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MINIREVIEW

Gut microbiome-mediated epigenetic regulation of brain disorder and application of machine learning for multi-omics data analysis[1]

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Abstract: The gut–brain axis (GBA) is a biochemical link that connects the central nervous system (CNS) and enteric nervous system (ENS). Clinical and experimental evidence suggests gut microbiota as a key regulator of the GBA. Microbes living in the gut not only interact locally with intestinal cells and the ENS but have also been found to modulate the CNS through neuroendocrine and metabolic pathways. Studies have also explored the involvement of gut microbiota dysbiosis in depression, anxiety, autism, stroke, and pathophysiology of other neurodegenerative diseases. Recent reports suggest that microbe-derived metabolites can influence host metabolism by acting as epigenetic regulators. Butyrate, an intestinal bacterial metabolite, is a known histone deacetylase inhibitor that has shown to improve learning and memory in animal models. Due to high disease variability amongst the population, a multi-omics approach that utilizes artificial intelligence and machine learning to analyze and integrate omics data is necessary to better understand the role of the GBA in pathogenesis of neurological disorders, to generate predictive models, and to develop precise and personalized therapeutics. This review examines our current understanding of epigenetic regulation of the GBA and proposes a framework to integrate multi-omics data for prediction, prevention, and development of precision health approaches to treat brain disorders.

Key words: gut–brain axis, epigenetics, neurodegenerative diseases, Alzheimer's disease, machine learning.

Résumé : L'axe intestine–cerveau (GBA pour "gut–brain axis") est une signalisation biochimique qui lie le système central nerveux (CNS) avec le système nerveux entérique (ENS). Des évidences cliniques et expérimentales suggèrent que le microbiote intestinal serait un régulateur clé du GBA. Les microbes qui vivent dans l'intestin interagissent non seulement localement, avec les cellules intestinales et l'ENS, mais ils modulent également le CNS via des voies de signalisation neuroendocrines et biochimiques. Des études ont également exploré l'implication de la dysbiose du microbiote intestinal dans la dépression, l'autisme, les accidents vasculaires cérébraux, ainsi que dans la pathophysiologie d'autres maladies neurodégénératives. Des rapports récents suggèrent que des métabolites dérivés des microbes influenceraient le métabolisme de l'hôte en agissant comme des régulateurs épigénétiques. Le butyrate, un métabolite bactérien intestinal, est un inhibiteur connu de l'histone désacétylase qui peut améliorer l'apprentissage et la mémoire chez des modèles animaux. En raison de la grande variabilité des maladies au sein de la population, une approche multi-omique qui fait appel à l'intelligence artificielle et à l'apprentissage machine pour analyser et intégrer les données "omiques" est nécessaire pour mieux comprendre le rôle du GBA dans la pathogenèse des désordres neurologiques, générer des modèles prédictifs et développer des thérapies précises et personnalisées. Cette synthèse examine les connaissances actuelles sur la régulation épigénétique du GBA et propose un cadre analytique pour intégrer

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les données multi-omiques pour la prédiction, la prévention et le développement d'approches de médecine personnalisée pour traiter les désordres du cerveau.

Mots-clés : axe intestin–cerveau, épigénétique, maladies neurodégénératives, maladie d'Alzheimer, apprentissage machine.

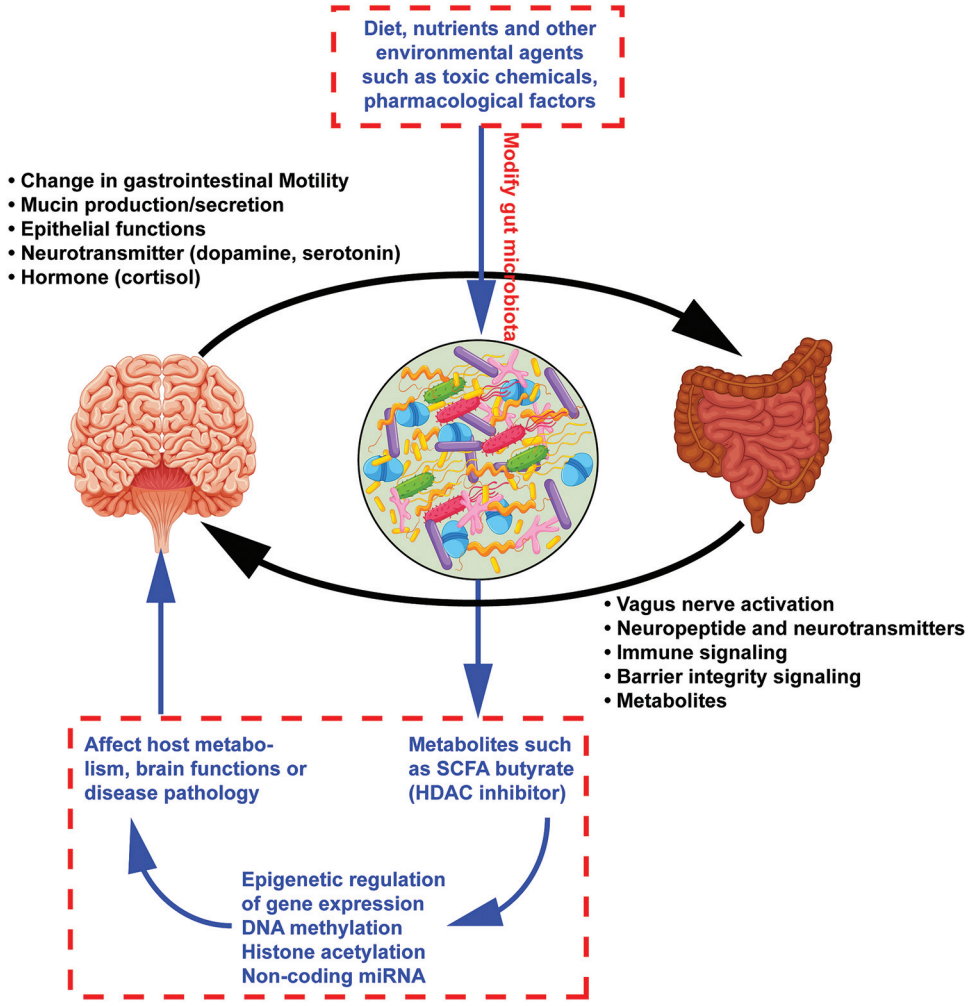
Introduction

A biochemical link exists between the central nervous system (CNS) and the enteric nervous system (ENS) of the body, which is known as the gut–brain axis (GBA) (Sharon et al. 2016). In fact, the ENS is often referred to as the "second brain" based on its size, complexity, and similarity in producing neurotransmitters and signaling molecules alike the brain (Mayer 2011). The ENS produces more than 30 neurotransmitters, most of which are identical to the ones found in the CNS, such as acetylcholine, dopamine, and serotonin. This complex network of communication consists of the CNS, ENS, and the autonomic nervous system (ANS), where the ANS with sympathetic and parasympathetic limbs drives both afferent signals, arising from the lumen and transmitted though enteric, spinal, and vagal pathways to the CNS, and efferent signals, arising from the CNS to the intestinal wall (Carabotti et al. 2015). The ENS also consists of a complex peripheral neural circuit embedded within its wall comprising sensory neurons, motor neurons, and interneurons (Foster et al. 2017). It is estimated that the human ENS consists of some 500 million neurons, which is about five times the number of neurons present in the human spinal cord. It can independently regulate basic gastrointestinal functions (i.e., motility, mucous secretion, and blood flow), but the central control of gut functions is provided by vagal and, to a lesser extent, spinal motor inputs that serve to coordinate gut functions with the general homeostatic state of the organism (Mertz 2003). It is believed that the brain can influence the ENS indirectly, via changes in gastrointestinal motility and secretion and intestinal permeability, or directly, via the signaling molecules that are released from intestinal cells (enterochromaffin cells, neurons, immune cells) into the gut lumen (Rhee et al. 2009). Likewise, there are multiple ways by which the gut can influence the brain, most commonly through neural, endocrine, immune, and humoral pathways (Sherman et al. 2015) (Fig. 1). Multiple studies have suggested the role of the GBA in health and disease ranging from psychiatric disorders (e.g., anxiety, depression) to neurological and neurodevelopmental disorders including autism, Alzheimer's disease (AD), and Parkinson's disease (PD). Gut microbiota also plays a significant role in epigenetic modifications such as DNA methylation and histone modifications, which have been known to influence the brain and behavior. Recent advancements in multi-omics data integration and analysis provides an opportunity for comprehensive investigation of gut microbiota and its contribution to host health (Tilocca et al. 2020).

Gut microbiome

Research in the past decade has shown that gut microbiota is a key regulator of the GBA (de la Fuente-Nunez et al. 2018; Mittal et al. 2017). Gut microbiota refers to the total of all microorganisms that live in the gastrointestinal tract and includes not just bacteria but also other microbes such as fungi, archaea, viruses, and protozoans (Sekirov et al. 2010). It is considered as the largest reservoir of microbes in the human body, containing about 10^{14} microbes (Sender et al. 2016). These microbes maintain a symbiotic relationship with gut mucosa and impart metabolic, immunological, and gut protective functions in the host. The collective genomes of the microorganisms in any given environment, i.e., microorganism plus their set of genes, is known as the microbiome (Ursell et al. 2013). But the term microbiome is often used interchangeably with microbiota (Ursell et al. 2012). Among the four most dominant phyla in the gut microbiota, Firmicutes and Bacteroidetes account for 90% of the total population and Actinobacteria and Proteobacteria account for less than 1%–5%, and the alteration in the balance of these phyla is known as dysbiosis, which has been suggested to be linked to several diseases including intestinal and extra-intestinal diseases (Rogers et al. 2016; Tamboli et al. 2004). In recent times, interest within the scientific community has focused on the role of gut microbiota in human diseases ranging from inflammatory bowel diseases and irritable bowel syndrome, to metabolic diseases such obesity and diabetes, to allergic diseases, to neurodevelopmental illnesses (Jandhyala et al. 2015). In recognition of fundamental involvement of commensal microbes in important functions such as human development, immunity, and nutrition, the National Institutes of Health (NIH) launched the Human Microbiome Project in 2007. The primary goal of the Human Microbiome Project was to study the functional contributions of microbial communities to human health and disease. This 10 year project was divided into two phases, where phase I (HMP 1) was designed to conduct a survey of microbial communities from five major habitats of the human body (oral, skin, nares, gastrointestinal tract, and urogenital tract) and to evaluate whether a characteristic microbial community was associated with a specific host health status (NIH Human Microbiome Portfolio Analysis Team 2019). The second phase of the human microbiome project (HMP 2) was designed to create an integrated dataset of the biological properties of both the microbiome and host over time, in a series of disease cohorts, as a resource for the broader research community. Analysis of the Human Microbiome Project

Fig. 1. Bidirectional communication between the brain and gut mediated via the gut microbiota (shown in black). The effect of gut microbiome on brain functions via epigenetic regulation of gene expression (shown in blue).



data has revealed a high degree of microbial community specialization as well as considerable variation in overall microbiome composition between individuals. The study provided a vast knowledge in the field, including development of reference sequences, multi-omic datasets, computational and statistical tools, and analytical and clinical protocols as resources for the broader research community (Integrative 2019). Advances in omics technologies and better understanding of host–microbe interactions from the Human Microbiome Project has led to attempts to use microbiome data-guided precise and personalized medicine approaches for developing diagnostics and therapeutic interventions (individualized microbiome alterations to improve clinical outcomes) (Petrosino 2018).

