

REVIEW

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Ischemic stroke and intestinal flora: an insight into brain–gut axis

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Abstract

Stroke is a type of cerebrovascular disease that significantly endangers human health and lowers quality of life. This understandably places a heavy burden on society and families. In recent years, intestinal flora has attracted increasing attention from scholars worldwide, and its association with ischemic stroke is becoming a hot topic of research amongst researchers in field of stroke. After suffering from a stroke, intestinal microbial dysbiosis leads to increased intestinal permeability and activation of the intestinal immune system, which in turn leads to ectopic intestinal bacteria and pro-inflammatory cells that enter brain tissue through the damaged blood-brain barrier. This exacerbates ischemia-reperfusion injury. Interestingly, after a stroke, some metabolites produced by the intestinal flora attenuate ischemia-reperfusion injury by suppressing the post-stroke inflammatory response and promotes the repair of neurological function. Here we elucidate the changes in gut flora after occurrence of a stroke and highlight the immunomodulatory processes of the post-stroke gut flora.

Keywords: Ischemic stroke, Intestinal flora, Brain–gut axis, Dysbiosis, Metabolites

Background

Stroke is an acute cerebrovascular disease, which can be caused by either sudden rupture of cerebral vessels or vascular occlusion; this is also referred to as hemorrhagic stroke (HS) and ischemic stroke (IS), respectively [1]. The incidence of ischemic stroke is significantly higher than that of hemorrhagic stroke, accounting for roughly 80% of the total incidence of cerebrovascular injury. The interruption of blood supply to brain, accompanied by hypoxia, further cause IS related nerve damage. Ischemic stroke was caused by a variety of risk factors and brought a heavy burden upon the patients' family as well as society in general [2]. The most important risk factors are hypertension, diabetes and atherosclerosis. Ischemic stroke is also a complex disease caused by a variety of environmental and genetic factors. Long-term domestic

and foreign studies have shown that the risk factors of IS are made up of two categories, namely, non-modifying risk factors [3] (gender, age, genetic factors, family history and race.) as well as modifying risk factors [4] (hypertension, abnormal blood glucose, hyperlipidemia, atrial fibrillation, high homocysteine, and bad living habits.) Intervention refers to the ability of controlling the risk factors, especially the most dangerous, which are hypertension and diabetes, to reduce the incidence and mortality of this disease.

Intestinal flora refers to all microorganisms in the human gastrointestinal tract, comprising of between 15,000~36,000 bacterial species, which represents mostly the Firmicutes and Bacteroidetes phyla [5–7]. Beside bacteria, Archaea and eukaryotes, viruses as well as bacteriophages are also included in intestinal flora [8]. Intestinal flora has the ability to regulate the metabolic activity of the host, as well as regulate the intestinal immune and biological barriers [9]; thus, it has a role of maintaining the health state of the host [10]. The total number of bacteria and species that makes up the intestinal flora can be affected by many factors, such as

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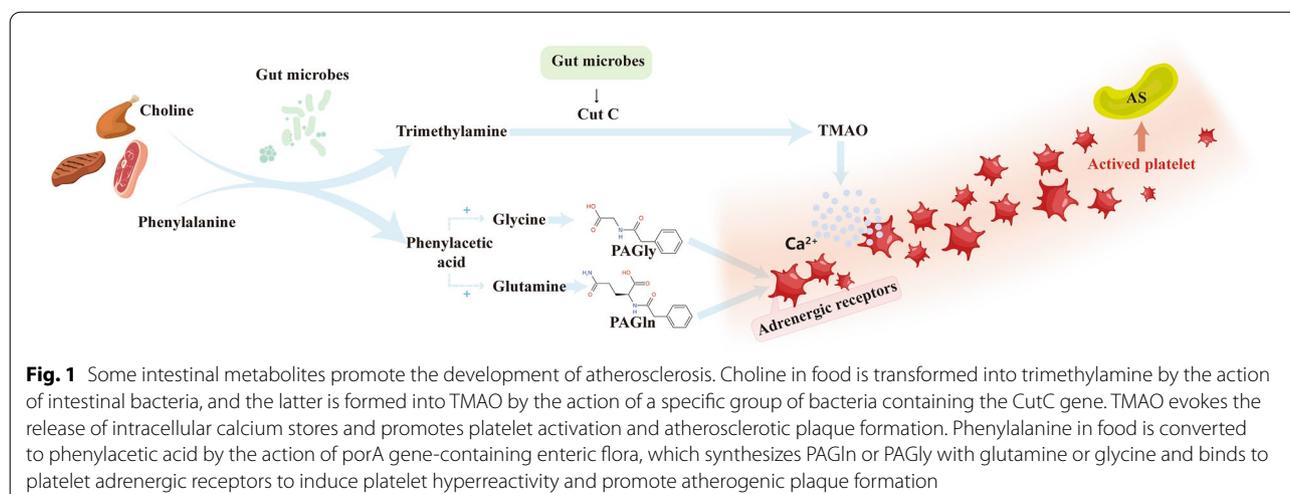
environment [11], diet [12], medications and genetics. Intestinal flora and its surrounding intestinal environment are collectively referred to as the intestinal microecosystems, which functions to maintain the homeostasis of the internal environment under normal conditions for humans and animals. Once the intestinal microecosystems lose its homeostasis, various diseases occur, which may also involve the central nervous system [13–15]. Intestinal microecosystem disorders can change the microenvironment of the intestine, affect the function of intestinal absorption and metabolism, subsequently affecting the risk factors of IS [16] directly or indirectly. In addition, the enteric nervous system, known as the human “second brain”, can interact with the central nervous system, autonomic nervous system [17], hypothalamus-pituitary-adrenal axis and other structures to form a two-way regulatory axis, the brain-gut axis. Intestinal flora can also decompose fermented food ingredients and produce a series of metabolites [18–20] that play an important role in the brain-gut axis. It can form a network of nerve, immune and endocrine regulation by stimulating neuroendocrine and conduction pathways, which is the “flora-gut-brain” axis. Changes in intestinal flora can alter the intestinal defence function and intestinal permeability [21], which affects both the enteric nervous system and central nervous system.

