. Clos-Garcia *et al*. identified altered concentrations in 228 of 2000 measured metabolites in FM patients, with 88 identified, of which 7 were amino-acids (36). No differences were noted for selected cytokines and miRNA analysis, but significant quantitative correlations were observed between the relative abundance of some bacterial taxa and the severity of some of the symptoms associated with FM.

Finally, in light of the observed alterations in the abundance of butyrate-metabolism-related bacteria, our group utilised a directed metabolomic approach to quantify the concentrations of certain organic acids in the sera of FM patients. Higher levels of butyric acid, lower levels of propionic acid, and similar levels of lactic acid were observed for FM patients compared to controls (47).

Putative mechanisms of action

While appreciation of significant alterations in gut microbiome composition and function among patients with FM

is growing, it is still unclear whether these changes have a causal role in the pathogenesis of the syndrome or are merely its harbinger. It has been suggested that gut bacteria could modulate nociception at multiple levels, ranging from direct effects on peripheral nociceptors to modulation of conduction and perception in the CNS (48). Animal studies demonstrate several putative mechanisms which could allow the gut microbiome to affect nociception. The two main mechanisms are the secretion of biologically active metabolites, which include SCFA, bile acids, neurotransmitters and toxins, and pathogen-associated molecular pattern (PAMP), which act by interacting with the host immune system. PAMPs include cell-wall molecules, nucleic acids and other antigens (48).

SCFA such as butyrate, propionate and lactate have been linked to pain sensation via activity on free-fatty-acid-receptor (FFAR) 2/3, and their regulatory effect on leukocytes (49, 50). Intriguingly, butyrate induced visceral hypersensitivity in mice, whereas it reduced visceral pain in humans (51). The effect of butyrate on the colonic mucosa is thought to be mediated by its capacity to activate luminal transient receptor potential cation channel 1 (TRPV1) (48). Individuals with FM show significantly altered composition of SCFA metabolising bacteria, as well as alterations in the serum and urine levels of SCFA (45, 47).

Bacterial-derived bile acids have effect in metabolic (52), inflammatory and oncologic processes (40). Several gut bacterial species known to be involved in the metabolism of secondary bile acids were found differentially abundant in FM patients (47). Mediated by bile-acid receptors, these effects include bile-acid induced itch and analgesia via the G-protein coupled bile-acid receptor TGR5 in the DRG (42, 53, 54). A nuclear bile-acid receptor, farnesoid X receptor (FXR), was shown to be involved in the bile-acid induced visceral sensitivity in IBS, via the expression of nerve growth factor (NGF) and TRPV1 (43). FXR activation by bile- acids was also associated with the pathogenesis of depression via the expression of brain- derived neurotrophic factor (BDNF) (55). Serine protease cathepsin G, secreted by gut bacteria decreases the excitability of dorsal root ganglia (DRG) neurons, and their response to capsaicin, an effect recapitulated by *Faecalibacterium prausnitzii* (56), a species significantly depleted among patient with FM (47).

Gut bacteria have been shown to produce a wide range of neurotransmitters (57). *P. merdae* and *A. muciniphila* were shown to increase the GABA/glutamate ratio in the brains of mice, thus decreasing over all CNS excitability and excreting a protective anti-seizure effect (58). Interestingly, *P. merdae* was one of the species for which the highest fold increase in FM patients was observed, possibly explaining such symptoms as fatigue and cognitive dysfunction (47). PAMPs have the capacity to modulate the activity of the host immune system (48, 49). Lipopolysaccharide, a membrane derived PAMP, plays a critical role in the development of neuropathic pain in a mouse model of chemotherapy induced neuropathy (25).

The finding that most bacterial species, known to be increased in inflammatory rheumatic diseases were found deficient in FM (47), is in line with the overall notion that FM is not a peripheral inflammatory disease (although neuroinflammation cannot be discounted). Nonetheless, the hypothesis that bacteria-derived antigens have a role in invoking an immune response in FM cannot be refuted at this point (59).

Implications for clinical care of FM patients

Knowledge of differences in microbiome composition in many diseases and specifically FM must prompt one to question the clinical implications for these findings. Future study will help determine the role of the microbiome in human disease, may even serve as a biomarker for disease, and will allow for better understanding of the complex interplay of the microbiome and genetic factors. With increasing scientific and media coverage of the microbiome, patients and health care providers will be eager to explore potential opportunities to address the microbiome with hope that disease may be influenced. The way to clinically apply the novel insights of the gut-microbiome role in health and disease typically involves three steps: the discovery of a correlation between specific microbiome compositions and a clinical phenotype; the establishment of a causal relationship between the changes in the composition and function of the gut microbiome and the pathogenesis of the clinical condition at hand; and finally the use of targeted manipulation of the gut microbiome as a treatment modality. Recently, important first steps have been taken in establishing a correlation between gut-microbiome composition and fibromyalgia. These may allow for the development of objective diagnostic aids to this challenging syndrome. The next necessary step is to establish the direction of causality – are microbiome alterations causing FM symptoms or are they caused by the syndrome? Answering this question may allow us to improve our mechanistic understanding of FM. If the gut microbiome is found to have a role in the pathogenesis of FM, then the road could be paved to the development of new treatment modalities aimed at modulating this community. These prospects are exciting for patients and clinicians alike, and are fueled by anecdotal reports on symptomatic amelioration following the adoption of certain dietary habits. Despite the fact that the composition of the gut microbiome is amenable to change by means of either dietary interventions, probiotics and fecal microbiota transplantation (FMT), we must acknowledge that at this point evidence-based advice is limited for several reasons: first, the human gut microbiome is so variable that a "favourable" composition cannot be determined. Second, while it may be tempting to "normalise" the abundance of bacterial species which are altered in FM, we must bear in mind the complexity of this eco-system. For example, *P. copri* is depleted in FM, but is increased in inflammatory arthritis, so one should apply caution manipulating its abundance. Finally, even if we did have an ideal microbiome composition

to which to aspire, the available modalities to alter the composition of the gut microbiome are still not refined enough to allow directed changes (60).

While there is insufficient study at this time to know whether manipulation of the microbiome could have impact on symptoms of FM, it could be cautiously suggested that a move closer to a Neolithic diet that was consumed ~10,000 years before present (BP) could offer global health benefits. An argument could therefore be developed as follows: eating habits over the last 10,000 years have changed considerably; there has been a progressive increase in diseases of civilisation including obesity, heart disease, cancers and also chronic pain; and the Western diet is associated with changes in the microbiome with less diversity observed. It is therefore plausible that diet has impact on expression of disease. A diet that was consumed by preagricultural hominins was characterised by fresh fruit and vegetables, less fatty meat, and devoid of refined sugars, vegetable oils, and refined cereals (61, 62, 63).

While not suggesting that we recommend our patients to return to the hunter-gatherer diet, we can make suggestions to reduce fats and sugars and increase fiber. Tentative advice for dietary adjustment towards a globally healthier diet will do no harm, help towards patient empowerment and perhaps could have health related benefits for those with FM.

In summary, growing evidence links alterations in gut microbiome to mechanisms involved in pain, depression, irritable bowel syndrome and other symptoms associated with FM. There is hope that gut microbiome modulation could offer promising prospects for better understanding of basic mechanisms of FM, for new diagnostic aids and for new treatment modalities.

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