Gut microbiota-brain communication (via vagus nerve, neuroendocrine, and neuroimmune pathways)

It still remains to be ascertained how gut microbes affect brain functions. Experimental data suggest that the microbiota may send direct signals to the brain by activating afferent sensory neurons of the vagus nerve via neuroimmune and neuroendocrine pathways (Forsythe et al. 2014; Kennedy et al. 2017; Mohajeri et al. 2018; Sampson and Mazmanian 2015). The vagus nerve is the 10th cranial nerve, extending from its origin in the brainstem through the neck and the thorax down to the abdomen, which links the viscera with the brain and consists of both afferent as well as efferent neurons. It represents the main component of the parasympathetic nervous system, which oversees a vast array of crucial bodily functions, including control of mood, immune response, digestion, and heart rate. Efferent neural signals are conducted from the CNS to the gastrointestinal tract and can modulate gastrointestinal motility, secretion, and epithelial permeability, which modify the physical environment that the microbiota inhabit, thus affecting its composition. The vagus afferent nerves transmit signals from the gastrointestinal tract to the CNS and can recognize microbial products or cell wall components (Cox and Weiner 2018). Stimulation of the

vagus nerve is known to stabilize the abnormal electrical activity in the brain and is widely used in the treatment of epilepsy and other neurological conditions. The role of the vagus nerve has been very well studied in psychiatric (anxiety, mood disorders) and gastrointestinal disorders (Breit et al. 2018; Browning et al. 2017). Ablated gut-related vagal communication in animal studies has shown to affect the adult neurogenesis, stress reactivity, and anxiety- and fear-related behavior, as well as cognition, which are all indicative of brain changes observed in psychiatric disease (Fulling et al. 2019).

Gut bacteria are known to influence the cells of the gastrointestinal track that produce neurotransmitters and digestive hormones or peptides in the gut that in turn alter the brain and behavior. All components that can influence the gut bacteria, such as diet, environment, probiotics, and drugs, such as antibiotics, are also known to affect vagus nerve activity (Breit et al. 2018). Moreover, using electrophysiological studies, it has been shown that vagal afferent nerve fibers respond to a variety of stimuli including cytokines, nutrients, gut peptides, and hormones (Bonaz et al. 2018; Lal et al. 2001). The vagal receptors present at the visceral afferent endings of the vagus nerve in the intestine recognize the regulatory gut peptides, inflammatory molecules, dietary components, and bacterial metabolites to relay signals to the CNS (de Lartigue et al. 2011). Hence, altered gut microbiota composition or changes in any of the gut microbiota products (microbial signals) can be directed by the vagus nerve to alter CNS outputs, suggesting an active role of gut microbiota in mediating neurological functions. Various vagotomy studies in animal models have confirmed the vagus nerve as the most probable and direct route for gut-to-brain signaling (Bercik et al. 2011; Bravo et al. 2011). Bravo et al. 2011 has shown that 28-days of probiotic *Lactobacillus rhamnosus* treatment in mice resulted in reduced stress-induced corticosterone and anxiety- and depression-related behavior that was not found in vagotomized mice, thus identifying the vagus as a major modulatory constitutive communication pathway between the bacteria exposed to the gut and the brain (Bravo et al. 2011). Another recent study showed that *Lactobacillus intestinalis* and *Lactobacillus reuteri* caused depression- and anhedonia-like phenotypes in antibiotic-treated mice via the vagus nerve. They demonstrated this by performing subdiaphragmatic vagotomy in mice, which blocked depression- and anhedonia-like phenotypes and reduced brain inflammation, and in antibiotic-treated mice after the ingestion of *L. intestinalis* and *L. reuteri* (Wang et al. 2020). Modulating the gut bacteria using probiotics, *L. reuteri* has been shown to target the ion channels in enteric sensory neurons leading to increased action potential and excitability, suggesting gut bacteria can communicate with other components of the ENS (Kunze et al. 2009).

The neuroendocrine system is classically defined as an organized set of cells with neural determination, which produce hormones or neuropeptides. The major neuroendocrine system that controls various body processes in response to stress is also called the hypothalamic–pituitary–adrenal (HPA) axis. Studies have identified a potential link between the gut microbiota and the neuroendocrine system in various psychiatric as well as gastrointestinal diseases (Dinan and Cryan 2012; Mukhtar et al. 2019).

Immunomodulation by the microbiota is emerging as an important pathway that orchestrates microbiota–gut–brain communication (Rea et al. 2016). Gut microbiota is well known to regulate inflammation, autoimmunity, and immune cell trafficking (Fung et al. 2017). It is also involved in microglial maturation and function, and altered microbial community composition has been reported in neurological disorders with known microglial involvement in humans (Abdel-Haq et al. 2019). Moreover, germ-free mice have been shown to have compromised microglial maturation and morphology that can be normalized following the treatment of these animals with complex microbial community, suggesting active microbial signaling is required throughout adulthood to preserve microglial maturation (Erny et al. 2015).

Gut metabolites (short-chain fatty acids)

Gut microbiota is a complex microbial ecosystem that produces a wide range of metabolites such as neurotransmitters, hormones, vitamins, and short-chain fatty acids (SCFAs), which play an important role in host defense (training the host immune system) (Kau et al. 2011; Reijnders et al. 2016; Sampson and Mazmanian 2015; Strandwitz et al. 2019). Gut microbiota can affect neuronal functions directly or indirectly via these molecules. The fermentation of non-digestible dietary fibers by commensal bacteria (anaerobic) in the gut results in the production of key bacterial metabolites and SCFAs such as acetate, propionate, butyrate, and valerate, which are known to regulate gut integrity and immune responses and maintain overall host homeostasis (Kasubuchi et al. 2015). The major SCFAs and other metabolites produced from carbohydrates and protein by gut bacteria are listed in a review by Macfarlane and Macfarlane (2003). About 95% of these SCFAs are absorbed by colonocytes as an energy source, and it has been suggested that around 60%–70% of their energy need is fulfilled from the oxidation of SCFAs (den Besten et al. 2013). Along with this, SCFAs also act as signaling molecules that are mainly involved in systemic lipid metabolism and glucose/insulin regulation (den Besten et al. 2013). SCFAs can cross the blood–brain barrier via monocarboxylate transporters located on endothelial cells (Silva et al. 2020). They are essential for maintaining the intestinal barrier permeability by regulation of tight junction proteins in the gut. Moreover, evidence also suggests the involvement of SCFAs in maintaining the blood–brain barrier permeability (Obermeier et al. 2013; Silva et al. 2020). They modulate the mammalian cell functions by serving as an energy substrate or by signaling through G-protein-coupled receptors (GPCRs, GPR41, and GPR43) (W. Wu

et al. 2017). In the brain, SCFAs produced by gut microbiota have been shown to influence the CNS immune system by regulating microglial maturation and function. Microglia are the primary innate immune cells of the brain, and dysfunction of these cells has been shown to be involved in the initiation or progression of multiple CNS diseases, including AD, PD, and even autism spectrum disorder (ASD) and depression (Colonna and Butovsky 2017).

Role of gut microbiota in brain disorders

Despite the significant influence of gut bacteria on development or progression of various extra-intestinal diseases, it is only in the last few years that the involvement of gut microbiota has become the focus of interest for neurologists particularly in understanding the pathophysiology of neurodegenerative diseases and neuropsychiatric diseases such as depression, anxiety, and cognitive deficit disorders. Several reports from the last decade have evidenced the role of gut microbiota dysbiosis in depression, anxiety, autism, neurodegenerative diseases, and stroke and have proposed the possibility of using gut microbiota composition as a biomarker for the CNS (Zhu et al. 2020). The brain alters the intestinal microenvironment by regulating the gut motility and secretion as well as mucosal immunity via the neuronal glial–epithelial axis and visceral nerves (Furness 2012; Matteoli and Boeckxstaens 2013). On the other hand, the gut reacts via changing the bacterial metabolites such as neurotransmitters, neuromodulators like SCFAs, and gut hormones such as leptin or ghrelin.

Manipulation of commensal gut microbiota using probiotics and (or) antibiotics in animals has shown to impact behavioral responses in rodents, suggesting the involvement of gut bacteria in altering brain functions. Germ-free models provide a direct way to study how gut microbiota regulates behavior (Cryan and O'Mahony 2011). Previously, researchers have transplanted microbiota from adult germ-free BALB/c mice (a highly anxious strain) into adult germ-free NIH Swiss mice (a low anxious strain), and the BALB/c mice received the microbiota of the NIH Swiss mice and showed that colonization of germ-free BALB/c increased exploratory behavior and hippocampal levels of BDNF, whereas colonization of germ-free NIH Swiss mice with BALB/c microbiota reduced exploratory behavior, suggesting the direct influence of altered gut microbiota on behavior in mice (Bercik et al. 2011). Various preclinical and clinical studies provide the evidence of involvement of gut microbiota in the development of anxiety-like disorders and improvement of anxiety symptoms by regulating the intestinal microbiota, which has been summed in a recent review (Yang et al. 2019).

Neuropsychiatric symptoms could be defined as psychiatric manifestations of cerebral (neuropsychiatric) disorders. Cerebral disorders cause various psychiatric symptoms such as dementia, mood disorder, stress, anxiety, movement disorder, personality disorder, and other behavioral disorders. One of the most enduring and replicated findings in biological psychiatry is the high correlation of the HPA axis with the neurobiology of various mood disorders and other brain illnesses, including anxiety disorder, bipolar disorder, insomnia, posttraumatic stress disorder, borderline personality disorder, major depressive disorder, burnout, chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, and alcoholism (Drevets et al. 2008). Traditional approaches to treat most of these brain disorders is a combination of talk therapy, such as cognitive-behavioral therapy, and an antidepressant medication. These antidepressants, which are routinely prescribed, directly regulate HPA axis function (Barden 2004). Data from various animal and human studies suggest a role of microbiota in influencing several psychological disorders, mood disorders, anxiety, depression, and autism. Gut microbiota has also been found to play an important role in the development of the HPA axis (Sudo et al. 2004). Disruption occurring in the GBA determines changes in intestinal motility and secretion and causes visceral hypersensitivity, leading to cellular alterations of the entero-endocrine and immune systems. The state of the gut microbiome has been found to be associated with various mental illnesses including stress, anxiety, and depression (de Weerth 2017; Foster and McVey Neufeld 2013; Gur et al. 2015; Jiang et al. 2015; Moloney et al. 2014; Yang et al. 2019). A recent review highlights the findings published in all areas of biological psychiatry research and microbiome (Bastiaanssen et al. 2019).

ASD refers to a group of complex neurodevelopment disorders characterized by repetitive and characteristic patterns of behavior and difficulties with social communication and interaction. Along with genetics, many environmental factors may modify and (or) trigger psychiatric conditions including ASD. Immune system abnormalities have been hypothesized to play a crucial role in the pathophysiology of ASD (Edmiston et al. 2017; Fluegge 2017; Goines and Van de Water 2010; Morgan et al. 2010; Wong and Hoeffer 2018). Gut microbiota can impact the development and function of the immune, metabolic, and nervous systems and has been shown to play a role in ASD (Sharon et al. 2019). It has been more than two decades since a potential link between the microbiota and ASD was first suggested. An altered gut microbiota has been observed in both a mouse model of ASD as well as an individual with ASD (Coretti et al. 2018; De Angelis et al. 2013; Finegold et al. 2010; Kang et al. 2018; Kushak et al. 2017; F. Liu et al. 2019; Wang et al. 2019), as well as in mouse models of ASD (Buffington et al. 2016; Coretti et al. 2017; Liu et al. 2018). In fact, a recent study has reported colonization of germ-free wild-type mice with fecal microbiota from a human with ASD was enough to induce hallmark autistic behaviors relative to those colonized with typically developing control microbiota (Sharon et al. 2019).

