TRUST YOUR GUT:
THE RELEVANCE OF THE GUT MICROBIOME IN HEALTH AND DISEASE

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Abstract

The gut microbiome contains 100 trillion microorganisms inhabiting the gastrointestinal tract. Research into the gut microbiome began in the 16th century, with some accounts that date even earlier. Increasing interest in the gut microbiome has led to the founding of multiple international consortiums like the Human Microbiome Project and the American Gut Project. The gut microbiome confers multiple benefits to the host due to the synthesis of essential vitamins and regulation of immunity, gut health, as well as modulation of behaviour and mood through the gut-brain axis, which is a network that connects the nervous system to the gastrointestinal tract. Dysbiosis of the microbiome is associated with several diseases such as gastric and non-gastric cancers, inflammatory diseases, neurodegenerative, and neuropsychiatric disorders. Current therapies, mainly for gut-based diseases, include faecal microbiota transplantation and the use of prebiotics and probiotics either through diet or as supplements. Future lines of research include studying the molecular mechanisms underlying dysbiosis and screening the microbiome for diagnostic purposes, as well as establishing therapies for gut-brain linked diseases.

Introduction

The gut microbiome, which inhabits the gastrointestinal tract, is a collection of archaea, eukarya, and bacteria [1]. It is estimated that the gut microbiome has 100 trillion microorganisms, consisting of 150-400 species, depending on ethnicity [1, 2]. It is also estimated that the microbiome encompasses 2-20 million genes compared to the 20,000 genes in the human genome, hence being called the last organ of the human body [3, 4].

Dysregulation of the gut microbiome is associated with many diseases such as Alzheimer's disease, depression, cardiovascular disease, diabetes, obesity, and autoimmune disorders [5]. Undoubtedly, the interest in the human microbiome has increased rapidly over the years, with almost US $1.7 billion being spent on medical research for the human microbiome [4]. This has led to swift advancements not only in terms of laboratory screening techniques - for identification and labelling every microbe in the gut but also therapeutically - with poop pills, faecal transplantation, probiotics, and prebiotics for certain diseases [5].

This review aims to elucidate the role of the gut microbiome in health and disease: the history behind gut microbiome research; its association with various diseases such as cancer, neurological disorders, neurodegenerative disorders, and gut disorders; the current therapies ranging from faecal microbiota transplants (FMTs) to poop pills leading on to what the future holds for gut microbiome research.

A brief history of gut microbiome research

In scientific research, one of the early descriptions of the human gut microbiome was documented by a Dutch scientist, Antonie van Leeuwenhoek in 1683. He examined microbes under the microscope, from his own mouth and stool, and concluded the presence of more than 1000 'animalcules' which differed based on their origin [3]. In the 16th century, Li Shizhen, a pharmacologist, successfully treated patients suffering from an abdominal disease with a soup containing fresh, dry, or fermented stool [6]. Escherich and Metchnikoff, doctor and zoologist respectively, laid the foundations for understanding host-microbe interactions. Escherich proposed the potential role of endogenous microbes in the physiopathology of intestinal disease. He was later known for discovering the bacteria *Escherichia coli* [3]. In the 18th and 19th centuries, scientists from Britain and Germany documented the presence of microorganisms in the stomach and intestines of men [3]. The German botanist Uffelman also documented the presence of a large number of microorganisms in normal faeces of breast-fed babies [3].

Henry Tissier, a paediatrician, used a pure culture of *Bacillus acidiparalactici,* known to have strong fermentative power, to treat children with gastrointestinal disease. He noticed the restoration of the normal flora of his patients when they recovered [3].

Hermann Senator, a doctor of internal medicine, postulated that poisons generated in the gastrointestinal tract could enter the vascular system and cause a systemic effect [3]. The theory of autointoxication proposed by Charles Bouchard states that toxic metabolites produced by microorganisms, such as from the intestinal waste within the body, can lead to poisoning and is a major contributor to diseases [7, 8]. Furthermore, he suggested that one of the common ways to prevent autointoxication was through colon resection. However, Metchnikov suggested the introduction of harmless or beneficial microorganisms to replace harmful flora. He demonstrated its efficacy by popularising the age-old remedy of fermented milk to treat diarrhoea and claimed to have found a cure for ageing [3].