At the same time, intestinal flora plays an important role in the development of central nervous system [22]. Studies have shown that gut microbiota can regulate a series of neurotrophic factors or proteins that are involved in brain development and plasticity, such as brain-derived neurotrophic factors [23], synaptophysin and postsynaptic dense region proteins. The sterile state of sterile animals can lead to changes in the nervous system, such as increased permeability of blood-brain

barrier (BBB). Microglial cells are different from traditional bacterial colonization animals in morphology and function [24]. In addition, intestinal flora is also involved in the regulation of central nervous system activities, such as anxiety, depression and stress response [25]. Normal and steady intestinal flora plays a very important role in maintaining normal brain function and repair. When the balance of the intestinal flora changes, it can increase the risk of stroke through different mechanisms.

Intestinal flora and its products cause stroke by inducing atherosclerosis

Platelet activation, aggregation and atherosclerotic plaque formation are important pathogenesis of ischemic stroke (Fig. 1). Recent studies have shown that intestinal flora play an important role in the occurrence of atherosclerotic plaques. Intestinal flora can affect the occurrence of atherosclerosis in three different ways: (1) Bacterial infections activates the immune system [26] by influencing various immune cells [27]. Moreover, TLR expression by macrophages further leads to the increase of proinflammatory cytokines and chemokines, which accelerates the progression of atherosclerotic plaques and leads to the formation of vulnerable plaque. Microbes that have been shown to promote atherosclerosis include *Porphyromonas gingivalis* [28], *Aggregatibacter actinomycetemcomitans*, *Chlamydia pneumoniae* [29, 30] in addition to others. (2) Intestinal flora metabolism of food such as cholesterol and fat affect the formation of atherosclerotic plaque [31]. Transplantation of pro-inflammatory microorganisms can reduce the types of microorganisms that produce short-chain fatty acids (SCFA) in mice, enhance the inflammatory response and promote the formation of atherosclerosis [32]. Certain



kinds of bacteria such as *L. rhamnosus* GG (LGG) or pharmaceuticals telmisartan (TLM) supplements can alter bacterial genera and reduce α -diversity, which has significant correlations to atherosclerotic plaque size, plasma A-FABP and cholesterol level [33]. (3) Certain metabolites such as trimethylamine N-oxide (TMAO), which is produced by intestinal flora, promotes atherosclerotic plaque formation by activating platelet activity. The TMAO pathway is considered to be the most direct pathway, where intestinal flora influences the process of atherosclerosis [34, 35].

Choline from the diet is metabolized by intestinal microorganisms to produce trimethylamine, which is oxidized to TMAO after entering the liver via liver-gut circulation. TMAO promotes the release of intracellular calcium ions extracellularly in a platelet activator-dependent manner, which thereby mediates the high reactivity of platelets and increases the risk of thrombosis [36, 37].

In addition to animal experiments, clinical studies have also shown that TMAO is involved in the occurrence of atherosclerosis, which is significantly associated with the risk of cardiovascular and cerebrovascular events. In a study conducted by Tang et al. [38] which involved 4007 subjects who were followed up for 3 years to study the relationship between the concentration of plasma TMAO and the risk of cardiovascular/cerebrovascular events. The results showed that TMAO was positively correlated with the risk of thrombosis in a dose-dependent manner, and this effect was independent of traditional cardiovascular and cerebrovascular disease risk factors. Yet in the study of Yin et al. [39] did not find elevated plasma TMAO levels in stroke patients or transient ischemic attack (TIA) patients. Their study also analyzed the differences in intestinal flora composition and TMAO levels in asymptomatic patients with atherosclerosis, stroke and TIA. The results showed that the levels of TMAO and the composition of intestinal flora were similar in asymptomatic atherosclerosis patients, with or without carotid plaques. However, the composition of intestinal flora in patients with stroke or TIA was significantly different from that of patients with asymptomatic atherosclerosis. Notably, even though the TMAO level was not as high as expected, the level was still lower than that observed in patients with asymptomatic atherosclerosis. Tang et al. [38] explained that the medications used to treat stroke may reduce the level of TMAO. Therefore, the correlation between intestinal flora product, TMAO, and ischemic stroke needs further confirmatory research. The microbial cut C gene was found to mediate the TMA/TMAO conversion, as well as increase infarction size; thus this gene can be

thought to promote impaired neurological function by genetically engineering modified bacterial transplants in germ-free mice. In other words, gut microbes can exacerbate infarcts by producing TMAO [40].

In addition to TMAO, other intestinal flora metabolites that can activate platelets include PAGln and PAGly [41]. They represent phenylacetic acid, which is consumed from the diet, subsequently converted into phenylalanine by intestinal flora and ultimately into glutamine and glycine, respectively. PAGln and PAGly are similar in structure to adrenergic receptors and can, therefore, bind to platelet β 2 receptors in the body, able to activate platelets to promote thrombosis. However, some studies have found that PAGly can activate the Gai/PI3K/AKT signal cascade by stimulating β 2AR, thereby inhibiting cell apoptosis and reducing the area of myocardial infarction caused by I/R injury. However, high-dose treatment will cause a higher mortality rate [42]. It can be observed that the role of PAGly in the body is closely related to its dose. However, the role of PAGly after ischemic stroke, and the mechanism by which it functions, have not yet been reported, and needs further exploration (Fig. 1).

It is worth mentioning that *Porphyromonas gingivalis*, located in the oral cavity, is also found to be associated with the development of stroke [43, 44].

Changes in intestinal flora can affect brain repair after stroke

The “flora-intestine-brain” axis is a new concept. It is a prerequisite hypothesis, which showed that in the model of middle cerebral artery occlusion (MCAO), intestinal flora has a significant impact on stroke prognosis. The study of Benakis et al. [45] declared that flora imbalance, caused by antibiotics, could reduce the α -diversity of intestinal flora and improve prognosis; the histology showed a decrease in the volume of ischemic tissue. This effect is mainly due to the decrease of IL-17⁺ γ δ T cells and the increase of Treg cells in the small intestine, thereby limiting the infiltration of harmful substances into the brain membrane of IL-17⁺ γ δ T cells. Sun et al. [46] found that butyric acid bacteria can reduce cerebral I/R injury in diabetic mice by regulating intestinal flora. 16S rRNA gene sequencing, combined with LC-MS analysis, showed that in rats with IS, the intestinal flora and plasma metabolites changed. Moreover, it showed that the abundance of Proteobacteria Firmicutes and Deferribacteres was significantly different between Sham and IS groups. The gut microbiota was strongly correlated with the dysregulated metabolites [47]. Xu et al. [48] found that MCAO mice extended rapid and dynamic dysbiosis. The increase of Enterobacteriaceae bacteria aggravates cerebral infarction by enhancing systemic inflammation. Related studies have shown that dysregulation of

microflora is one of the reasons for poor prognosis of patients with primary stroke. The use of aminoguanidine or superoxide dismutase to reduce nitrate production, or by using tungstate to inhibit nitrate respiration, can inhibit the overgrowth of Enterobacteriaceae bacteria, reduce systemic inflammation and reduce risk of cerebral infarction. These therapeutic effects are dependent on the gut microbiota, which indicates the translational value of the brain-gut axis in the treatment of stroke. Wang et al. [49] proved that in patients with T2D, after AIS, the serum levels of lipopolysaccharide (LPS) and D-lactate (DLA) clearly increased; moreover, she showed that butyrate-producing bacteria including *Lachnospira*, *Blautia*, and *Butyricoccus* decreased. After BS was replenished the mice showed lower levels of proinflammatory cytokine and exhibited a significantly smaller infarction volume. It also showed that fecal transplantation could attenuate ischemic stroke injury by protecting the BBB. MCAO models of pigs [50], after 1 day of stroke, also showed a reduction in the microbial diversity, and on the third day the lesion volume was negatively correlated with microbial diversity. In relation to the models, the abundance of Proteobacteria was significantly increased, while Firmicutes and lactic acid bacteria, *Lactobacillus*, decreased on the third day poststroke. The aforementioned results (from a pig model) suggest the plasticity of the gut microbiome during the acute period of stroke and its influence on brain damage.

Changes in intestinal mucosal permeability affect stroke outcome

An intact intestinal mucosal barrier is an important defensive line for the body to protect against adverse external factors. When acute ischemic stroke occurs, the intestinal mucosal permeability is altered for various reasons, generally manifesting as increased permeability. This results in a large number of toxic products that enter the blood circulation through the intestinal mucosa, which then enter the nervous system causing damage. The impairment of intestinal barrier function in patients with cerebral infarction may be related to the following factors, outlined in the next three paragraphs.

Ischemic stroke leads to reduced expression of intestinal junction proteins

The intestinal mucosa, including the structure of epithelial tight junctions (TJs), are composed of multiple protein subunits [51], of which Claudins and occludins are particularly important because of their key structural roles. Many studies [52, 53] have examined their expression levels as a marker of altered mucosal permeability. There is a reduced expression of zonula occludens-1 (ZO-1), occludin, and claudin-1 after stroke [54]. Cerebral

infarction decreases the expression of intestinal mucosa tight junction proteins, Occludin, which leads to the destruction of tight junctions, damages the intestinal barrier, and increases intestinal permeability. Xia et al. [55] found that compared with the Sham group, the expression of ZO-1, VE-cadherin, Occludin and Claudin-5 in the rats from the MCAO group appeared to be reduced in different degrees. Shengui Sansheng Pulvis (SSP) administration restored the expression of these proteins in the intestinal mucosal epithelium while reducing MCAO-induced brain edema, and increased VIPR1/2 expression in the OGD blood-brain barrier models, reducing endothelial injury.

Increased intestinal epithelial permeability induced by microRNA after stroke

MicroRNA is a kind of small non-coding ribonucleic acid that participates in various pathophysiological processes of the body. MiR-21-5p is one type of miRNAs. Wu et al. [56] found that miR-21-5p was significantly increased in the serum of patients with cerebral infarction. Studies have found that miR-21-5p can increase intestinal epithelial permeability by up-regulating small GTPase-ADP-ribosylation factor 4 (ARF4) [57]. The ability of miR-21-5p to increase vascular permeability has been similarly demonstrated in studies of colorectal cancer and may be related to its targeting of Krev interaction trap protein 1 (KRIT1) and activation of the β -catenin signaling pathway.

Dysregulated intestinal flora, after stroke, produces toxic metabolites acting on the intestinal mucosal epithelium

Kurita et al. [58] detected LPS and K99 pili protein localization in the brain 24 h after stroke, existing in the Iba-1 positive microglia, neurons as well as endothelial cells. The result indicated that ischemia-induced Enterobacteriaceae proliferation led to increasing luminal LPS concentration, weakened the tight junction of epithelial cells and promoted LPS circulatory system entry. Singh et al. [59] found that stroke could affect the composition of intestinal flora. When intestinal flora is imbalanced, opportunistic pathogens can produce a variety of harmful substances, such as lipopolysaccharide. Lipopolysaccharide is the cell wall component of Gram-negative bacteria, also known as endotoxin, which can affect the tight junction of intestinal epithelium and increase intestinal permeability by mediating the Toll-like receptor (TLR)4/MyD88 signal transduction pathway. TLR-4 positive cells started to increase in number 1 h after MCAO and continued until 22 h. Specific knockdown of TLR-4 was able to produce a protective effect against ischemic stroke. It is evident that TLR-4 is an important target in stroke [60]. Gut microbiota disruption could cause

cerebral endothelial dysfunction through eNOS activity decrease [61]. Stroke can lead to increased abundance of Gram-negative Enterobacteriaceae bacteria and further increased circulatory LPS levels [58, 62], which can trigger inflammation via TLR-4 [63] and alter intestinal mucosal ligand protein expression levels leading to a leaky gut. Meanwhile, LPS induces an inflammatory response, which further aggravates stroke injury. This suggests that stroke and altered intestinal flora are biphasic. In the cerebral artery lysates of antibiotic-treated rats, the eNOS-P/total eNOS ratio was decreased compared to the control subjects. Using antibiotics cause the disruption of gut microbiota and as a result lead to cerebral endothelial dysfunction. However, this study is opposite to the study of Benakis et al. [45]. The intestinal barrier

is one of the basic defence lines of the body against the external environment, which plays an important role in ensuring the stability of the body internal environment. Blood DAO (diamine oxidase), D-LAC (Dlactate) and endotoxins [64] are reliable indicators that reflect the function of the intestinal barrier. Mice with hyperuricemia were found to possess a damaged intestinal barrier as well as an enhanced intestinal permeability, which lead to an induced inflammatory process. Elevated serum uric acid levels were seen to be associated with an increased risk of acute ischemic stroke; however, the mechanism is not clear. Potentially, by combining the changes to the characteristics of intestinal permeability acute ischemic stroke and hyperuricemia, we could elucidate the correlation. In fact, Crapser et al. [65] had the similar results

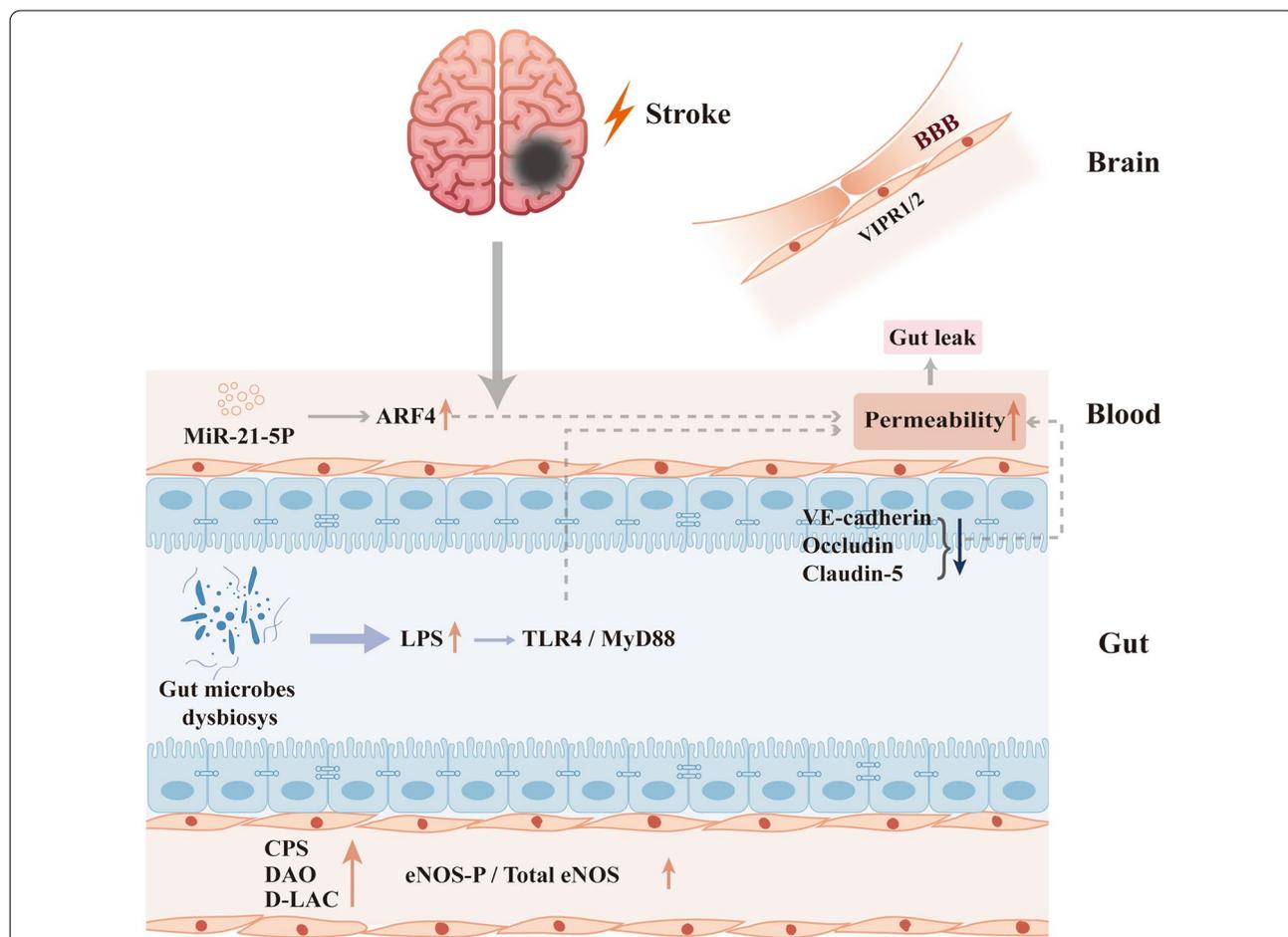


Fig. 2 Post-stroke intestinal changes and their impacts on cerebral organization. Stroke causes a reduction in the expression of intestinal epithelial tight junction proteins including VE-cadherin, Occludin and Claudin-5; more LPS is produced by post-stroke intestinal flora, which induces damage by binding to TLR4/MyD88 in the downstream inflammatory response; LPS also contributes to an increase in eNOS-P/total eNOS, causing vascular endothelial damage; stroke causes an increase in miR-21-5p and further upregulated ARF4; the aforementioned factors combined lead to increased intestinal mucosal permeability and leaky gut. The blood LPS, DAO and D-LAC elevated after vascular endothelial injury and BBB endothelial injury accompanied by VIPR1/2 decreasing

in animal studies. However, several studies [66] have concluded there is insufficient evidence for changes in the morphology and expression of permeability proteins in the intestinal mucosal epithelium after MCAO (Fig. 2).

Cytokines released by gliocytes and other cell type, post ischemic stroke, can either aggravate or relieve brain damage

Ischemia and hypoxia in brain tissue from various causes trigger a series of cascade reactions, including glial cell activation and release of inflammatory mediators, leading to the activation of endothelial cells, which express adhesion molecules and recruit inflammatory and immune cells from the circulation to the site of stroke injury. The simultaneous release of DAMPs/cytokines as well as the activation of the vagus nerve results in intestinal motility disorders, intestinal disorders and increased intestinal permeability. Vila et al. [67] found that serum concentrations of IL-6 and TNF- α , at the time of admission, in stroke patients are strongly associated with early neurological deterioration. However, the specific sources of IL-6 and TNF- α were not mentioned in this study. Several studies [68–70] have shown that the period after an episode of a stroke can cause increased expression of pro-inflammatory inflammatory factors in the serum and within brain tissue. This exacerbates local or systemic inflammatory responses and further aggravates brain tissue damage. Primary astrocytes were seen to express only low levels of TLR2, TLR4, TLR5 and TLR9 under resting culture conditions, but their mRNA expression levels were significantly upregulated when cells were exposed to specific bacteria-derived ligands [71]. TREM1 is produced by Ly6C⁺MHCII⁺ macrophages in the lamina propria of the intestinal mucosa after a stroke; its ability to increase mucosal epithelial permeability promotes bacterial translocation across the intestinal barrier into brain tissue [72]. This reflects the fact that peripheral TREM1 induces enhanced pro-inflammatory responses to brain-derived and gut-derived immunogenic components. Inhibition of TREM1 is able to reduce brain damage through this specific innate immune pathway. Early activation of PMN in ischemic brain tissue may be caused by the rapid release of danger-associated molecular patterns (DAMPs) and eventually leads to the secretion of IL-1b [73–75]. This is a process that promotes the onset of inflammatory vesicle formation by activating immune cell surface receptors and further activating the NLRP3 pathway. If the infiltration of the penumbra PMN is removed after the onset of stroke, the initial brain damage does not have a significant impact on the behavioral performance of the animal [76].

It is not difficult to discover that an inflammatory state is critical to evoke the neurotoxic potential of the

invader. Resting-state PMN showed no neurotoxic activity in brain slices without ischemic pre-injury, and only lipopolysaccharide-activated PMN exhibited this effect. A similar increase in TREM-1 expression can occur during intestinal ischemia–reperfusion, but the use of the inhibitor, LP17, delays death in experimental animals [77]. The intestinal tract is the main immune organ that is equipped with the largest immune cell pool, accounting for more than 70% of the whole immune system [78]. Displaced intestinal microorganisms can: (1) stimulate intestine-related lymphoid tissue, stimulate the differentiation of immune cell subsets; (2) promote the occurrence of inflammatory response; and (3) aggravate the possibility of systemic inflammatory response and multiple organ dysfunction.

Microglia are derived from the myeloid cells of the yolk sac, which are localized to the central nervous system early in individual development and are resident immune cells of the central nervous system [79]. The dendritic and axonal morphology of germfree mouse neurons is affected during development and such developmental defects are often associated with an immature microglial phenotype. This means gut microbial colonization during the development of the brain is crucial [80, 81]. Microglia are capable of proliferation and polarization and can change when in a pathological situation from a branching resting state to an amoeboid activated state [82–84].

In addition, T lymphocytes play an important role in the stroke process. The dysbiosis, induced by the acute phase of stroke, promotes pro-inflammatory Th1 and Th17-mediated immune responses derived from intestinal Peyer's lymph nodes and contributes to brain injury [59, 85]. When intestinal microecological homeostasis is achieved after FMT, the number of Treg increases within the ischemic brain region [86]. In chronic colitis, combined with stroke, intestine-derived CD4⁺T cells migrate from the intestine to the meninges and may interact with meningeal macrophages, leading to non-intestine-derived CD4⁺T cell infiltration and M1 and M2 microglia/macrophage imbalance, exacerbating brain injury in ischemic stroke [87]. At the same time, it can also promote the migration of immune cells from the intestine to the injury site of the cerebral infarction, and aggravate local injury. This may provide an insight into the positive correlation between the degree of intestinal barrier dysfunction and the degree of neurological deficit in patients with cerebral infarction.

Bacterial ectopic location after stroke leading to the occurrence of infections in other tissues and organs

Stroke can lead to ectopic bacterial infections. A neuro-central injury such as a stroke can lead to a disruption of the original balance between the CNS and the immune system, secondary immunodeficiency or immunosuppression. Ultimately this leads to the development of infection [88, 89]. The bacteria belonging to ectopic infections are almost always species pertaining to bacteria native to the intestinal flora that enter the blood circulation and invade other tissues after stroke. One reason for this is due to increased permeability of the intestinal mucosa, colonizing and causing infection. The study of Wen SW et al. [90] demonstrates that exacerbated dysfunction of the intestinal barrier in advanced age promotes translocation of gut-derived bacteria and contributes to the increased risk to post-stroke bacterial infection. Tascilar et al. [91] found that in the animal MCAO model, there is post-stroke intestinal mucosal barrier disruption and bacterial translocation, which includes lung, liver, spleen and mesenteric lymph nodes. The most common pathogen is coagulase-negative *Staphylococcus aureus*. The impaired intestinal barrier function creates favourable conditions for intestinal microbial translocation.

However, Oyama [66] suggested no significant differences in intestinal mucosal changes in animals during the acute phase of stroke; moreover, their lung bacterial colonization may be related to the inadvertent aspiration of intestinal flora into the trachea and subsequently into the lungs during the operation of gavage. In addition, pro-stroke stress is also associated with bacterial translocation from the colon into other tissues (e.g. mesenteric lymph nodes, liver, and the spleen.), increases the inflammatory phenotype of the intestinal mucosa (e.g. COX-2, iNOS.) and reduces the amount of local secretion of IgA [92]. This pro-stroke stress is linked to stroke outcome [93, 94]. Regardless, post-stroke infection is the most common complication, as well as being the most serious complication; its mechanisms need to be further explored.

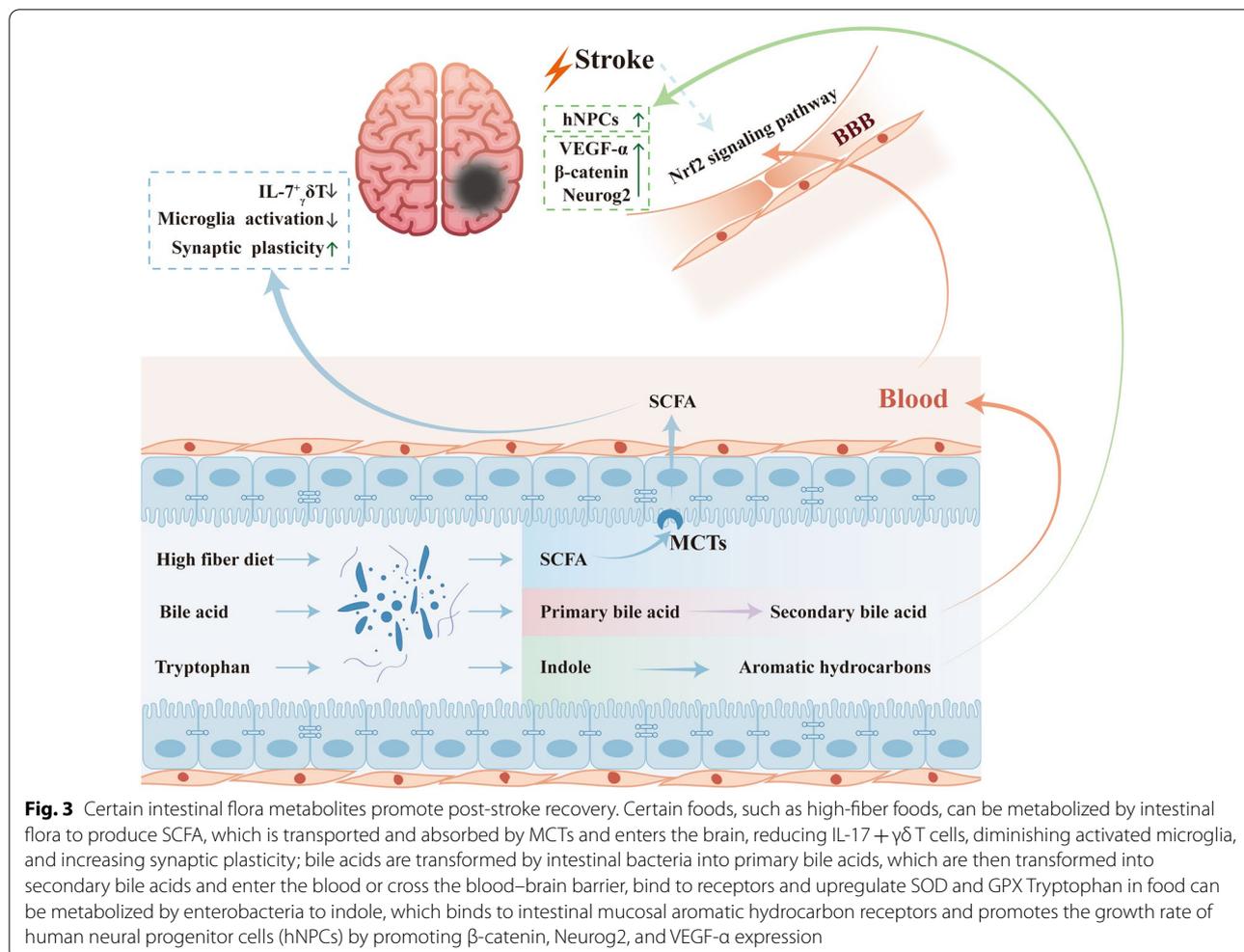
Changes of intestinal flora are closely related to post-stroke depression

The brain-gut axis is a two-way regulatory axis of the interaction between the brain and the gastrointestinal tract. Gastrointestinal discomfort is often accompanied by emotional reactions, which in turn can activate the neural activities of the related central nervous system parts. At the same time, the regulatory information is transmitted down to the gastrointestinal tract through the brain-gut axis, changing its dynamic and secretive functions, activating

intestinal mucosal immunity and affecting the intestinal mucosal barrier function. For example, in patients with gastroesophageal reflux, there is a strong correlation between anxiety and depression as well as gastrointestinal symptoms, such as gastric mucosal erosion. Furthermore, psychological or antidepressant treatment is effective for some patients [95, 96]. In psychiatric patients, depression and generalized anxiety disorder are often accompanied by gastrointestinal discomfort [97], and many patients with generalized anxiety disorder are often first diagnosed with a gastro-enterologic issue [98]. Thus, brain-gut axis dysfunction may play a role in the development of mental illness. However, regarding the underlying mechanism, current research tends to point towards the involvement of the gut flora [99, 100]. Under pathological conditions, the permeability of the BBB changes [101], various inflammatory factors enter the central nervous system. The inflammatory signal is transmitted to the central nervous system, and glial cells are activated through the NF- κ B pathway to promote the occurrence of depression [102, 103].

Post-stroke depression is very common in the post-stroke population [104]. Patients suffering from the post-stroke phase, combined with cognitive impairment and depression, tend to have dysbiosis of the intestinal flora. PSCCID patients, compared to non-PSCCID patients, exhibit increased abundance of Proteobacteria, including Gammaproteobacteria, Enterobacteriales and Enterobacteriaceae, and decreased abundance of several short-chain fatty acid producing bacteria [105].

The administration of LPS was found to mimic depression-like behaviour in experimental animals. A significant inflammatory response in the central nervous system was observed, suggesting inflammatory responses induced by bacterial products such as LPS can affect the central nervous system and promote the development of depression [106, 107]. Chronic mild stress causes elevated IL-1 β , COX-2 and PGE2 in blood and decreased 15d-PGJ2 expression in brain tissue. The use of antibiotics can reduce inflammation by inhibiting the TLR signalling pathway, so this target can be studied for depression [108]. Blocking or inhibiting toll-like receptors involved in central nervous system inflammation, and depression-like behaviour, induced by chronic mild stress, can both lead to improvement of inflammation and animal behavior [109–111]. Depression and post-stroke depression have similar clinical manifestations. Current studies have concluded that the pathogenesis of both are similar. However, recent studies also have shown the association between depression and gut flora are not specific to post-stroke depression, advising that research in this area needs to be further investigated. Therefore, further animal experiments and clinical studies are needed to explore the effect of intestinal flora on post-stroke depression.



Mechanisms that can exert a protective effect against stroke through intestinal flora

The intestinal flora is also capable of producing metabolites that facilitate stroke recovery, of which SCFA is one of the most widely and intensively studied molecule. SCFA in humans includes high levels of acetic, propionic, and butyrate [112], as well as low levels of formate, valerate and caproate [113]. SCFA is actively absorbed into the circulation via monocarboxylate transporters (MCTs) [114] and can cross the blood–brain barrier [115, 116]. Clinical studies have found that lower SCFA levels are strongly associated with stroke and stroke-associated pneumonia (SAP) [117]. Fecal transplantation or SCFA supplementation improve stroke prognosis, with butyric acid having the most significant effect, increasing the abundance of beneficial lactobacilli and reducing intestinal mucosal permeability [118]. The intestinal microbiota of young and older mice was examined separately. We identified a high concentration of SCFA, and its producing strains, in the stool of young mice. SCFA-producing

bacteria (*Bifidobacterium longum*, *Clostridium symbiosum*, *Faecalibacterium prausnitzii* and *Lactobacillus fermentum*) transplantation resulted in increased intestinal mucosal integrity, increased SCFA in the blood and brain tissue, increased Treg in brain tissue, decreased IL-17⁺ $\gamma\delta$ T cells, reduced neuro-inflammation, and significantly improved behavioural scores [119]. Sadler et al. [120] reported a number of notable findings: (1) SCFA levels in the blood decreased after stroke; (2) artificial SCFA supplementation reduced the expression of CD68 in Iba-1⁺ microglia, as well as decreased the number of microglia activation, which reduced the inflammatory response in the brain group after stroke. This in turn was stipulated to enhance synaptic plasticity in the cortical semidark zone and improve stroke prognosis and cortical reconstruction. This suggests that SCFA, produced by intestinal flora, may serve as the basis of metabolites for the brain–gut axis to function. Not only complex short-chain fatty acids, but also SCFA species such as butyrate alone can exert neuroprotective effects [121–123].

Once bile is secreted in the intestine, bile acids are metabolized into a pool of bile acid by the action of intestinal flora. After metabolism, primary BAs such as CA, CDCA and UDCA are formed, and further secondary BAs including DCA and LCA are produced. These metabolites can bind to various receptors in the brain, such as FXR [124], TGR5 [125], NMDAR [126], and PXR [127], subsequently these molecules exert biological activities. TUDCA injection 1 h after ischemia increased intracerebral bile acid levels, reduced infarction size, and decreased neuronal apoptosis by increasing mitochondrial stability. This protective effect was maintained for at least 7 days [128]. TUDCA can reduce serum glutamate, TG, TC, and LDL-C levels, decrease inflammatory factor expression, increase SOD and GPX expression, reduce oxidative stress damage, and down-regulate the Nrf2 signalling pathway and apoptotic protein levels in cerebral ischemic rats. Thus, these effects exert neuroprotective effects [129]. Considering the wide variety of metabolites of bile acids and their ongoing discovery, the role of other species of bile acids in ischemic stroke needs further investigation. In addition, the neuroprotective effect of beneficial bile acid species can be induced by modulating the intestinal flora.

Tryptophanase expressing microorganisms in the intestine converts tryptophan to indole, which upon binding to aromatic hydrocarbon receptors promotes the expression of β -catenin, Neurog2, and VEGF- α and promotes neurogenesis in the hippocampus [130]. This is highly consistent with the findings of Möhle et al. [131], which found that antibiotic treatment reduced hippocampal neurogenesis and memory formation in adult mice; however, adoptive transfer of Ly6C(hi) monocytes rescued this injury. Physiological levels of SCFA can promote the growth rate of human neural progenitor cells (hNPCs), and induce increased mitosis [132]. Promoting neurogenesis or neural stem cell regeneration can facilitate neurological recovery after stroke, thus intestinal flora may further improve stroke outcomes by promoting neural stem cell regeneration (Fig. 3).

In addition to the aforementioned, factors such as age at onset of stroke and gender [16, 133–135] can also influence the outcome by affecting the gut flora. MCAO using SD rats of different genders revealed that male SD rats had a more pronounced increase in intestinal mucosal permeability, more elevated pro-inflammatory cytokines in the blood, and possessed a higher mortality and neurological deficits compared to female SD rats [136]. Compared to bacteria, the role of fungi [137, 138] in the gut has been poorly studied. In addition, the so-called intestinal dark matter, i.e. viruses [139, 140]

(including phages), are also supposed to have a very high importance in the disease and less research has been done in this area. Hence factors that are able to alter gut flora need to be refined and subsequently integrated at an overall level in future studies. After all, each organism is inextricably linked to one another rather than independent of each other, and this is where the microbial-brain-gut axis is specifically presented. In short, the research on intestinal flora and ischemic stroke is still in its infancy. Intestinal flora is expected to become a new target for nerve protection through many pathways in post-stroke injury repair. It is believed that the treatment mode targeting intestinal flora in the future will play an important role in primary prevention and secondary prevention of ischemic stroke.

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Author contributions

Wenjie Hu, Xiangyi Kong and Hui Wang wrote the main manuscript text, Yunqing Li revised the text, Yimin Luo prepared all figures and conceived the outline. All authors read and approved the final manuscript.

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Availability of data and materials

These data were derived from the following resources available in the public domain: <https://clarivate.com/webofsciencegroup/solutions/web-of-science/>; <https://pubmed.ncbi.nlm.nih.gov/>.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish.

Competing interests

The authors declare no conflicts of interest.

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References

1. Tuttolomondo A. Ischemic stroke pathogenesis: genetics, epigenetics and inflammation. *Curr Pharm Des.* 2020;26(34):4207–8.
2. Ziai WC, Al-Kawaz M. Blood pressure management after endovascular therapy. *Lancet Neurol.* 2021;20(4):248–9.

3. Singh K, Chandra A, Sperry T, Joshi PH, Khara A, Virani SS, et al. Associations between high-density lipoprotein particles and ischemic events by vascular domain, sex, and ethnicity a pooled cohort analysis. *Circulation*. 2020;142(7):657–69.
4. Singhal S, Bevan S, Barrick T, Rich P, Markus HS. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain*. 2004;127:2031–8.
5. Qin JJ, Li RQ, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59–U70.
6. Dinan TG, Cryan JF. The microbiome-gut-brain axis in health and disease. *Gastroenterol Clin North Am*. 2017;46(1):77.
7. Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome and brain function. *Nutr Rev*. 2018;76(7):481–96.
8. Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P, et al. Metagenomic analyses of an uncultured viral community from human feces. *J Bacteriol*. 2003;185(20):6220–3.
9. Nakai H, Murosaki S, Yamamoto Y, Furutani M, Matsuoaka R, Hirose Y. Safety and efficacy of using heat-killed *Lactobacillus plantarum* L-137: High-dose and long-term use effects on immune-related safety and intestinal bacterial flora. *J Immunotoxicol*. 2021;18(1):127–35.
10. Yang L, Luo H, Tan DC, Zhang SY, Zhong ZF, Wang SP, et al. A recent update on the use of Chinese medicine in the treatment of inflammatory bowel disease. *Phytomedicine*. 2021;92:153709.
11. Zhou Y, Zhang MH, Zhao X, Feng JH. Ammonia exposure induced intestinal inflammation injury mediated by intestinal microbiota in broiler chickens via TLR4/TNF- α signaling pathway. *Ecotoxicol Environ Saf*. 2021;226:12832.
12. Kurekci C, Ozsoy B, Hassan E, Ozkan H, Gundogdu A, Ozsoy SY, et al. Effect of essential oil supplementation to diet on meat quality, fatty acid composition, performance parameters and intestinal microbiota of Japanese quails. *J Anim Physiol Anim Nutr*. 2021;105(5):927–37.
13. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. *Cell*. 2016;167(6):1469.
14. Yang XQ, Yu DK, Xue L, Li H, Du JR. Probiotics modulate the microbiota-gut-brain axis and improve memory deficits in aged SAMP8 mice. *Acta Pharmaceutica Sinica B*. 2020;10(3):475–87.
15. Sharon G, Cruz NJ, Kang DW, Gandall MJ, Wang B, Kim YM, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell*. 2019;177(6):1600.
16. Szychala MS, Venna VR, Jandzinski M, Doran SJ, Durgan DJ, Ganesh BP, et al. Age-related changes in the gut microbiota influence systemic inflammation and stroke outcome. *Ann Neurol*. 2018;84(1):23–36.
17. Brunetti V, Vollono C, Testani E, Pilato F, Della MG. Autonomic nervous system modifications during wakefulness and sleep in a cohort of patients with acute ischemic stroke. *J Stroke Cerebrovasc Dis*. 2019;28(6):1455–62.
18. Yan XF, Jin JJ, Su XH, Yin XL, Gao J, Wang XW, et al. Intestinal flora modulates blood pressure by regulating the synthesis of intestinal-derived corticosterone in high salt-induced hypertension. *Circ Res*. 2020;126(7):839–53.
19. Wang Q, Hao CJ, Yao WH, Zhu DF, Lu HF, Li L, et al. Intestinal flora imbalance affects bile acid metabolism and is associated with gallstone formation. *Bmc Gastroenterol*. 2020;20(1):59.
20. Needham BD, Adame MD, Serena G, Rose DR, Preston GM, Conrad MC, et al. Plasma and fecal metabolite profiles in autism spectrum disorder. *Biol Psychiat*. 2021;89(5):451–62.
21. Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, et al. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *Am J Physiol Gastrointest Liver Physiol*. 2017;312(6):G559–71.
22. Li D, Ke YL, Zhan R, Liu CJ, Zhao MM, Zeng AP, et al. Trimethylamine-N-oxide promotes brain aging and cognitive impairment in mice. *Aging Cell*. 2018;17(4):e12768.
23. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry*. 2013;18(6):666–73.
24. Angelucci F, Cechova K, Amlerova J, Hort J. Antibiotics, gut microbiota, and Alzheimer's disease. *J Neuroinflammation*. 2019;16:108.
25. Evrensel A, Unsvaler BO, Ceylan ME. Psychobiotics. *Front Psychiatry*. 2019;1192:565–81.
26. Chistiakov DA, Kashirskikh DA, Khotina VA, Grechko AV, Orekhov AN. Immune-inflammatory responses in atherosclerosis: the role of myeloid cells. *J Clin Med*. 2019;8(11):1798.
27. Haghikia A, Li XMS, Liman TG, Bledau N, Schmidt D, