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The guide through brain-gut-enteric microbiota axis – regulation pathways and related disorders

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ABSTRACT

The brain-gut axis is a complex system of connections between the central nervous system (CNS) and the digestive tract. Information within the axis is transmitted through many pathways (neural, endocrine, metabolic, immunological) and is strongly dependent on the microbiota inhabiting the gut. Therefore, the brain-gut axis is also called the "brain-gut-enteric microbiota axis". Many studies are carried out to determine how it functions but the exact mechanism is still poorly understood. Unraveling the mystery of brain-gut-enteric microbiota axis seems to be crucial in understanding of many diseases and processes governing the human body.

***Keywords*:** brain-gut axis, enteric microbiota, gastrointestinal system

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List of abbreviations:

2BAs - secondary bile acids

CNS – central nervous system

ECC – enterochromaffin cells

EEC – enteroendocrine cells

ENS – enteric nervous system

FMT – fecal microbiota transplant

GABA – gamma-aminobutyric acid

GALT – gut-associated lymphoid tissues

GF mice – germ-free mice

GI – gastrointestinal

GLP-1 – glucagon-like peptide-1

HPA – hypothalamic-pituitary-adrenal axis

IBD – inflammatory bowel disease

IBS – irritable bowel syndrome

KYNA – kynurenine acid

NMDAr – NMDA receptors

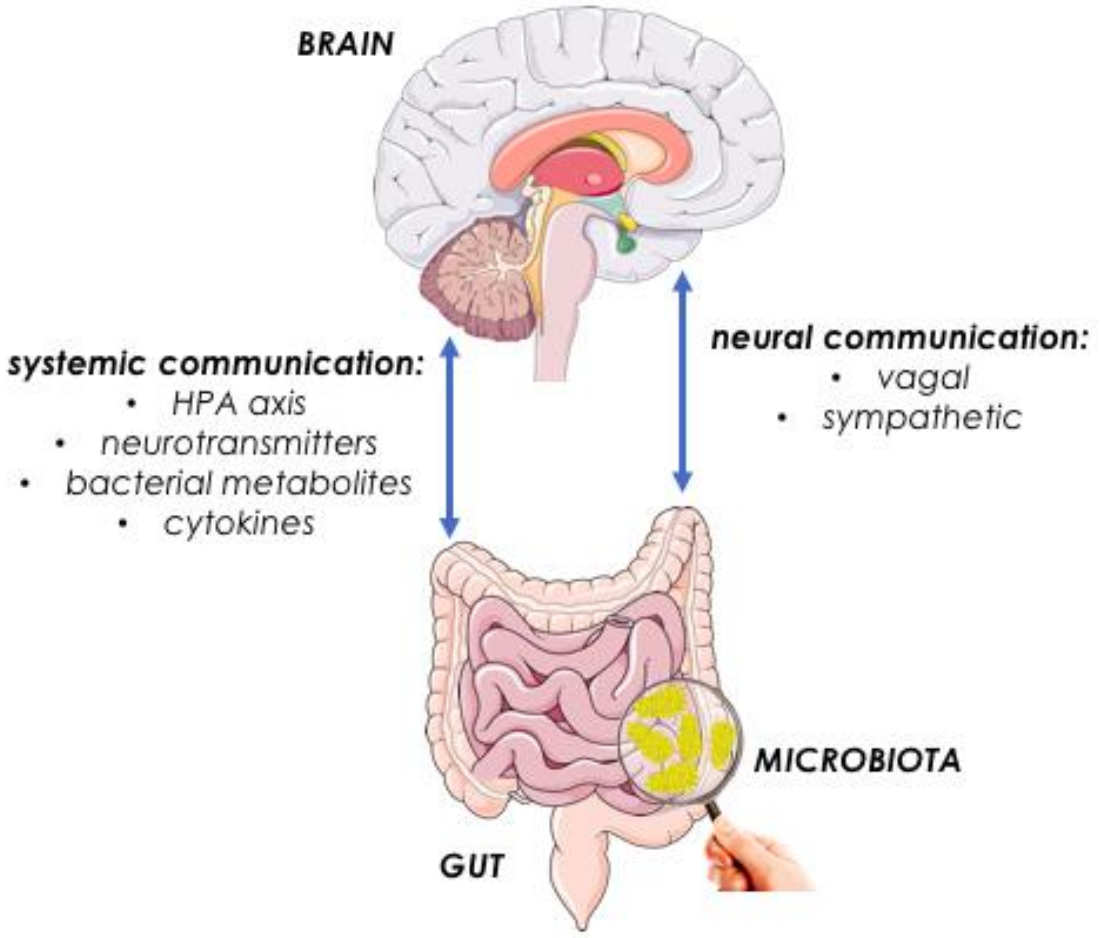
SCFAs – short-chain fatty acids

VIP – vasoactive intestinal polypeptide

1. INTRODUCTION

The brain-gut axis is a complex system of connections between the central nervous system (CNS) and the digestive tract. Information within the axis is transmitted through many different pathways. Neural pathway is the basis and it consists of the central nervous system (CNS) and enteric nervous system (ENS) elements. The other ways of communication include: endocrine, metabolic and immunological pathways. Information transmitted within the axis is bi-directional and strongly dependent on the microbiota inhabiting the gut.

Therefore, the brain-gut axis is also called the "*brain-gut-enteric microbiota axis*" (Scheme 1). Many studies are carried out to determine how it functions but the exact mechanism is still poorly understood. Unraveling the mystery of brain-gut-enteric microbiota axis seems to be crucial in understanding of many diseases and processes governing the human body.



Scheme 1. The brain-gut-enteric microbiota axis.
Based on websites: http://www.jneurosci.org/content/34/46/15490/tab-figures-data and https://smart.servier.com

2. GASTROINTESTINAL TRACT

Proper function of gastrointestinal tract is the basis of healthy human body. GI tract consists of many elements which cooperate precisely to provide food digestion, absorption, defecation and detoxification. A unique feature of the digestive system (in comparison to other human systems) is its extensive connection with the external environment. Every day many products from the outside enter the gastrointestinal tract. According to the latest reports, the total mean of interior mucosal surface of the digestive tract, and thus the surface of contact with the external environment, reaches about $32m^2$ (1). For comparison, the total area of human skin ranges from 1.5 to $2m^2$. Such a vast intestinal mucosa area determines the efficiency of digestion and absorption but also carries a lot of dangers. One of them is a huge risk of infection. Performing such a responsible function as the integration of the external and internal environment is reflected in the anatomic structure of the digestive system. Namely, it contains an extremely extensive immune and nervous system which both allow precise control of its work.