Global research initiatives

Increasing revelations connect the gut flora to many vital physiological functions. However, the entire repertoire of gut microbes remains undefined. DNA sequencing techniques such as the 16S rRNA gene-based sequence analysis and metagenomic analysis are being used to identify new species [9]. Hence, countries have come up with multiple global projects like the Human Microbiome Project, MyNewGut funded by the EU, the British Gut Project, and the

American Gut Project to study, understand, and characterise gut microbial diversity as well as the human microbiome [10].

Factors that influence the gut microbiome

The origin of the development of the gut microbiome is a controversial debate [10]. Some studies suggest that the placenta and the womb are sterile, and the GI tract of the infant is colonised at birth. However, recent studies report the presence of microorganisms in the placenta, umbilical cord, amniotic fluid and foetal membranes [11]. The mode of delivery also influences the gut and faecal microbiota composition in infants. An infant delivered through C-section has lower Bacteroides composition and higher levels of *Clostridium* species, resembling only 41% of the mother's faecal microbiome [1]. Infants delivered through vaginal birth contain a high abundance of *Lactobacilli* (due to the *Lactobacillus* present in the vagina), and their microbiota resembles 72% of the mother's faecal microbiome [1].

The composition of the microbiome evolves depending on the infant's diet and environmental exposure. After birth, two phyla temporarily dominate the microbiome, namely, *Actinobacteria* and *Proteobacteria*. Thereafter, lactic acid bacteria and *Bifidobacterium* are present in the gut till the infant is introduced to solid food. From then on, adult-type microbes such as *Bacteroides*, *Clostridium*, *Ruminococcus*, *Veilonella* and *Prevotella* dominate the infant's intestine [11]. An adult-like gut microbiota is established by about three years of age [11]. The diversity of the microbiome alters due to multiple factors such as illness, antibiotics exposure, diet, and other environmental factors such as smoking, geographical location, and living arrangements (urban or rural) [1].

For instance, in the case of antibiotic treatment, a study performed in humans found that β-lactam antibiotics (like ampicillin, cefazolin etc.) affect the microbiome by hampering the production of important cellular metabolites like acetyl CoA and acetyl phosphate that regulate cell metabolism [1]. Investigations in mice whose microbiome was depleted by antibiotic treatment showed that not only secondary bile acid and serotonin metabolism were affected in the colon but also that the mice were more susceptible to pathogenic infection [1].

Diet also plays an important role in determining microbial composition in the gut [12]. A study performed in 2018 involving immigrants originating from Thailand who migrated to the United States showed that the immigrants displayed a 'westernised' gut flora compared to their original microbiome [13]. Differences in populations that lead agricultural or early hunter-gatherer lifestyles versus urban populations are mostly in the number of fibre-digesting bacteria, with the former communities having 60% of their microbiome composed of these bacteria. In another study, mice that were fed a low-fibre diet were shown to have a loss in microbial diversity as opposed to mice fed with a high-fibre diet [12].

The gut microbiome in health and disease

The gut microbiome offers multiple benefits to the host by supporting many physiological functions such as regulation of immunity, protection against pathogens, and maintaining the intestinal epithelium [14]. It also produces vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and cobalamin (vitamin B12), which are not produced by the host and are supplied through diet. [14]. The microbiota performs these functions by fermenting non-digestible substances like dietary fibres, thereby supporting the growth of microbes that produce short-chain fatty acids (SCFAs) like acetate, butyrate, and propionate [1, 14].

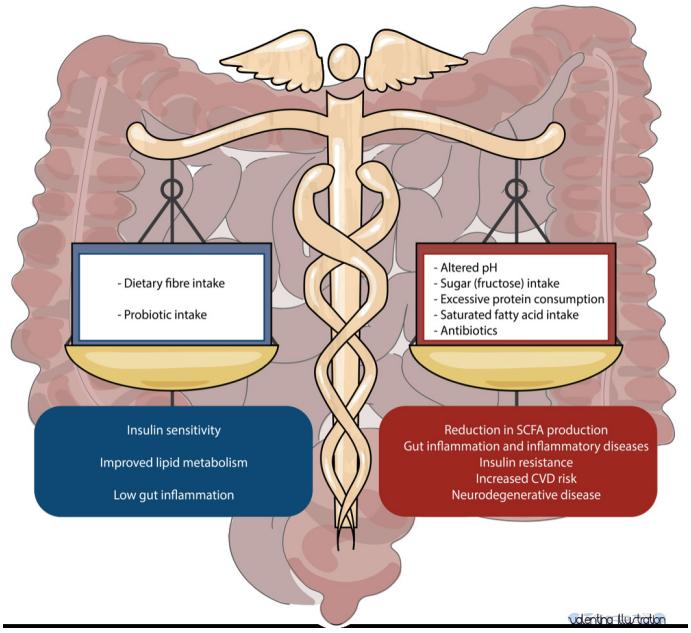

Figure 1: *Illustration displaying the factors that influence the gut microbiome and the consequences during dysbiosis. The balance depicts the health effects when there is adequate fibre and probiotic intake (blue box) vs when there is an intake of foods with high sugar, protein and saturated fatty acid content, which alters the gut microbiome and increases the risk of various diseases (red box)[14].*

The most abundant short-chain fatty acid, acetate, regulates appetite and is important for lipogenesis and cholesterol metabolism [14]. Butyrate plays an important role for colon cells by not only being their main energy source but also being anti-inflammatory and anti-cancer by inducing apoptosis in colon cancer cells [14]. It also regulates glucose metabolism and is responsible for maintaining a state of hypoxia in the intestine to prevent dysbiosis. Propionate also plays a role in glucose metabolism and in satiety signalling.

Disturbance in the microbial composition causes dysbiosis which leads to conditions such as altered gut hormone production and immune dysregulation [15]. Dysbiosis of the gut can occur due to increased sugar, fat, and protein intake and low dietary fibre intake, which alters pH levels. Other factors include lack of sleep, frequency of exercise, and exposure to antibiotics [14]. Dysbiosis is linked to multiple diseases and disorders such as obesity, inflammatory bowel disease, cancer, metabolic disorders, neurological disorders, and neurodegenerative diseases (figure 1) [15].

Gut microbiota and cancer

Dysbiosis of the gut microbiome has been reported to contribute to the development and progression of cancer as well as influencing the efficacy of anti-cancer therapies [16]. For instance, bowel cancer is the world's third most commonly occurring cancer [17]. *Bacteroides fragilis*, a strain of bacteria found in the gut, produces a toxin- Bacteroides fragilis toxin- which is implicated in the tumorigenesis of colon cancer as it triggers inflammation through common molecular pathways involving NF-κB and Stat3 in colon epithelial cells [18]. Similarly, a strain of *E. coli* (*E. coli* NC101) was found to promote colorectal cancer due to the production of a DNA-damaging toxin that leads to an inflammatory and tumorigenic environment [19]. Besides certain strains of bacteria directly causing tumorigenesis, even just the presence of certain strains indicates trouble. Similarly, the presence of *Fusobacterium nucleatum* in patients with bowel tumours is linked to worse survival outcomes as it directly interacts with

cancer signalling and immune pathways and promotes resistance to chemotherapy [20]. Besides intestine-related cancers, the gut microbiome also influences the prognosis and treatment response in other cancers such as liver, breast, and pancreatic cancers [14]. Animal experiments have shown that the presence of specific gut microbes (*Blautia producta*, *Clostridium saccharogumia*) influences the protective effect of phytoestrogens in breast cancer [14].

The gut microbiome may also improve the efficacy of anti-cancer drugs such as chemotherapy and immune checkpoint inhibitors [21]. Germ-free mice that do not have a gut microbiome are resistant to a common chemotherapy drug, cyclophosphamide (CTX). Similarly, these mice resist CTLA-4 blockade, a common immune checkpoint treatment, until introducing a certain *Bacteroides* strain that restores the response to CTLA-4 treatment by inducing and promoting the maturation of dendritic cells [21].

Gut microbiota and immune-related disorders

Multiple studies have documented altered gut microbiome composition in inflammatory diseases such as Crohn's disease, rheumatoid arthritis, ulcerative colitis, asthma, diabetes mellitus, and obesity [22]. Short-chain fatty acids such as butyrate play an important role in the suppression of the NF-kB pathway and IFN-γ, regulating inflammatory and innate immune responses. SCFA's bind to G-protein coupled receptors present on immune cells such as macrophages, thereby conferring their anti-inflammatory effect [22]. Taking the example of inflammatory bowel disease, certain species of bacteria are reduced, causing a reduction in the production of SCFAs. This further causes an imbalance in the number of Th17 and T-reg cells which are vital for anti-inflammatory processes and, as a consequence, increase proinflammatory cytokine production [23].

Gut microbiota and neurological and neurodegenerative diseases

Alteration in gut microbiota is associated with having consequences for the central nervous system [24]. The gastrointestinal tract and the nervous system communicate via the gut-brain axis, a bidirectional network that connects the autonomic nervous system, enteric nervous system, central nervous system and the hypothalamic-pituitary-adrenal axis, to the gastrointestinal tract [25]. The gut-brain axis regulates behaviour, cognition, and even brain development [26].

Recently, it was found that 90% of serotonin, a neurotransmitter responsible for the regulation of behaviour, sleep, and mood, is produced in the gut [24]. Neuronal physiology can be influenced by microbes such as *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Bifidobacteria* that produce neurotransmitters [24]. Patients suffering from neurodegenerative diseases like Alzheimer's, Parkinson's, multiple sclerosis, and neuropsychiatric disorders had altered microbial compositions when compared to healthy individuals [24]. A change in microbe composition can lead to neuroinflammation and changes in permeability in the blood-brain barrier. A similar observation was made in patients suffering from neuropsychiatric disorders such as depression. In another study concerning Alzheimer's disease, animal models that were germ-free, transgenic and susceptible to Alzheimer's showed lower levels of β-amyloid precursor protein when compared to non-germ free, transgenic, Alzheimer's susceptible animal models, which suggested the role of the gut microbiome, with over-representation of certain bacteria, in Alzheimer's disease [24].

Therefore, the gut microbiome plays a role in many neuropsychiatric disorders and neurodevelopmental disorders [26]. Germ-free mice present depression-like and anxiety-like behaviour, exhibit social avoidance, and show fatigue. These symptoms were reduced by supplementing them with prebiotics and fermented foods, indicating potential targeted therapies in this field [12, 26].

Current therapies and future perspectives

Since the dysbiosis of the gut microbiome is linked to multiple diseases, various therapies have been developed that target certain microbial species linked to specific diseases, thereby restoring healthy microbial composition [27]. These therapies are more established for gut diseases rather than gut-brain linked disorders. One of the first therapies developed is faecal microbial transplantation (FMT). Even though this treatment was documented to be first used in Ancient Chinese medicine, in 1958, Ben Eiseman's team used faecal microbial transplantation as a method to treat patients suffering from pseudomembranous colitis. FMT is performed by transferring the gut microbiota from a healthy donor individual to the patient who has a dysbiotic microbiome via capsules, either orally or via a surgical colonoscopy. FMT was performed successfully for patients who suffered from recurrent *Clostridoides difficile* infection when antibiotic treatments failed. The cure rate was reported to be 80-90% [28, 29, 30].

FMT has not been restricted as a treatment only for *C. difficile* infection and shows promise in treating inflammatory bowel disease, gastric and non-gastric cancers, obesity, and neurodegenerative disorders [23, 26, 31]. Due to potential safety and regulatory issues, FMT is now also being tested in the form of capsules commonly known as poop pills [32].

Moreover, prebiotics and probiotics are well-established strategies for modulating the gut microbiome [33]. Prebiotics are non-digestible carbohydrates that serve as substrates for fermentation in the gut and hence lead to an increase in beneficial bacteria. Probiotics are live bacteria and yeasts that, when ingested, are beneficial to gut health [14]. Multiple animal studies have been performed that demonstrate the efficacy of these treatments for various diseases. However, there are limited numbers of clinical trials [33].

Future research involves targeting the gut microbiome in a specific manner, understanding the molecular interactions that occur due to dysbiosis, and advancement in technology for screening and diagnostics to prevent diseases.

Conclusion

This review elaborated on multiple roles of the gut microbiome in the human body in health and disease pathophysiology. Targeting the gut microbiome as a form of therapy is a promising approach and is already being implemented in clinical trials. Future research involving the use of the microbiome for diagnostic purposes is therefore warranted.

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