AD is one of the most common forms of dementia in older adults; a disorder that slowly destroys memory, cognitive skills, and, eventually, the ability to carry out the simplest of tasks. It is an irreversible, progressive brain disorder that mainly appears after 60 years of age. One of the greatest challenges faced by neuropsychologists over the past 50 years is to understand the cognitive and behavioral manifestations of dementia and their relationship to underlying brain pathology. The characteristic neuropathological hallmarks of AD are the progressive accumulation of amyloid beta in the brain and abnormal intracellular accumulation of hyper-phosphorylated Tau protein inside neurons, leading to neuroinflammation and neuronal degeneration in multiple brain regions (Kaur et al. 2020*b*; Serrano-Pozo et al. 2011). Several theories have been proposed and multiple hypothesis have been tested using various animal models of AD, which has shaped the mechanistic research and drug development for AD over the past decades. Multiple factors are known to contribute to the development of AD, including, but not limited to, lifestyle habits such as diet, exercise, education history, cognition and aging, genetics, immune-senescence, chronic infections, chronic inflammation, latent infections, and sleep problems (Edwards et al. 2019; van Praag 2018). However, the failure of drugs in clinical trials and the unsuccessful development of AD therapies suggests a lack in understanding of the pathophysiology of this disease. Recently, the role of gut microbiota has been documented in pathogenesis of AD, where several groups have observed altered gut microbial composition to be associated with an increased intestinal permeability and increased intestinal inflammation in various models of AD (Harach et al. 2017; Kaur et al. 2020*b*; Minter et al. 2016; S.C. Wu et al. 2017).

Gut metabolites as epigenetic regulators

For the last two decades, human gut microbiota has been studied extensively because of compelling evidence that suggests human health not only depends upon its own genome but also on microbes and their genome. The total number of microbial cells present in a human is 10 times more than the total number of human body cells. In fact, a metagenomic study of the human microbiome has demonstrated that there are three to four million unique genes present in the human gut, 100–150 times more genes than our own genome (Qin et al. 2010; Zhu et al. 2010). Many of these genes encode proteins that perform metabolic functions and produce metabolites exclusive to the microbiome. These intestinal bacterial metabolites not only exert local effects in the gut, but some are also absorbed into the systemic circulation and reach distant organs, including the brain. Some of these metabolites are biologically active, whereas others are further metabolized by host enzymes and serve as a mediator of microbial influence on the host. Several studies have shown that manipulation of gut microbiota composition affects microbial metabolome that in turn affects the host metabolism and genetics, suggesting the existence of dynamic crosstalk between the host and its microbiota, which is important for achieving and maintaining homeostasis (Rooks and Garrett 2016).

Gut microbiota and their metabolites are considered as important epigenetic regulators that can influence host gene expression (can affect epigenetic processes of the host as well). Epigenetics is often defined as the study of phenotypic changes secondary to alterations in gene expression that do not directly arise from changes in the underlying DNA sequence (Prasher et al. 2020) The genetic information in our body is stored in DNA which holds the instructions for building the proteins that carry out a variety of functions in a cell. The epigenome is the set of chemical modifications to the DNA and DNA-associated proteins in the cell, which alter gene expression, and are trans-generationally heritable (via meiosis and mitosis). Microbiota can influence the host genome through the host epigenome. The molecular mechanisms by which gut microbiota interact with the host cells and regulate the gene expression is not completely understood; however, certain microbial-derived metabolites have been shown to interact at DNA, RNA, and histone levels. These metabolites can induce epigenetic alterations of key genes that modulate the initiation and progression of diseases (Yuille et al. 2018). Epigenetic regulation is commonly mediated through DNA methylation, post-transcriptional histone modifications, chromatin restructuring and associated DNA methyltransferases (DNMTs), DNA hydroxylases, histone acetyltransferases, histone deacetylases, histone methyltransferases, and histone demethylases (Paul et al. 2015; Prasher et al. 2020).

DNA methylation is a heritable epigenetic mark involving the covalent transfer of a methyl group (-CH3) on the carbon-5 of the cytosine ring in cytosine–guanine dinucleotide-rich (CpG) regions by enzyme DNMTs (Jin et al. 2011). DNA methylation is generally associated with the suppression of gene transcription by regulating accessibility of the transcriptional machinery, transcription factors, and histone modifiers to the chromatin. There are several factors that can affect the DNMTs and hence can affect the epigenetics. Diet, nutrients, and other environmental agents such as toxic chemicals and pharmacological factors (can modify microbiota) have been found to be epigenetic modifiers of DNA and histone proteins, and have been known to induce susceptibility to many different chronic diseases (Kumar et al. 2014; Lavebratt et al. 2012). It has been documented that the complete absence of microbiota or any changes in microbiota composition can establish long-lasting epigenetic modifications that can ultimately affect behavior. Little is known of how metabolic activity of gut microbiota regulates the host tissue epigenetics.

Gut microbiota, particularly *Clostridia*, a highly polyphyletic class of Firmicutes, process dietary fibers into SCFAs, particularly butyrate. Butyrate is a known

histone deacetylase (HDAC) inhibitor that blocks the HDAC-mediated histone deacetylation (removal of acetyl group from the positively charged histone lysine residues) and effectively hyper-acetylates histones, thereby enhancing chromatin accessibility and activating gene expression (Bourassa et al. 2016; Louwies et al. 2020; Rooks and Garrett 2016). On the other hand, histone acetylation regulated by histone acetyltransferases results in a more relaxed chromatin confirmation, supporting a more transcriptionally active state of chromatin and enhanced gene expression. Likewise, the methylation of histones at different lysine residues has variable effects; methylation at lysine 4 of histone H3 is known to be associated with an increase in gene activity while methylation at lysine 9 results in repression of transcription. Both DNA methylation and histone modifications can act interchangeably to contribute to the overall chromatin state and its epigenetic control of numerous cellular processes (Tollefsbol 2011).

Alteration of this chromatin state is suggested as a possible mechanism by which gut microbiota affects the host metabolism. Not only does butyrate produced from gut bacteria alter DNA methylation and gene expression, molecules like acetate, propionate, and folate also participate in epigenetic processes. SCFAs such as acetate and propionate also have HDAC inhibitory activity (Bolduc et al. 2017; Silva et al. 2018). Regulation of DNA and histone methylation may be driven by complex microbiota–host metabolism interactions involving S-adenosyl methionine (SAM), derived from the essential amino acid methionine through diet using enzyme L-methionine S-adenosyltransferase (MAT) (Miro-Blanch and Yanes 2019). DNMTs transfer the methyl group from SAM to the carbon at position 5 of the cytosine residue of the dinucleotide CpG (regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5′→3′ direction) site results in gene silencing. 5-methylcytosine (5mC), also described as the fifth base of DNA, is considered as a chemically stable modification that is known so far to be the most reliable way of transmitting epigenetic information. Indeed, more than 4% of the cytosines present in the human genome have been reported to be methylated (Breiling and Lyko 2015). On the other hand, gene activation occurs through DNA demethylation; the enzymatic process that removes or modifies the methyl group from 5mC. Demethylation is catalyzed by dioxygenases of the TET (ten-eleven translocation) protein family composed of TET1, TET2, and TET3 enzymes (Kumar et al. 2018). These TET enzymes oxidize 5-mC to 5-hydroxymethylcytosine (5-hmC, so called 6th base), which can be further oxidized by TET oxidases to 5-formylcytosine (5-fC) and subsequently to 5-carboxycytosine (5-caC) (Ito et al. 2011). Thus, the interplay between DNMT and TET proteins sculpts the DNA methylation landscape and enables the flow of epigenetic information across cell generations.

Several studies have shown a role for gut bacteria in regulating the host gene expression via epigenetic modification. These bacteria may enhance or suppress the recruitment of enzymes mediating methylation and demethylation reactions (Takahashi et al. 2011). Another recent study from the same laboratory has documented the DNA methylation status of a specific population of genes in intestine was altered by gut microbiota, i.e., commensal bacteria-induced methylation of CpG motifs in the overlapping 5′ region of *Tmem267* and *3110070M22Rik* suppressed the expression of these genes (Takahashi et al. 2020). However, further studies are needed to understand the molecular mechanisms of how gut microbiota affects physiological functions of the host by regulating the gene expression in intestinal epithelial cells via DNA methylation. An altered gut microbiota is also found to influence SAM levels and, as a result, alter the methylation status of DNA and histones. Folate, generally produced by *Bifidobacterium* spp., is a methyl donor and is necessary for generation of SAM that in turn is a methyl-donating substrate for DNA methyltransferases (Paul et al. 2015). In fact, several strains of *Bifidobacterium* along with *Lactobacillus plantarum* bacteria, both are common probiotic bacteria, are known to produce folate in the gut, which can be utilized by the host and might affect DNA methylation pattern (Rossi et al. 2011). The epigenetic mechanisms regulated by gut microbiota and its metabolites is summarized in Table 1.

Further, the direct involvement of the vagus nerve in epigenetic regulation has been reported. In male Sprague Dawley rats vagus nerve stimulation (VNS) alters the hippocampal, cortical, and blood epigenetic transcriptomes and epigenetically modulates neuronal plasticity and stress-response signaling genes (Sanders et al. 2019). Another suggested mechanism is that glucose affects a cell-specific epigenetic regulation of memory-related genes such as *Bdnf* and *Fgf-1*, and also glucose-mediated secretion of gut hormones may induce VNS to enhance memory processes in the brain (Hossain et al. 2020). Disorders such as anxiety, trauma-related disorders, autism, and neurodegenerative diseases are complex and multi-factorial, and an intricate interplay between the genome, epigenome, and environment is thought to contribute to the development of these disorders (Stilling et al. 2014). Anxiety and depressive-like symptoms in animal models have been associated with epigenetic mechanisms such as altered HDAC activity (Nagy et al. 2018). Moreover, an epigenetic mechanism has also been implicated in the etiology of ASD, as comprehensively reviewed by Grafodatskaya et al. (2010). These epigenetic changes observed in neurological disorders have been suggested to be linked to gut microbiome changes; however, it is still not clear how these epigenetic changes are associated with microbiota changes. There exists a complex interaction between the host genetics, subsequent altered functional pathways, and the composition of the microbiome. It has been known that the host

Table 1. Epigenetic mechanisms regulated by gut microbiota and its metabolites.

Epigenetics-associated effects	Gut microbiota and metabolites	Epigenetic mechanisms	Source
Functional interactions between diet, gut microbiota, and host health	Microbe colonization, treatment with the SCFAs acetate, propionate, and butyrate	Global histone acetylation and methylation, chromatin modification	Krautkramer et al. 2016
Antidepressant-like effect, influence of gut microbes in gut–brain axis, influence of regulatory T cells in the colon	Butyrate	DNA methylation changes mediated by demethylation-facilitating enzymes like TET1 and HDAC inhibitor	Bourassa et al. 2016; Furusawa et al. 2013; Louwies et al. 2020; Rooks and Garrett 2016; Wei et al. 2014
HIV-1 replication by epigenetic regulatory mechanisms, regulation of host transcriptional response	Acetate, propionate	HDAC, histone acetylation	Bolduc et al. 2017; Lukovac et al. 2014; Silva et al. 2018; Soliman and Rosenberger 2011
Anxiolytic and antidepressant	Lactate	Altered HDAC activity (HDAC2/3, HDAC5)	Carrard et al. 2018; Karnib et al. 2019
Development and functions of cholinergic neurons	S-adenosylmethionine/diet	A major methyl donor for histone and DNA methylation	Mellott et al. 2007; Zeisel 2007
Impact on different stages throughout the life course	Folate (produced by *Bifidobacterium* spp.)	DNA methylation	Crider et al. 2012; Paul et al. 2015
Host immune maturation	Direct interaction of gut microbiota with TLR4 genes	DNA methylation, alteration of chromatin state	Miro-Blanch and Yanes 2019; Seeley et al. 2018; Takahashi et al. 2011
Memory dysfunction	Probiotics	Decreased H3K27me3 (trimethylation of histone H3 Lys 27)	Xiao et al. 2020
Affect gut microbiota composition and modulate gene expression involved in microbial recognition by different intestinal cell types	Fecal miRNA (noncoding RNA)	Modulate gut microbiota	Liu et al. 2016
Gut microbiota is an important source of B-vitamins	B-vitamins: biotin, cobalamin, folate, niacin, pantothenate, pyridoxine, riboflavin, and thiamin	Regulates global DNA and histone methylation, transposable element repression	Magnusdottir et al. 2015
Induces differentiation and senescence in colon cancer cells	Ursodeoxycholate (UDCA), a secondary bile acid produced by gut bacteria	Modulates chromatin by inducing histone hypoacetylation	Akare et al. 2006

genotypes play a role in shaping microbiota composition (Bongers et al. 2014; Campbell et al. 2012; Hildebrand et al. 2013). To further investigate this, a study was conducted on the heritability of the gut microbiome using monozygotic and dizygotic twins. Controlling for environmental factors proved that the host genetic variation has an influence on microbial composition (Turnbaugh et al. 2009; Yatsunenko et al. 2012). Genome-wide association studies (GWAS) have been conducted to find the genetic loci that influence the gut microbiota composition (Bonder et al. 2016; Goodrich et al. 2016; Malan-Muller et al. 2018).

Above, we discussed the possible routes for microbiota–gut–brain signaling and diet-influenced gut–brain communication and common epigenetic modification by gut microbiota. Here, we focus on how gut microbiota influence the epigenetics in brain disorders, especially in neuropsychiatric disorders such as anxiety, depression, and neurodegenerative disease. Both microbiome and epigenetic events are highly dynamic and could be influenced by nutrient availability. Histone-modifying enzymes regulate transcription and are sensitive to the availability of endogenous small-molecule metabolites, allowing chromatin to respond to changes in environment (Fan et al. 2015). Gut microbiota produces a variety of metabolites detectable in host circulation, including bile acids, vitamins, choline metabolites, and lipids including SCFAs (Kaur et al. 2020*a*, 2020*b*; Nicholson et al. 2012). Alteration of chromatin state is a possible mechanism by which gut microbiota induces host immune maturation (Seeley et al. 2018). SCFAs are consider as the key mediators of systemic microbiota-inducing changes in host chromatin state. They have been found to regulate MHC gene (involved in immune response) expression by coordinating the activity of enzymes that acetylate and methylate histones and DNA, thus allowing chromatin accessibility (Ting and Trowsdale 2002).

Previously, it has been documented that microbial colonization regulates the global histone acetylation and methylation in various host tissues in a diet-dependent manner, suggesting that any change in diet resulting in change in microbial composition could affect the epigenetics (Krautkramer et al. 2016). The direct connection between gut and brain is the vagus nerve, and it has been speculated that altered gut motility or neuroactive signals secreted by bacteria may result in changes in the epigenetics in host cells. The role of the gastrointestinal tract in cysteine absorption and glutathione production and its influence over DNA and histone methylation provides a perspective on how the microbiome exerts effect on brain development (Eshraghi et al. 2018). Interestingly, mice with an intestinal epithelial cell-specific deletion of HDAC3 exhibited extensive dysregulation of intestinal epithelial cell-intrinsic gene expression, including decreased basal expression of genes associated with antimicrobial defense, suggesting a direct connection between microbiota and epigenetic gene regulation (Alenghat et al. 2013). A comparative NMR-based metabolome analysis from mice models suggests that the luminal concentrations of gut metabolites positively correlates with the number of Treg (T-regulatory) cells in the colon. And among all SCFAs, butyrate is a very well-known anti-inflammatory molecule, which is also a HDAC inhibiter that has shown to induce the differentiation of colonic Treg cells (Furusawa et al. 2013; Kaisar et al. 2017). There is compelling evidence that the microbial composition is directly responsible for triggering epigenetic changes (Eshraghi et al. 2018). For example, a Japanese study showed that butyrate (a histone deacetylase inhibitor), a by-product of the digestion of dietary fiber by gut microbes, acts as an epigenetic switch that boosts the immune system by inducing production of regulatory T cells in the colon (Furusawa et al. 2013).

Gut microbiota influences bioavailability of dietary agents and provides energy metabolites as cofactors of epigenetic reactions. Choline is an essential nutrient that is required for normal development of the brain, and is known to participate in the synthesis of S-adenosylmethionine, a major methyl donor for histone and DNA methylation. Long-term dietary choline deficiency results in substantial alterations to epigenetic regulation such as DNA methylation (Pogribny and Beland 2009; Romano et al. 2017). Previous reports suggest the involvement of choline in learning and memory, possibly via modulating the hippocampal cholinergic system. In fact, it has been reported that maternal choline deficiency results in global DNA methylation in fetal hippocamps and alters the development and functions of cholinergic neurons (Mellott et al. 2007; Zeisel 2007). A recent study has shown the effect of bacterial choline metabolism on host metabolism, epigenetics, and behavior (Romano et al. 2017). They showed that choline-utilizing bacteria compete with the host for this nutrient (choline), significantly impacting (lowering) plasma and hepatic levels of methyl-donor metabolites (Romano et al. 2017). Gut microbiota dysbiosis has been shown to contribute to the development of AD or other cognition-related psychiatric disorders (Dickerson et al. 2017; Hsiao et al. 2013). In a recent study, manipulating the gut bacteria in animals using probiotics has been shown to restore the decreased H3K27me3 (trimethylation of histone H3 Lys 27) found in the adult hippocampus and partially prevented the memory dysfunction suggesting that the memory dysfunction could be ameliorated via epigenetic modulators or altering gut microbiota (Xiao et al. 2020). miRNAs are a class of small, noncoding RNAs that epigenetically modulate gene expression. A recent study showed that miRNA produced by gut epithelial cells regulated bacterial gene expression, which can be used as a tool to manipulate microbiome to benefit the host (Liu et al. 2016). However, further studies are required to confirm the interconnection between neuronal epigenetics and alteration in gut microbiome that

would result in development of novel microbiota-oriented strategies such as probiotic, diet in treatment of the psychiatric conditions or slowing down the progression of neurodegenerative diseases.

Application of machine learning in multi-omics data analysis

Based on the hypothesis that the GBA and gut microbiome influence health by interacting with the host immune system, identifying microbial signaling pathways could lead to novel approaches in tackling the disease (microbiome multi-omics to identify changes in gut microbiome and its metabolites). In the following section, we propose a framework to integrate multi-omics data to move forward from just prediction to prevention and personalized therapeutics (Ferretti et al. 2018) (Fig. 2). We focused on the integration of -omics approaches (genomics, metabolomics, connectomics, and gut microbiome multi-omics) that carry complimentary information about disease emergence and progression. Multi-modal brain imaging data (e.g., structural MRI, resting state fMRI, and PET) can be used to track neurodegeneration and other network impairments (connectomics; brain connectivity datasets produced by functional and structural brain imaging). Blood, saliva, and cerebrospinal fluid samples can provide a global small-molecule snapshot that is sensitive to ongoing pathological processes (metabolomics to identify unique metabolite panels). GWAS and next-generation genome sequencing can be used to identify common-variants and rare-variants genetic risk factors associated with the disease (genomics to identify polygenic risk score).

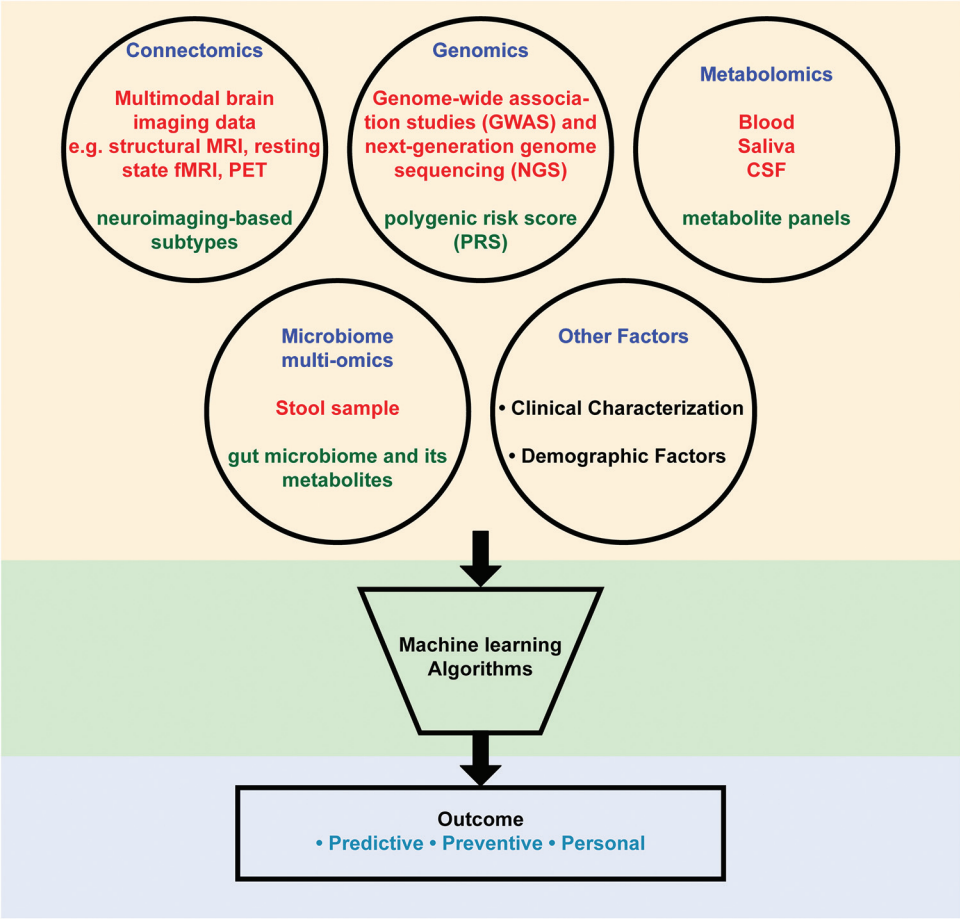
Due to the multi-factorial nature of diseases such as AD and the presence of inter-individual variation in response (clinical and pathological heterogeneity) among populations (mainly due to genetics and environmental factors/epigenetics), there is a strong need to use an integrative omics approach for finding the patterns of pathogenesis as well as to find the correct therapeutic strategies for an individual or to develop a tailored medicine. In the last decade with the advancement in machine learning and the availability of high computing resources there has been a radical shift in understanding the pathology of diseases. Earlier it was a "top-down" approach wherein the disease phenotype was labeled by the clinicians, nowadays it has become "bottom-up" wherein pathological signatures are derived using supervised and unsupervised machine learning algorithms and high-throughput omics metrics. These data-driven approaches provide a new insight at multiple levels (e.g., network, cellular, and molecular) to the complex etiology of neurodegenerative and psychiatric disorders and an opportunity to identify biomarker signatures with high diagnostic and prognostic value (Abraham et al. 2017; Badhwar et al. 2020; Huang et al. 2018; Jack et al. 2018; P. Liu et al. 2019; Orban et al. 2018). Machine learning algorithms have been extensively used for imaging and genetic and electrophysiological data in early detection, diagnosis, and treatment, as well as outcome prediction and prognosis evaluation in various diseases including cancer, nervous system disease, and cardiovascular disease (Jiang et al. 2017).

Machine learning is an approach to recognize, classify, and predict patterns of data (Tarca et al. 2007; Zhou and Gallins 2019). Both supervised and unsupervised algorithms are used for biomarker identification in neurodegenerative and psychiatric disorders. Unsupervised machine learning algorithms such as hierarchical clustering and k-means clustering are important exploratory tools to identify patterns in the unlabeled data. While supervised machine learning algorithms use the labeled data as an input to train the model to identify the feature characteristics associated with the disease. Large datasets are required to train the model for better prediction of underlying disease condition, because if the number of features exceed the sample size then over-fitting of complex models to the data are a concern, thus leading to false predictions. This problem is of interest when using just the microbiome data as predictor of various diseases, it is in part due to the high dimensionality of microbiome data (strain-level gene markers), which can induce sparsity of the data in the feature space. Efficient dimensionality reduction techniques can solve this problem by transforming this high-dimensional microbiome data into a robust low-dimensional representation, and then applying machine learning classification algorithms on the learned representation will increase the prediction accuracy (Mossotto et al. 2017; Statnikov et al. 2013; Thaiss et al. 2016; Thomas et al. 2019; Topcuoglu et al. 2020; Wirbel et al. 2019).

Previously, the field of genomics has been the main engine to the precision medicine movement, as the disease pathology in an individual is largely determined by the genetic makeup of the person and (or) the variability in their genes (gene mutations, unique footprints). Numerous studies have been undertaken in the fields of cognition, AD, and precision medicine using genetic, phenotypic, and psychosocial characteristics of individuals (Ferretti et al. 2018; Reitz 2016). However, lately with an enhanced understanding in the field of microbiome, more researchers and physicians have begun considering microbiome as one of the critical components and a new target in precision medicine.

Despite advances in data collection methods such as brain imaging and gut microbiome sequencing, the assortment and analysis of large amounts of patient data remains a challenge. This prevents researchers and physicians from understanding trends and causal links between the gut microbiome and brain function. In a precision health setting, clinicians were unable to develop predictive models, resulting in incomplete

Fig. 2. Framework for multi-omics (host genomics, connectomics, transcriptomics, proteomics, and metabolomics) data integration and use of advanced machine learning approaches to predict the heath or diseased state or to find the right therapeutic strategy for an individual person (Ferretti et al. 2018). Blue text represents the multi-omics dataset type, red text represents the data collection method or technique, and green text represents the information extracted from the dataset.



diagnosis (De Santis et al. 2019). The emergence of machine learning and artificial intelligence, however, has provided a solution to the "big data challenge". It not only aids physicians in sifting through patient data but also develops tools to better understand underlying biological mechanisms. It is clear that the development of experimental and machine learning approaches has made data collection robust, and its analysis thorough. The applications are endless; the response of the human gut microbiome to drugs, diet, and environmental factors such as stress can be correlated with neurodegenerative diseases. Ultimately, this knowledge will aid the development of predictive models and better diagnosis.

Discussion

The GBA is a biochemical link between the CNS and ENS where gut microbiota is the main contributor to variable functions of this axis. Understanding the mechanistic connection between the gut microbiome and brain function has been critical in developing a working understanding of the role of the GBA. The notion that the gut microbiota can influence the brain through neural, endocrine, immune, and humoral pathways is supported by extensive research in microbiology, epigenetics, and computational biology. Gut microbiota produces a wide-range of metabolites, most notably SCFAs, which play an important role in host defense, act as signaling molecules involved in lipid metabolism, and glucose/insulin regulation. This is essential for maintaining the intestinal and blood–brain barrier permeability and is known to modulate mammalian cell functions by serving as an energy substrate or by signaling through G-protein-coupled receptors. In addition, SCFAs can directly or indirectly stimulate the vagal nerve thereby influencing the GBA. The role of diet, nutrients, environment, probiotics, and drugs in impacting gut microbiota composition has been well documented—primarily via epigenetic mechanisms. Metabolic by-products from microbiota such as SCFAs act as HDAC inhibitors, play a role in epigenetic regulation through DNA methylation, post-transcriptional histone

modification, and chromatin restructuring to alter gene expression. Recent advances in omics and computational biology have led to the identification of biological factors and mechanisms that connect the onset and progression of neurological diseases to gut microbiome. In the future, microbiome multi-omics could be used in machine learning modeling for disease risk prediction and precision medicine in humans (Fig. 2). However, several more microbiome-sequencing studies are required with larger sets to train the model for better prediction of underlying disease conditions based on microbiome data.

Conclusion

Several studies have highlighted the impact of microbial metabolites on neural and endocrine signaling. Understanding the involvement of gut microbiota metabolites' in epigenetic regulation of immune, metabolic, and nervous systems will elucidate the pathophysiology of complex brain disorders. Further, identifying microbial signaling pathways could lead to novel approaches in tackling the disease. In a clinical setting, machine learning approaches can not only help in integrating phenotypic, genotypic, epigenetic, connectomics, and microbiome data but also develop predictive models of disease. This will initiate novel precision health approaches for treatment of brain disorders, through the development of targeted therapy, using prebiotics and probiotics in combination with dietary changes to restore normal interaction between the CNS and gut.

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References

Abdel-Haq, R., Schlachetzki, J.C.M., Glass, C.K., and Mazmanian, S.K. 2019. Microbiome-microglia connections via the gut–brain axis. J. Exp. Med. **216**(1): 41–59. doi:10.1084/jem.20180794. PMID:30385457.

Abraham, A., Milham, M.P., Di Martino, A., Craddock, R.C., Samaras, D., Thirion, B., et al. 2017. Deriving reproducible biomarkers from multi-site resting-state data: An Autism-based example. NeuroImage, **147**: 736–745. doi:10.1016/j.neuroimage.2016.10.045. PMID:27865923.

Akare, S., Jean-Louis, S., Chen, W., Wood, D.J., Powell, A.A., and Martinez, J.D. 2006. Ursodeoxycholic acid modulates histone acetylation and induces differentiation and senescence. Int. J. Cancer, **119**(12): 2958–2969. doi:10.1002/ijc.22231. PMID:17019713.

Alenghat, T., Osborne, L.C., Saenz, S.A., Kobuley, D., Ziegler, C.G., Mullican, S.E., et al. 2013. Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. Nature, **504**(7478): 153–157. doi:10.1038/nature12687. PMID:24185009.

Badhwar, A., McFall, G.P., Sapkota, S., Black, S.E., Chertkow, H., Duchesne, S., et al. 2020. A multiomics approach to heterogeneity in Alzheimer's disease: focused review and roadmap. Brain, **143**(5): 1315–1331. doi:10.1093/brain/awz384. PMID:31891371.

Barden, N. 2004. Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. J. Psychiatry Neurosci. **29**(3): 185–193. PMID:15173895.

Bastiaanssen, T.F.S., Cowan, C.S.M., Claesson, M.J., Dinan, T.G., and Cryan, J.F. 2019. Making Sense of ... the Microbiome in Psychiatry. Int. J. Neuropsychopharmacol. **22**(1): 37–52. doi:10.1093/ijnp/pyy067. PMID:30099552.

Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., et al. 2011. The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut–brain communication. Neurogastroenterol. Motil. **23**(12): 1132–1139. doi:10.1111/j.1365-2982.2011.01796.x. PMID:21988661.

Bolduc, J.F., Hany, L., Barat, C., Ouellet, M., and Tremblay, M.J. 2017. Epigenetic Metabolite Acetate Inhibits Class I/II Histone Deacetylases, Promotes Histone Acetylation, and Increases HIV-1 Integration in CD4(+) T Cells. J. Virol. **91**(16): e01943-16. doi:10.1128/JVI.01943-16. PMID:28539453.

Bonaz, B., Bazin, T., and Pellissier, S. 2018. The Vagus Nerve at the interface of the Microbiota–Gut–Brain Axis. Front. Neurosci. **12**: 49. doi:10.3389/fnins.2018.00049. PMID:29467611.

Bonder, M.J., Kurilshikov, A., Tigchelaar, E.F., Mujagic, Z., Imhann, F., Vila, A.V., et al. 2016. The effect of host genetics on the gut microbiome. Nat. Genet. **48**(11): 1407–1412. doi:10.1038/ng.3663. PMID:27694959.

Bongers, G., Pacer, M.E., Geraldino, T.H., Chen, L., He, Z., Hashimoto, D., et al. 2014. Interplay of host microbiota, genetic perturbations, and inflammation promotes local development of intestinal neoplasms in mice. J. Exp. Med. **211**(3): 457–472. doi:10.1084/jem.20131587.

Bourassa, M.W., Alim, I., Bultman, S.J., and Ratan, R.R. 2016. Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? Neurosci. Lett. **625**: 56–63. doi:10.1016/j.neulet.2016.02.009. PMID:26868600.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., et al. 2011. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. U.S.A. **108**(38): 16050–16055. doi:10.1073/pnas.1102999108. PMID:21876150.

Breiling, A., and Lyko, F. 2015. Epigenetic regulatory functions of DNA modifications: 5-methylcytosine and beyond. Epigenet. Chromatin, **8**: 24. doi:10.1186/s13072-015-0016-6. PMID:26195987.

Breit, S., Kupferberg, A., Rogler, G., and Hasler, G. 2018. Vagus nerve as modulator of the Brain–Gut Axis in psychiatric and inflammatory disorders. Front. Psychiatry, **9**: 44. doi:10.3389/fpsyt.2018.00044. PMID:29593576.

Browning, K.N., Verheijden, S., and Boeckxstaens, G.E. 2017. The Vagus Nerve in Appetite Regulation, Mood, and Intestinal Inflammation. Gastroenterology, **152**(4): 730–744. doi:10.1053/j.gastro.2016.10.046. PMID:27988382.

Buffington, S.A., Di Prisco, G.V., Auchtung, T.A., Ajami, N.J., Petrosino, J.F., and Costa-Mattioli, M. 2016. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. Cell, **165**(7): 1762–1775. doi:10.1016/j.cell.2016.06.001. PMID:27315483.

Campbell, J.H., Foster, C.M., Vishnivetskaya, T., Campbell, A.G., Yang, Z.K., Wymore, A., et al. 2012. Host genetic and environmental effects on mouse intestinal microbiota. ISME J. **6**(11): 2033–2044. doi:10.1038/ismej.2012.54. PMID:22695862.

Carabotti, M., Scirocco, A., Maselli, M.A., and Severi, C. 2015. The gut–brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann. Gastroenterol. **28**(2): 203–209. PMID:25830558.

Carrard, A., Elsayed, M., Margineanu, M., Boury-Jamot, B., Fragniere, L., Meylan, E.M., et al. 2018. Peripheral administration of lactate produces antidepressant-like effects.

Mol. Psychiatry, **23**(2): 392–399. doi:10.1038/mp.2016.179. PMID:27752076.

Colonna, M., and Butovsky, O. 2017. Microglia function in the central nervous system during health and neurodegeneration. Annu. Rev. Immunol. **35**(1): 441–468. doi:10.1146/annurev-immunol-051116-052358. PMID:28226226.

Coretti, L., Cristiano, C., Florio, E., Scala, G., Lama, A., Keller, S., et al. 2017. Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder. Scientific Rep. **7**(1): 45356. doi:10.1038/srep45356. PMID:28349974.

Coretti, L., Paparo, L., Riccio, M.P., Amato, F., Cuomo, M., Natale, A., et al. 2018. Gut microbiota features in young children with autism spectrum disorders. Front. Microbiol. **9**: 3146. doi:10.3389/fmicb.2018.03146. PMID:30619212.

Cox, L.M., and Weiner, H.L. 2018. Microbiota Signaling Pathways that Influence Neurologic Disease. Neurotherapeutics, **15**(1): 135–145. doi:10.1007/s13311-017-0598-8. PMID:29340928.

Crider, K.S., Yang, T.P., Berry, R.J., and Bailey, L.B. 2012. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv. Nutr. **3**(1): 21–38. doi:10.3945/an.111.000992. PMID:22332098.

Cryan, J.F., and O'Mahony, S.M. 2011. The microbiome–gut–brain axis: from bowel to behavior. Neurogastroenterol. Motil. **23**(3): 187–192. doi:10.1111/j.1365-2982.2010.01664.x. PMID:21303428.

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D.I., et al. 2013. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One, **8**(10): e76993. doi:10.1371/journal.pone.0076993. PMID:24130822.

de la Fuente-Nunez, C., Meneguetti, B.T., Franco, O.L., and Lu, T.K. 2018. Neuromicrobiology: How Microbes Influence the Brain. ACS Chem. Neurosci. **9**(2): 141–150. doi:10.1021/acschemneuro.7b00373. PMID:29220570.

de Lartigue, G., de La Serre, C.B., and Raybould, H.E. 2011. Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. Physiol. Behav. **105**(1): 100–105. doi:10.1016/j.physbeh.2011.02.040. PMID:21376066.

De Santis, S., Moratal, D., and Canals, S. 2019. Radiomicrobiomics: advancing along the gut–brain axis through big data analysis. Neuroscience, **403**: 145–149. doi:10.1016/j.neuroscience.2017.11.055. PMID:29237568.

de Weerth, C. 2017. Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. Neurosci. Biobehav. Rev. **83**: 458–471. doi:10.1016/j.neubiorev.2017.09.016. PMID:28918360.

den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., and Bakker, B.M. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J. Lipid Res. **54**(9): 2325–2340. doi:10.1194/jlr.R036012. PMID:23821742.

Dickerson, F., Severance, E., and Yolken, R. 2017. The microbiome, immunity, and schizophrenia and bipolar disorder. Brain Behav. Immun. **62**: 46–52. doi:10.1016/j.bbi.2016.12.010. PMID:28003152.

Dinan, T.G., and Cryan, J.F. 2012. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology, **37**(9): 1369–1378. doi:10.1016/j.psyneuen.2012.03.007. PMID:22483040.

Drevets, W.C., Price, J.L., and Furey, M.L. 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct. Funct. **213**(1-2): 93–118. doi:10.1007/s00429-008-0189-x. PMID:18704495.

Edmiston, E., Ashwood, P., and Van de Water, J. 2017. Autoimmunity, autoantibodies, and autism spectrum disorder. Biol. Psychiatry, **81**(5): 383–390. doi:10.1016/j.biopsych.2016.08.031. PMID:28340985.

Edwards, G.A., III., Gamez, N., Escobedo, G., Jr., Calderon, O., and Moreno-Gonzalez, I. 2019. Modifiable risk factors for Alzheimer's disease. Front. Aging Neurosci. **11**: 146. doi:10.3389/fnagi.2019.00146. PMID:31293412.

Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al. 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. **18**(7): 965–977. doi:10.1038/nn.4030. PMID:26030851.

Eshraghi, R.S., Deth, R.C., Mittal, R., Aranke, M., Kay, S.S., Moshiree, B., et al. 2018. Early Disruption of the Microbiome Leading to Decreased Antioxidant Capacity and Epigenetic Changes: Implications for the Rise in Autism. Front. Cell. Neurosci. **12**: 256. doi:10.3389/fncel.2018.00256. PMID:30158857.

Fan, J., Krautkramer, K.A., Feldman, J.L., and Denu, J.M. 2015. Metabolic regulation of histone post-translational modifications. ACS Chem. Biol. **10**(1): 95–108. doi:10.1021/cb500846u. PMID:25562692.

Ferretti, M.T., Iulita, M.F., Cavedo, E., Chiesa, P.A., Schumacher Dimech, A., Santuccione Chadha, A., et al. 2018. Sex differences in Alzheimer disease — the gateway to precision medicine. Nat. Rev. Neurol. **14**(8): 457–469. doi:10.1038/s41582-018-0032-9. PMID:29985474.

Finegold, S.M., Dowd, S.E., Gontcharova, V., Liu, C., Henley, K.E., Wolcott, R.D., et al. 2010. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe, **16**(4): 444–453. doi:10.1016/j.anaerobe.2010.06.008. PMID:20603222.

Fluegge, K. 2017. Humoral immunity and autism spectrum disorders. Immunol. Lett. **185**: 90–92. doi:10.1016/j.imlet.2017.03.003. PMID:28288805.

Forsythe, P., Bienenstock, J., and Kunze, W.A. 2014. Vagal pathways for microbiome–brain–gut axis communication. Adv. Exp. Med. Biol. **817**: 115–133. doi:10.1007/978-1-4939-0897-4_5. PMID:24997031.

Foster, J.A., and McVey Neufeld, K.A. 2013. Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosci. **36**(5): 305–312. doi:10.1016/j.tins.2013.01.005. PMID:23384445.

Foster, J.A., Rinaman, L., and Cryan, J.F. 2017. Stress & the gut–brain axis: Regulation by the microbiome. Neurobiol. Stress. **7**: 124–136. doi:10.1016/j.ynstr.2017.03.001. PMID:29276734.

Fulling, C., Dinan, T.G., and Cryan, J.F. 2019. Gut microbe to brain signaling: what happens in Vagus. Neuron, **101**(6): 998–1002. doi:10.1016/j.neuron.2019.02.008. PMID:30897366.

Fung, T.C., Olson, C.A., and Hsiao, E.Y. 2017. Interactions between the microbiota, immune and nervous systems in health and disease. Nat. Neurosci. **20**(2): 145–155. doi:10.1038/nn.4476. PMID:28092661.

Furness, J.B. 2012. The enteric nervous system and neurogastroenterology. Nat. Rev. Gastroenterol. Hepatol. **9**(5): 286–294. doi:10.1038/nrgastro.2012.32. PMID:22392290.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., et al. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature, **504**(7480): 446–450. doi:10.1038/nature12721. PMID:24226770.

Goines, P., and Van de Water, J. 2010. The immune system's role in the biology of autism. Curr. Opin. Neurol. **23**(2): 111–117. doi:10.1097/WCO.0b013e3283373514. PMID:20160651.

Goodrich, J.K., Davenport, E.R., Beaumont, M., Jackson, M.A., Knight, R., Ober, C., et al. 2016. Genetic determinants of the gut microbiome in UK twins. Cell Host & Microbe, **19**(5): 731–743. doi:10.1016/j.chom.2016.04.017. PMID:27173935.

Grafodatskaya, D., Chung, B., Szatmari, P., and Weksberg, R. 2010. Autism spectrum disorders and epigenetics. J. Am. Acad. Child Adolesc. Psychiatry, **49**(8): 794–809. doi:10.1016/j.jaac.2010.05.005. PMID:20643313.

Gur, T.L., Worly, B.L., and Bailey, M.T. 2015. Stress and the commensal microbiota: importance in parturition and infant neurodevelopment. Front. Psychiatry, **6**: 5. doi:10.3389/fpsyt.2015.00005. PMID:25698977.

Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K.D., Frisoni, G., et al. 2017. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci. Rep. **7**(1): 41802. doi:10.1038/srep41802. PMID:28176819.

Hildebrand, F., Nguyen, T.L., Brinkman, B., Yunta, R.G., Cauwe, B., Vandenabeele, P., et al. 2013. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. Genome Biol. **14**(1): R4. doi:10.1186/gb-2013-14-1-r4. PMID:23347395.

Hossain, M.S., Oomura, Y., Fujino, T., and Akashi, K. 2020. Glucose signaling in the brain and periphery to memory. Neurosci. Biobehav. Rev. **110**: 100–113. doi:10.1016/j.neubiorev.2019.03.018. PMID:32111301.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell, **155**(7): 1451–1463. doi:10.1016/j.cell.2013.11.024. PMID:24315484.

Huang, X., Liu, H., Li, X., Guan, L., Li, J., Tellier, L., et al. 2018. Revealing Alzheimer's disease genes spectrum in the whole-genome by machine learning. BMC Neurol. **18**(1): 5. doi:10.1186/s12883-017-1010-3. PMID:29320986.

Integrative H.M.P.R.N.C. 2019. The Integrative Human Microbiome Project. Nature, **569**(7758): 641–648. doi:10.1038/s41586-019-1238-8. PMID:31142853.

Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., et al. 2011. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science, **333**(6047): 1300–1303. doi:10.1126/science.1210597. PMID:21778364.

Jack, C.R, Jr., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., et al. 2018. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's Dement. **14**(4): 535–562. doi:10.1016/j.jalz.2018.02.018. PMID:29653606.

Jandhyala, S.M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., and Nageshwar, R.D. 2015. Role of the normal gut microbiota. World J. Gastroenterol. **21**(29): 8787–8803. doi:10.3748/wjg.v21.i29.8787. PMID:26269668.

Jiang, F., Jiang, Y., Zhi, H., Dong, Y., Li, H., Ma, S., et al. 2017. Artificial intelligence in healthcare: past, present and future. Stroke Vasc. Neurol. **2**(4): 230–243. doi:10.1136/svn-2017-000101. PMID:29507784.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., et al. 2015. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav. Immun. **48**: 186–194. doi:10.1016/j.bbi.2015.03.016. PMID:25882912.

Jin, B., Li, Y., and Robertson, K.D. 2011. DNA methylation: superior or subordinate in the epigenetic hierarchy? Genes Cancer, **2**(6): 607–617. doi:10.1177/1947601910393957. PMID:21941617.

Kaisar, M.M.M., Pelgrom, L.R., van der Ham, A.J., Yazdanbakhsh, M., and Everts, B. 2017. Butyrate conditions human dendritic cells to prime type 1 regulatory T cells via both Histone Deacetylase inhibition and G Protein-coupled receptor 109A signaling. Front. Immunol. **8**: 1429. doi:10.3389/fimmu.2017.01429. PMID:29163504.

Kang, D.W., Ilhan, Z.E., Isern, N.G., Hoyt, D.W., Howsmon, D.P., Shaffer, M., et al. 2018. Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe, **49**: 121–131. doi:10.1016/j.anaerobe.2017.12.007. PMID:29274915.

Karnib, N., El-Ghandour, R., El Hayek, L., Nasrallah, P., Khalifeh, M., Barmo, N., et al. 2019. Lactate is an antidepressant that mediates resilience to stress by modulating the hippocampal levels and activity of histone deacetylases. Neuropsychopharmacology, **44**(6): 1152–1162. doi:10.1038/s41386-019-0313-z. PMID:30647450.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., and Kimura, I. 2015. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. Nutrients, **7**(4): 2839–2849. doi:10.3390/nu7042839. PMID:25875123.

Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., and Gordon, J.I. 2011. Human nutrition, the gut microbiome and the immune system. Nature, **474**(7351): 327–336. doi:10.1038/nature10213. PMID:21677749.

Kaur, H., Golovko, S., Golovko, M.Y., Singh, S., Darland, D.C., and Combs, C.K. 2020*a*. Effects of probiotic supplementation on short chain fatty acids in the AppNL-G-F mouse model of Alzheimer's disease. J. Alzheimer's Dis. **76**(3): 1083–1102. doi:10.3233/JAD-200436. PMID:32623399.

Kaur, H., Nagamoto-Combs, K., Golovko, S., Golovko, M.Y., Klug, M.G., and Combs, C.K. 2020*b*. Probiotics ameliorate intestinal pathophysiology in a mouse model of Alzheimer's disease. Neurobiol. Aging. **92**: 114–134. doi:10.1016/j.neurobiolaging.2020.04.009. PMID:32417748.

Kennedy, P.J., Cryan, J.F., Dinan, T.G., and Clarke, G. 2017. Kynurenine pathway metabolism and the microbiota–gut–brain axis. Neuropharmacology, **112**(Pt B): 399–412. doi:10.1016/j.neuropharm.2016.07.002. PMID:27392632.

Krautkramer, K.A., Kreznar, J.H., Romano, K.A., Vivas, E.I., Barrett-Wilt, G.A., Rabaglia, M.E., et al. 2016. Diet-microbiota interactions mediate global epigenetic programming in multiple host tissues. Mol. Cell, **64**(5): 982–992. doi:10.1016/j.molcel.2016.10.025. PMID:27889451.

Kumar, S., Chinnusamy, V., and Mohapatra, T. 2018. Epigenetics of modified DNA bases: 5-methylcytosine and beyond. Front. Genetics, **9**: 640. doi:10.3389/fgene.2018.00640. PMID:30619465.

Kumar, H., Lund, R., Laiho, A., Lundelin, K., Ley, R.E., Isolauri, E., et al. 2014. Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. mBio, **5**(6): e02113-14. doi:10.1128/mBio.02113-14. PMID:25516615.

Kunze, W.A., Mao, Y.K., Wang, B., Huizinga, J.D., Ma, X., Forsythe, P., et al. 2009. *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J. Cell. Mol. Med. **13**(8B): 2261–2270. doi:10.1111/j.1582-4934.2009.00686.x.

Kushak, R.I., Winter, H.S., Buie, T.M., Cox, S.B., Phillips, C.D., and Ward, N.L. 2017. Analysis of the duodenal microbiome in autistic individuals: association with carbohydrate digestion. J. Pediatr. Gastroenterol. Nutr. **64**(5): e110–e116. doi:10.1097/MPG.0000000000001458. PMID:27811623.

Lal, S., Kirkup, A.J., Brunsden, A.M., Thompson, D.G., and Grundy, D. 2001. Vagal afferent responses to fatty acids of different chain length in the rat. Am. J. Physiol. Gastrointest. Liver Physiol. **281**(4): G907–915. doi:10.1152/ajpgi.2001.281.4.G907. PMID:11557510.

Lavebratt, C., Almgren, M., and Ekstrom, T.J. 2012. Epigenetic regulation in obesity. Int. J. Obesity. **36**(6): 757–765. doi:10.1038/ijo.2011.178. PMID:21912396.

Liu, S., da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., et al. 2016. The host shapes the gut microbiota via fecal MicroRNA. Cell Host & Microbe, **19**(1): 32–43. doi:10.1016/j.chom.2015.12.005. PMID:26764595.

Liu, F., Horton-Sparks, K., Hull, V., Li, R.W., and Martinez-Cerdeno, V. 2018. The valproic acid rat model of autism presents with gut bacterial dysbiosis similar to

that in human autism. Mol. Autism, **9**: 61. doi:10.1186/s13229-018-0251-3. PMID:30555669.

Liu, F., Li, J., Wu, F., Zheng, H., Peng, Q., and Zhou, H. 2019. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. Transl. Psychiatry, **9**(1): 43. doi:10.1038/s41398-019-0389-6. PMID:30696816.

Liu, P., Peng, G., Zhang, N., Wang, B., and Luo, B. 2019. Crosstalk between the gut microbiota and the brain: an update on neuroimaging findings. Front. Neurol. **10**: 883. doi:10.3389/fneur.2019.00883. PMID:31456743.

Louwies, T., Johnson, A.C., Orock, A., Yuan, T., and Greenwood-Van Meerveld, B. 2020. The microbiota–gut–brain axis: An emerging role for the epigenome. Exp. Biol. Med. (Maywood), **245**(2): 138–145. doi:10.1177/1535370219891690. PMID:31805777.

Lukovac, S., Belzer, C., Pellis, L., Keijser, B.J., de Vos, W.M., Montijn, R.C., et al. 2014. Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. mBio, **5**(4): e01438-14. doi:10.1128/mBio.01438-14. PMID:25118238.

Macfarlane, S., and Macfarlane, G.T. 2003. Regulation of short-chain fatty acid production. Proc, Nutr. Soc. **62**(1): 67–72. doi:10.1079/PNS2002207. PMID:12740060.

Magnusdottir, S., Ravcheev, D., de Crecy-Lagard, V., and Thiele, I. 2015. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. Front. Genet. **6**: 148. doi:10.3389/fgene.2015.00148. PMID:25941533.

Malan-Muller, S., Valles-Colomer, M., Raes, J., Lowry, C.A., Seedat, S., and Hemmings, S.M.J. 2018. The gut microbiome and mental health: implications for anxiety- and trauma-related disorders. OMICS, **22**(2): 90–107. doi:10.1089/omi.2017.0077. PMID:28767318.

Matteoli, G., and Boeckxstaens, G.E. 2013. The vagal innervation of the gut and immune homeostasis. Gut, **62**(8): 1214–1222. doi:10.1136/gutjnl-2012-302550. PMID:23023166.

Mayer, E.A. 2011. Gut feelings: the emerging biology of gut–brain communication. Nat. Rev. Neurosci. **12**(8): 453–466. doi:10.1038/nrn3071. PMID:21750565.

Mellott, T.J., Kowall, N.W., Lopez-Coviella, I., and Blusztajn, J.K. 2007. Prenatal choline deficiency increases choline transporter expression in the septum and hippocampus during postnatal development and in adulthood in rats. Brain Res. **1151**: 1–11. doi:10.1016/j.brainres.2007.03.004. PMID:17399691.

Mertz, H.R. 2003. Overview of functional gastrointestinal disorders: dysfunction of the brain-gut axis. Gastroenterol. Clin. North Am. **32**(2): 463–476. doi:10.1016/S0889-8553(03)00019-0. PMID:12858602.

Minter, M.R., Zhang, C., Leone, V., Ringus, D.L., Zhang, X., Oyler-Castrillo, P., et al. 2016. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci. Rep. **6**(1): 30028. doi:10.1038/srep30028. PMID:27443609.

Miro-Blanch, J., and Yanes, O. 2019. Epigenetic regulation at the interplay between gut microbiota and host metabolism. Front. Genet. **10**: 638. doi:10.3389/fgene.2019.00638. PMID:31338107.

Mittal, R., Debs, L.H., Patel, A.P., Nguyen, D., Patel, K., O'Connor, G., et al. 2017. Neurotransmitters: the critical modulators regulating gut–brain axis. J. Cell. Physiol. **232**(9): 2359–2372. doi:10.1002/jcp.25518. PMID:27512962.

Mohajeri, M.H., La Fata, G., Steinert, R.E., and Weber, P. 2018. Relationship between the gut microbiome and brain function. Nutr. Rev. **76**(7): 481–496. doi:10.1093/nutrit/nuy009. PMID:29701810.

Moloney, R.D., Desbonnet, L., Clarke, G., Dinan, T.G., and Cryan, J.F. 2014. The microbiome: stress, health and disease. Mamm. Genome, **25**(1–2): 49–74. doi:10.1007/s00335-013-9488-5. PMID:24281320.

Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., et al. 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry. **68**(4): 368–376. doi:10.1016/j.biopsych.2010.05.024. PMID:20674603.

Mossotto, E., Ashton, J.J., Coelho, T., Beattie, R.M., MacArthur, B.D., and Ennis, S. 2017. Classification of paediatric inflammatory bowel disease using. Sci. Rep. **7**(1): 2427. doi:10.1038/s41598-017-02606-2. PMID:28546534.

Mukhtar, K., Nawaz, H., and Abid, S. 2019. Functional gastrointestinal disorders and gut–brain axis: What does the future hold? World J. Gastroenterol. **25**(5): 552–566. doi:10.3748/wjg.v25.i5.552. PMID:30774271.

Nagy, C., Vaillancourt, K., and Turecki, G. 2018. A role for activity-dependent epigenetics in the development and treatment of major depressive disorder. Genes, Brain, and Behavior, **17**(3): e12446. doi:10.1111/gbb.12446. PMID:29251832.

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. 2012. Host–gut microbiota metabolic interactions. Science, **336**(6086): 1262–1267. doi:10.1126/science.1223813. PMID:22674330.

NIH Human Microbiome Portfolio Analysis Team, Proctor, L., LoTempio, J., Marquitz, A., Daschner, P., Xi, D., et al. 2019. A review of 10 years of human microbiome research activities at the US National Institutes of Health, Fiscal Years 2007-2016. Microbiome, **7**(1): 31. doi:10.1186/s40168-019-0620-y. PMID:30808411.

Obermeier, B., Daneman, R., and Ransohoff, R.M. 2013. Development, maintenance and disruption of the blood–brain barrier. Nat. Med. **19**(12): 1584–1596. doi:10.1038/nm.3407. PMID:24309662.

Orban, P., Dansereau, C., Desbois, L., Mongeau-Perusse, V., Giguere, C.E., Nguyen, H., et al. 2018. Multisite generalizability of schizophrenia diagnosis classification based on functional brain connectivity. Schizophr. Res. **192**: 167–171. doi:10.1016/j.schres.2017.05.027. PMID:28601499.

Paul, B., Barnes, S., Demark-Wahnefried, W., Morrow, C., Salvador, C., Skibola, C., et al. 2015. Influences of diet and the gut microbiome on epigenetic modulation in cancer and other diseases. Clin. Epigenet. **7**(1): 112. doi:10.1186/s13148-015-0144-7. PMID:26478753.

Petrosino, J.F. 2018. The microbiome in precision medicine: the way forward. Genome Med. **10**(1): 12. doi:10.1186/s13073-018-0525-6. PMID:29471863.

Pogribny, I.P., and Beland, F.A. 2009. DNA hypomethylation in the origin and pathogenesis of human diseases. Cell. Mol. Life Sci. **66**(14): 2249–2261. doi:10.1007/s00018-009-0015-5. PMID:19326048.

Prasher, D., Greenway, S.C., and Singh, R.B. 2020. The impact of epigenetics on cardiovascular disease. Biochem. Cell Biol. **98**(1): 12–22. doi:10.1139/bcb-2019-0045. PMID:31112654.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., et al. MetaHIT Consortium. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature, **464**(7285): 59–65. doi:10.1038/nature08821. PMID:20203603.

Rea, K., Dinan, T.G., and Cryan, J.F. 2016. The microbiome: A key regulator of stress and neuroinflammation. Neurobiol. Stress, **4**: 23–33. doi:10.1016/j.ynstr.2016.03.001. PMID:27981187.

Reijnders, D., Goossens, G.H., Hermes, G.D., Neis, E.P., van der Beek, C.M., Most, J., et al. 2016. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. Cell Metab. **24**(1): 63–74. doi:10.1016/j.cmet.2016.06.016. PMID:27411009.

Reitz, C. 2016. Toward precision medicine in Alzheimer's disease. Ann. Transl. Med. **4**(6): 107–107. doi:10.21037/atm.2016.03.05. PMID:27127760.

Rhee, S.H., Pothoulakis, C., and Mayer, E.A. 2009. Principles and clinical implications of the brain–gut–enteric microbiota axis. Nat. Rev. Gastroenterol. Hepatol. **6**(5): 306–314. doi:10.1038/nrgastro.2009.35. PMID:19404271.

Rogers, G.B., Keating, D.J., Young, R.L., Wong, M.L., Licinio, J., and Wesselingh, S. 2016. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol. Psychiatry, **21**(6): 738–748. doi:10.1038/mp.2016.50. PMID:27090305.

Romano, K.A., Martinez-Del Campo, A., Kasahara, K., Chittim, C.L., Vivas, E.I., Amador-Noguez, D., et al. 2017. Metabolic, epigenetic, and transgenerational effects of gut bacterial choline consumption. Cell Host & Microbe, **22**(3): 279–290.e277. doi:10.1016/j.chom.2017.07.021. PMID:28844887.

Rooks, M.G., and Garrett, W.S. 2016. Gut microbiota, metabolites and host immunity. Nat. Rev. Immunol. **16**(6): 341–352. doi:10.1038/nri.2016.42. PMID:27231050.

Rossi, M., Amaretti, A., and Raimondi, S. 2011. Folate production by probiotic bacteria. Nutrients, **3**(1): 118–134. doi:10.3390/nu3010118. PMID:22254078.

Sampson, T.R., and Mazmanian, S.K. 2015. Control of brain development, function, and behavior by the microbiome. Cell Host & Microbe, **17**(5): 565–576. doi:10.1016/j.chom.2015.04.011. PMID:25974299.

Sanders, T.H., Weiss, J., Hogewood, L., Chen, L., Paton, C., McMahan, R.L., et al. 2019. Cognition-enhancing vagus nerve stimulation alters the epigenetic landscape. J. Neurosci. **39**(18): 3454–3469. doi:10.1523/JNEUROSCI.2407-18.2019. PMID:30804093.

Seeley, J.J., Baker, R.G., Mohamed, G., Bruns, T., Hayden, M.S., Deshmukh, S.D., et al. 2018. Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. Nature, **559**(7712): 114–119. doi:10.1038/s41586-018-0253-5. PMID:29950719.

Sekirov, I., Russell, S.L., Antunes, L.C., and Finlay, B.B. 2010. Gut microbiota in health and disease. Physiol. Rev. **90**(3): 859–904. doi:10.1152/physrev.00045.2009. PMID:20664075.

Sender, R., Fuchs, S., and Milo, R. 2016. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell, **164**(3): 337–340. doi:10.1016/j.cell.2016.01.013.

Serrano-Pozo, A., Frosch, M.P., Masliah, E., and Hyman, B.T. 2011. Neuropathological alterations in Alzheimer disease. Cold Spring Harbor Perspect. Med. **1**(1): a006189–a006189. doi:10.1101/cshperspect.a006189. PMID:26824647.

Sharon, G., Cruz, N.J., Kang, D.W., Gandal, M.J., Wang, B., Kim, Y.M., et al. 2019. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. Cell, **177**(6): 1600–1618.e1617. doi:10.1016/j.cell.2019.05.004. PMID:31150625.

Sharon, G., Sampson, T.R., Geschwind, D.H., and Mazmanian, S.K. 2016. The central nervous system and the gut microbiome. Cell, **167**(4): 915–932. doi:10.1016/j.cell.2016.10.027.

Sherman, M.P., Zaghouani, H., and Niklas, V. 2015. Gut microbiota, the immune system, and diet influence the neonatal gut–brain axis. Pediatr. Res. **77**(1–2): 127–135. doi:10.1038/pr.2014.161. PMID:25303278.

Silva, Y.P., Bernardi, A., and Frozza, R.L. 2020. The role of short-chain fatty acids from gut microbiota in gut–brain communication. Front. Endocrinol. **11**: 25. doi:10.3389/fendo.2020.00025. PMID:32082260.

Silva, L.G., Ferguson, B.S., Avila, A.S., and Faciola, A.P. 2018. Sodium propionate and sodium butyrate effects on histone deacetylase (HDAC) activity, histone acetylation, and inflammatory gene expression in bovine mammary epithelial cells. J. Anim. Sci. **96**(12): 5244–5252. doi:10.1093/jas/sky373. PMID:30252114.

Soliman, M.L., and Rosenberger, T.A. 2011. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. Mol. Cell. Biochem. **352**(1–2): 173–180. doi:10.1007/s11010-011-0751-3. PMID:21359531.

Statnikov, A., Henaff, M., Narendra, V., Konganti, K., Li, Z., Yang, L., et al. 2013. A comprehensive evaluation of multicategory classification methods for microbiomic data. Microbiome, **1**(1): 11. doi:10.1186/2049-2618-1-11. PMID:24456583.

Stilling, R.M., Dinan, T.G., and Cryan, J.F. 2014. Microbial genes, brain & behaviour - epigenetic regulation of the gut–brain axis. Genes. Brain. Behav. **13**(1): 69–86. doi:10.1111/gbb.12109. PMID:24286462.

Strandwitz, P., Kim, K.H., Terekhova, D., Liu, J.K., Sharma, A., Levering, J., et al. 2019. GABA-modulating bacteria of the human gut microbiota. Nat. Microbiol. **4**(3): 396–403. doi:10.1038/s41564-018-0307-3. PMID:30531975.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., et al. 2004. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. J. Physiol. **558**(1): 263–275. doi:10.1113/jphysiol.2004.063388. PMID:15133062.

Takahashi, K., Sugi, Y., Nakano, K., Kobayakawa, T., Nakanishi, Y., Tsuda, M., et al. 2020. Regulation of gene expression through gut microbiota-dependent DNA methylation in colonic epithelial cells. Immunohorizons, **4**(4): 178–190. doi:10.4049/immunohorizons.1900086. PMID:32295802.

Takahashi, K., Sugi, Y., Nakano, K., Tsuda, M., Kurihara, K., Hosono, A., et al. 2011. Epigenetic control of the host gene by commensal bacteria in large intestinal epithelial cells. J. Biol. Chem. **286**(41): 35755–35762. doi:10.1074/jbc.M111.271007. PMID:21862578.

Tamboli, C.P., Neut, C., Desreumaux, P., and Colombel, J.F. 2004. Dysbiosis in inflammatory bowel disease. Gut, **53**(1): 1–4. doi:10.1136/gut.53.1.1. PMID:14684564.

Tarca, A.L., Carey, V.J., Chen, X-W., Romero, R., and Drăghici, S. 2007. Machine learning and its applications to biology. PLoS Comput. Biol. **3**(6): e116. doi:10.1371/journal.pcbi.0030116. PMID:17604446.

Thaiss, C.A., Itav, S., Rothschild, D., Meijer, M.T., Levy, M., Moresi, C., et al. 2016. Persistent microbiome alterations modulate the rate of post-dieting weight regain. Nature, **540**(7634): 544–551. doi:10.1038/nature20796. PMID:27906159.

Thomas, A.M., Manghi, P., Asnicar, F., Pasolli, E., Armanini, F., Zolfo, M., et al. 2019. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. Nat. Med. **25**(4): 667–678. doi:10.1038/s41591-019-0405-7. PMID:30936548.

Tilocca, B., Pieroni, L., Soggiu, A., Britti, D., Bonizzi, L., Roncada, P., et al. 2020. Gut–brain axis and neurodegeneration: state-of-the-art of meta-omics sciences for microbiota characterization. Int. J. Mol. Sci. **21**(11): 4045. doi:10.3390/ijms21114045. PMID:32516966.

Ting, J.P., and Trowsdale, J. 2002. Genetic control of MHC class II expression. Cell, **109**(2): S21–33. doi:10.1016/s0092-8674(02)00696-7. PMID:11983150.

Tollefsbol, T.O. 2011. Advances in epigenetic technology. Methods Mol. Biol. **791**: 1–10. doi:10.1007/978-1-61779-316-5_1. PMID:21913067.

Topcuoglu, B.D., Lesniak, N.A., Ruffin, MTt., Wiens, J., and Schloss, P.D. 2020. A framework for effective application of machine learning to microbiome-based classification problems. mBio, **11**(3): e00434-20. doi:10.1128/mBio.00434-20. PMID:32518182.

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., et al. 2009. A core gut microbiome

in obese and lean twins. Nature, **457**(7228): 480–484. doi:10.1038/nature07540. PMID:19043404.

Ursell, L.K., Metcalf, J.L., Parfrey, L.W., and Knight, R. 2012. Defining the human microbiome. Nutr. Rev. **70**((Suppl 1): S38–S44. doi:10.1111/j.1753-4887.2012.00493.x. PMID:22861806.

Ursell, L.K., Van Treuren, W., Metcalf, J.L., Pirrung, M., Gewirtz, A., and Knight, R. 2013. Replenishing our defensive microbes. BioEssays, **35**(9): 810–817. doi:10.1002/bies.201300018. PMID:23836415.

van Praag, H. 2018. Lifestyle factors and Alzheimer's disease. Brain Plasticity, **4**(1): 1–2. doi:10.3233/BPL-120418. PMID:30564543.

Wang, S., Ishima, T., Zhang, J., Qu, Y., Chang, L., Pu, Y., et al. 2020. Ingestion of *Lactobacillus intestinalis* and *Lactobacillus reuteri* causes depression- and anhedonia-like phenotypes in antibiotic-treated mice via the vagus nerve. J. Neuroinflammation, **17**(1): 241. doi:10.1186/s12974-020-01916-z. PMID:32799901.

Wang, M., Wan, J., Rong, H., He, F., Wang, H., Zhou, J., et al. 2019. Alterations in gut glutamate metabolism associated with changes in gut microbiota composition in children with autism spectrum disorder. mSystems, **4**(1). doi:10.1128/mSystems.00321-18. PMID:30701194.

Wei, Y., Melas, P.A., Wegener, G., Mathe, A.A., and Lavebratt, C. 2014. Antidepressant-like effect of sodium butyrate is associated with an increase in TET1 and in 5-hydroxymethylation levels in the Bdnf gene. Int. J. Neuropsychopharmacol. **18**(2): pyu032. doi:10.1093/ijnp/pyu032. PMID:25618518.

Wirbel, J., Pyl, P.T., Kartal, E., Zych, K., Kashani, A., Milanese, A., et al. 2019. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. Nat. Med. **25**(4): 679–689. doi:10.1038/s41591-019-0406-6. PMID:30936547.

Wong, H., and Hoeffer, C. 2018. Maternal IL-17A in autism. Exp. Neurol. **299(**Pt A): 228–240. doi:10.1016/j.expneurol.2017.04.010. PMID:28455196.

Wu, S.C., Cao, Z.S., Chang, K.M., and Juang, J.L. 2017. Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in *Drosophila*. Nat. Commun. **8**(1): 24. doi:10.1038/s41467-017-00040-6. PMID:28634323.

Wu, W., Sun, M., Chen, F., Cao, A.T., Liu, H., Zhao, Y., et al. 2017. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. Mucosal Immunol. **10**(4): 946–956. doi:10.1038/mi.2016.114. PMID:27966553.

Xiao, J., Wang, T., Xu, Y., Gu, X., Li, D., Niu, K., et al. 2020. Long-term probiotic intervention mitigates memory dysfunction through a novel H3K27me3-based mechanism in lead-exposed rats. Transl. Psychiatry, **10**(1): 25. doi:10.1038/s41398-020-0719-8. PMID:32066679.

Yang, B., Wei, J., Ju, P., and Chen, J. 2019. Effects of regulating intestinal microbiota on anxiety symptoms: A systematic review. General Psychiatry. **32**(2): e100056. doi:10.1136/gpsych-2019-100056. PMID:31179435.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., et al. 2012. Human gut microbiome viewed across age and geography. Nature, **486**(7402): 222–227. doi:10.1038/nature11053. PMID:22699611.

Yuille, S., Reichardt, N., Panda, S., Dunbar, H., and Mulder, I.E. 2018. Human gut bacteria as potent class I histone deacetylase inhibitors in vitro through production of butyric acid and valeric acid. PLoS One, **13**(7): e0201073. doi:10.1371/journal.pone.0201073. PMID:30052654.

Zeisel, S.H. 2007. Gene response elements, genetic polymorphisms and epigenetics influence the human dietary requirement for choline. IUBMB Life, **59**(6): 380–387. doi:10.1080/15216540701468954. PMID:17613168.

Zhou, Y.H., and Gallins, P. 2019. A review and tutorial of machine learning methods for microbiome host trait prediction. Front. Genet. **10**: 579. doi:10.3389/fgene.2019.00579. PMID:31293616.

Zhu, S., Jiang, Y., Xu, K., Cui, M., Ye, W., Zhao, G., et al. 2020. The progress of gut microbiome research related to brain disorders. J. Neuroinflammation. **17**(1): 25. doi:10.1186/s12974-020-1705-z. PMID:31952509.

Zhu, B., Wang, X., and Li, L. 2010. Human gut microbiome: the second genome of human body. Protein Cell, **1**(8): 718–725. doi:10.1007/s13238-010-0093-z. PMID:21203913.