3. NEURAL CONTROL OF GASTROINTESTINAL TRACT

Many elements are responsible for the neural control of the digestive tract. They are arranged hierarchically. The highest level of control are the higher centers located within the brain. The next level consists of sympathetic and parasympathetic fibers. The parasympathetic fibers are derived from the parasympathetic nucleus of the vagus nerve and from the parasympathetic nuclei within the sacral spinal cord. They are responsible for promoting peristalsis, fluid secretion and sphincter relaxation. The sympathetic fibers have their centers within the intermediolateral nucleus of the spinal cord. Their preganglionic neurons reach the prevertebral ganglia of the sympathetic nervous system and travel further to autonomic plexuses located in close proximity to the innervated organs (celiac plexus, superior mesenteric plexus and inferior mesenteric plexus). Sympathetic fibers determine decreased peristalsis and reduced fluid secretion.

The lowest level of neural control is the system of internal nerve plexuses located in the intestinal wall. These neurons create so-called *enteric nervous system* (ENS) (2). It was first noticed in the middle of the 19th century by Leopold Auerbach and Georg Meissner. Decades later W.M. Bayliss and E.H. Starling noticed that after fragmental dissection of the dog's intestine and increasing the pressure within it, contraction of muscularis mucosae occurs. This discovery proved that enteric nervous system can function autonomously and its action can be preserved even after connections with CNS are damaged. This discovery fueled the development of neurogastroenterology (3).

The basic elements of the ENS are Auerbach's (myenteric) plexus located within the outer muscular layer (responsible for motor control) and Meissner's (submucous) plexus (responsible for secretion control). Meissner's plexus is formed by a network of neurons which start within the esophagus and end in the internal anal sphincter, while Auerbach's plexus is limited to the area of the small and large intestine. The size and complex structure of ENS are impressive, for that reason it is called "*the second brain*" or "*minibrain*". Sometimes, it is also classified as the third component of the autonomic nervous system - together with the parasympathetic and sympathetic system (4)(5). The ENS consists of 200 to 600 million of neurons, among which sensory neurons, interneurons and motor neurons may be distinguished. Their quantitative ratio is 2: 1: 1, respectively (6). The sensory neurons are responsible for receiving information from sensory receptors in the muscular layer and mucosa. Their stimuli can be mechanical (mainly stretching), thermal, osmotic and chemical. The effector cells for motoneurons are smooth muscle cells, secretory cells as well as blood vessels present in the gastrointestinal wall. Interneurons are responsible for coordination of received stimuli and reinforcement of the response they evoke.

The role of the central nervous system is to control the synchronous operation of muscle fibers (swallowing/chewing/defecation movement patterns) and regulate the internal environment of the stomach and its emptying through vagovagal reflexes (3). ENS's own work is based on numerous internal neuronal connections which create complex reflexes responsible for peristalsis, regulation of local blood flow and local fluid secretion / absorption. Synapses in the ENS, as in the CNS, are chemical. The list of neurotransmitters that participate in transmission is long and there are still new ones being reported. Currently, the most important intestinal neurotransmitters are: acetylcholine, serotonin, histamine, VIP, noradrenaline, neuropeptide Y, CCK, adenosine and somatostatin (7).

Control of the GI tract is complex and despite the huge amount of scientific research its full mechanism has not been fully understood. It was thought that connections between the brain and the intestines are one-directional and that brain supervises intestinal function. However, the latest scientific reports suggest that this relationship is bi-directional and ENS has the ability to influence the CNS. This relationship was named the *brain-gut axis* (8).

4. BRAIN-GUT-ENTERIC MICROBIOTA AXIS

The *brain-gut axis* is a network of bi-directional connections between the brain and the digestive tract. Interestingly, the neural pathway, which is based on a wide range of neurotransmitters, is not the only way of transmitting information within the axis. The other ways include: endocrine, metabolic and immunological pathways (9).

The endocrine pathway occurs mainly through the hypothalamic-pituitary-adrenal axis (HPA), which is responsible for secretion of cortisol - the most important stress hormone. The metabolic path consists in communication through some of the colonic fermentation products, mainly short-chain fatty acids (SCFAs), such as acetate, propionate or butyrate. Immunological communication is based mainly on cytokines, which can have a direct impact on CNS. It can be either through crossing the blood-brain barrier or by activation of afferent nerve fibers, for example those located within the vagus nerve (10). As the latest researches show, all of the above-mentioned pathways are strongly dependent on microorganisms inhabiting the human GI tract. The statement that gut flora has the ability to "communicate" with the body and can affect its functions is the discovery of recent decades; however, it has already become the subject of many studies (11). In fact, scientists found it so significant that in 2009 the term "*brain-gut axis*" was extended to "*brain-gut-enteric microbiota axis*" (12). Not so long ago, motility disorders, intestinal hypersensitivity and exposure to mental and physical stress factors were thought to be the basis of functional gastrointestinal disorders. Currently, according to the Rome IV criteria, disorders of brain-gut interactions are the fourth element taken into consideration (13). Better knowledge on this issue may be helpful in determining the precise pathogenesis of many diseases, for example mental disorders or inflammatory and functional bowel diseases (14)(15).

Many studies provide more and more information on the cooperation between the human body and the intestinal microbiota. According to the latest estimates, duodenum and jejunum houses 10^3-10^4 microorganisms per mL of content, ileum 10^8/mL and the colon 10^{11}/mL (16). In general, enteric microbiota is dominated by anaerobic bacteria belonging to four genera: *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* (17). However, despite the dominance of the above-mentioned genera, the intestinal flora is strongly diversified. Over a thousand different species and seven thousand subspecies of microorganisms were identified (18). Furthermore, fungi, viruses and bacteriophages are also components of gut flora (19). In order to fully understand the diversity of microbiota and determine its significance for the human body the Human Microbiome Project (HMP) has been carried out (20).

The main function of gut flora is assisting with food digestion and production of certain vitamins (vitamin B and K) (21). However, bacteria are assigned to much more functions. Starting from early childhood they can even modulate the nervous system development (22)(23).

As mentioned before, the main methods of communication between the intestines, microbiota and the central nervous system include: metabolic, endocrine, neurological and immunological pathways.

4. 1. Metabolic pathway

Transfer of information via the metabolic pathway is based mainly on bacterial metabolism products. These include: short-chain fatty acids (SCFAs), secondary bile acids (2BAs) and tryptophan metabolites (24). The exact mechanism of this connection has not been fully understood, although it is believed that metabolites stimulate enteroendocrine cells (EECs), enterochromaffin cells (ECCs) and elements of the immune system within the intestinal wall. Another possible way of communication is the direct action of metabolites on the CNS, after they cross the intestinal and the blood-brain barrier. However, it is still unclear whether these molecules act directly on specific brain centers or transmit information by stimulating vagus or sympathetic fibers at the corresponding level of nervous system (25)(26)(27).

4. 2. Endocrine pathway

Stimulation of the endocrine cells located within the digestive system (ECC and EEC), together with the regulation through the HPA axis, form the endocrine component of the brain-gut-enteric microbiota axis. Interestingly, stimulation of enteric endocrine cells was found to be dependent on bacterial metabolites (SCFA and 2BAs). ECCs and EECs have huge secretory potential and can synthesize more than 20 different signaling molecules with local and systemic effects. The systemic action includes controlling of ingestive behavior by regulation of hunger and satiety.

Thus, by ability to stimulate endocrine cells microorganisms have impact on ingestive behavior. What is more, enteric microbiota seems to affect the nutritional preferences of its host (28). Bacteria may generate a demand for products favoring their existence or act harmful to competing organisms. They may also lower the mood of their host, until the desired food is consumed.

Scientists suggest that the level of bacterial metabolites is strictly dependent on the diet. Consuming small amounts of fiber reduces the SCFAs production and the low-fat diet reduces the concentration of 2BAs. Decrease in SCFAs and 2Bas concentration results is the decrease of GLP-1 production which is normally responsible for inducing satiety (24)(29)(30). The impact of diet on the composition of intestinal microbiota has been repeatedly confirmed. De Filippo et al. conducted a study comparing the composition of intestinal flora of children in Europe and children in Africa. They found that microbiota of children living in rural areas of Africa differs significantly from microbiota of European children. Diet of African children, which was rich in low-processed products, supported the development of the *Prevotella, Bacteroides* and *Xylanibacter* bacterial genera (31).

Moreover, Li et al. noticed that change of diet affects not only the composition of intestinal microbiota but also the nervous system function. During the study mice were divided in two groups. One of them was fed with classic rodent food and the second one with a blend containing 50% beef. Mice receiving beef-enriched diet had more diverse microbiota, increased activity and better memory than mice fed with standard rodent food (32).

The above data indicate that the relationship between diet, enteric microbiota composition and CNS function is complex and still requires many further studies.

4. 3. Neural pathway

The main substance produced by ECCs is serotonin. It is a biogenic amine, which acts both as tissue hormone and neurotransmitter in the CNS. Around 90% of body serotonin is produced and accumulated within the enterochromaffin cells in the gut. The rest is accumulated within the CNS (33).

Serotonin is synthesized from tryptophan, which is an essential amino acid. Tryptophan from digested proteins serves as a substrate for serotonin production in the gut and CNS (34). Enteric microbiota probably influences blood tryptophan concentration and the way it is used (7). Consequently, bacterial ability to stimulate ECCs and regulate tryptophan metabolism gives them control over the microenvironment in which they exist and over the concentration of tryptophan available for CNS.

There are two main metabolic pathways in which tryptophan is involved: the serotonin synthesis pathway and the kynurenine pathway. Serotonin plays an important role in the regulation of gastrointestinal motility and secretion control (35). The kynurenine pathway leads to the formation of many active metabolites, which seem to antagonize each other. So far, kynurenine acid (KYNA) is considered to be the most important element of kynurenine pathway. It is the only one known endogenous antagonist of NMDA receptors (NMDAr) in the mammalian brain. NMDAr assist in cognitive and learning processes as well as in pain transmission and motor activity. What is more, it is already known that functional disorders of NMDAr play a key role in the pathogenesis of neurodegenerative diseases (36). The function of KYNA in the intestines is still unclear, although it probably reduces muscle tone and inhibits inflammation (37). Keszthelyi et al. indicate the clinical implication of equilibrium between serotonin and KYNA. The scientists have found that in patients suffering from IBS intestinal serotonin and KYNA concentrations are higher and plasma levels are lower, than in patients from the control group (38).

Some microorganisms are able to produce their own serotonin. This ability was found among bacterial genera: *Streptococcus*, *Escherichia* and *Enterococcus*. Furthermore, members of the genus *Escherichia*, *Bacillus* and *Saccharomyces* can produce dopamine or noradrenaline, some strains in the genus *Lactobacillus* produce acetylcholine, and *Lactobacillus* and *Bifidobacterium* synthesize gamma-aminobutyric acid (GABA) (39)(40)(41). All of the aforementioned substances act as neurotransmitters in the CNS, and thus are potential carrier of information in the brain-gut-enteric microbiota axis.

4. 4. Immune pathway

Correlation between the intestinal microbiota and the immune system is complex. Bacteria can be both an element of physiological flora and a pathogen capable of causing infection. It depends on host's immune system, the location of the microorganism within the digestive tract and the presence of other microorganisms. Interestingly, bacteria can affect the condition and composition of intestinal microbiota. It can happen either directly or indirectly. The direct mechanism includes competition for nutrients and production of antibacterial substances, which act against unwanted bacteria. The indirect mechanism includes the

stimulation of intestinal epithelial cells for production of antibacterial molecules and sealing up the connections between enterocytes.

Bacteria also have the ability to regulate the activation of lymphocytes and dendritic cells to fight against other microorganisms. Incorrect function of above-mentioned mechanisms is a potential source of autoimmune and functional bowel disorders (42). The current hypothesis concerning pathogenesis of irritable bowel syndrome (IBS) suggests that alteration of intestinal microbiota causes an increase in inflammation, intestinal permeability and nociceptive pain fibers stimulation. As a consequence, the enteric nervous system becomes dysregulated and bowel movements are disturbed (43). Verdú et al. confirmed that microbiota affects the intestinal immune system. Several groups of mice were identified in the study - including control group and groups in which mice received antibiotics. In mice treated with antibiotic therapy local intestinal inflammation, increase in substance P and myeloperoxidase concentrations as well as hypersensitivity of the intestinal wall muscles were observed. Interestingly, the supplementation of *Lactobacillus paracasei* bacterium resulted in normalization of inflammation parameters and decreased muscle hypersensitivity (44). Furthermore, intestinal bacteria are thought to influence the development of intestinal immune system. It was found that lack of commensals in the GI tract results in disruption of GALT development (45). This influence has been found in many independent scientific studies (46)(47)(48).

Interestingly, recent reports suggest that intestinal microbiota is also important in the regulation of immune system's work outside the digestive tract. Ichinohe et al. noticed that reduction of microbiota after broad-spectrum antibiotic therapy resulted in impaired B and T lymphocyte response to influenza A virus infection (49). The above-mentioned reports are promising for the possible regulation of immunological responses by affecting the microbiome composition.

5. BRAIN-GUT-ENTERIC MICROBIOTA AXIS – DISEASES

Disturbances in the function of brain-gut-enteric microbiota axis seem to have serious clinical implications. There are many reports suggesting the relationship of axis disorders with other diseases, such as: mental, neurological, metabolic or functional gastrointestinal disorders. Initially, researchers believed that gut flora should contain a set of basic bacterial species, so-called "*core microorganisms*", which could be found universally among healthy population. However, some reports point that even among healthy people the composition of enteric microbiota varies significantly. In a MetaHIT cohort study approximately 1000-1150 species of bacteria were cultured from human fecal samples, with an average of only 160 species identified in one person (50). It can therefore be assumed that healthy microbiota is not composed of specific bacterial species. The basis for its proper function is balanced but diverse set of bacteria, which present functional and metabolic properties consistent with the host's needs (51)(52)(53). The composition of intestinal microbiota changes throughout life and depends strongly on environmental factors, such as: diet, antibiotic therapy or exposure to stress. A healthy, high-fiber diet increases the diversity of intestinal flora and contributes to intestinal epithelium integrity and proper function of the immune system. An unhealthy diet, based on simple sugars and saturated fats, predisposes to reduced microbiota diversity and increased inflammation (54).

5. 1. Mental disorders – depression and anxiety

Many studies have been performed to state if enteric microbiota influences mood and emotions (55). Desbonnet et al. evaluated the effect of *Bifidobacteria infantis* supplementation on depressive behavior in rats exposed to external stress factors. Research showed that rats receiving the probiotic had reduced levels of inflammatory factors and increased plasma concentration of tryptophan (a serotonin precursor) and kynurenic acid. These findings led to suggestion that probiotics may have antidepressant effects (56).

Similar conclusions can be drawn from a study in which FMT (*fecal microbiota transplant*) between depressed people and GF mice (*germ-free mice*) was performed. Scientists observed that mice with a new set of intestinal microorganisms showed depressive behavior, including anhedonia and anxiety-like behaviors (57).

In 2016 *Kato-Kataoka et al.* performed an interesting study among medical students. For 8 weeks prior to the examination session volunteers were given fermented milk with the *Lactobacillus casei Shirota* bacterial strain. One day before the exam, students in the control group had higher aggression rate, higher salivary cortisol and serum L-tryptophan levels, than their probiotic receiving colleagues. Moreover, students who were given the probiotic reported less stress related symptoms (e.g. abdominal pain) (58).

As the reports on the possible use of probiotics in mental disorders therapy emerged, the term “psychobiotic” was introduced to medical nomenclature(59). It is assumed that in the future, after completing all necessary clinical trials, these products will become a standard element of mental disorders therapy.

5. 2. Neurological discorders – autism, Parkinson’s disease

Patients with autism often suffer from digestive tract disorders. Moreover, many studies have found differences in microbiota composition between them and healthy population. Children with autism were reported to have increased *Clostridium* species concentration in the stool (60)(61). However, it is still unknown if these microbiota alterations are the cause of neurological disorders, or whether they just coexist and share common pathogenesis. (62).

Similar findings are reported in Parkinson’s disease. Patients with Parkinson’s seem to have decreased *Prevotellaceae bacteria* concentration in gut flora. Some research also suggests that there is a positive correlation between the quantity of *Enterobacteriaceae* species and the severity of clinical symptoms. However, it is still uncertain whether these changes are the cause or the consequence of Parkinson's disease (63)(64).

5. 3. Metabolic disorders – obesity

There is an interesting suggestion that certain changes in gut microbiota predispose to obesity. Animal model study showed that FMT between obese and slim GF mice causes hyperphagia and weight gain in the latter (65). This finding implicates that gut flora has influence on hunger and satiety and also regulates food intake pattern.

Bariatric surgery is considered to be the last and the most radical element of obesity treatment. Surprisingly, it has been reported that after such surgery the enteric microbiota composition changes (66). What is more, scientists made attempts to transplant the microbiota of patients who underwent this procedure to GF mice and noticed that mice with new gut flora showed reduction in food intake and weight loss (67).

The above-mentioned reports are promising; however, further clinical trials are still needed to assess the potential use of microbiota-based therapies (e.g. probiotics, FMT) in obesity (68).

5. 4. Bowel disorders

As mentioned above, disorders of the enteric microbiota composition were found among patients suffering from irritable bowel syndrome - IBS (69). However, this is not the only intestinal disorder in which such changes are noticed. Microbiota alterations are also reported in patients with inflammatory bowel diseases (IBDs) – such as *Leśniowski-Crohn's disease* and *ulcerative colitis* (70). The most probable cause of inflammatory bowel diseases is an aberrant immune response against the gut microbiota. This exaggerated immunological reaction seems to result from compilation of environmental factors and genetic susceptibility of the host (71). Some studies show that antibiotic therapy may induce remission of IBD, although it is unclear how it affects the further course of the disease (72). What is more, it is undefined which substances, in what doses and in what sequences would be the best therapeutic option. Despite the potential profits, the side effects of prolonged antibiotic administration (e.g. negative changes in the microbiota composition, increasing bacteria resistance, diarrhea, allergic reactions) are not to be ignored and may even outweigh possible benefits (73).

An alternative to antibiotherapy is the use of probiotics and fecal microbiota transplantation. The FMT procedure is the transfer of healthy donor's feces to the recipient's digestive tract, mainly in the form of frozen capsules taken orally (74). It is a recommended method in the treatment of antibiotic-resistant and recurrent *Clostridium difficile* infections, although clinical trials assessing its usage in the treatment of IBD are also conducted. So far, there is no clear opinion on its effectiveness (75)(76).

In the ongoing 2-phase randomized controlled trial, no changes in the diversity of microbiota were observed in the group of IBD patients who responded positively to FMT. However, there were some quantity differences, e.g. an increase in butyrate-producing bacteria. Butyrate is a short fatty acid which takes part in enhancing intestinal epithelial cell integrity and influences cooperation between gut and brain (77). It may suggest the possible effect of bacteria produced SCFAs in the pathogenesis of IBD.

Nevertheless, the correlation between microbiota alterations and the brain-gut-enteric microbiota axis function in IBD is still poorly understood. Therefore, further studies are necessary to determine clinical implications of microbiota-based therapies in the IBD treatment.

6. CONCLUSIONS

With an increasing number of studies on brain-gut-enteric microbiota axis, the knowledge of intestinal microorganisms is constantly growing. However, many long-term and multi-center studies are still needed to fully understand the bi-directional correlation between intestinal microbiota and human body. It is a matter of great importance to know the impact of gut flora on physiological and pathophysiological processes, as it could be the key to understanding pathogenesis and treatment options of many diseases.

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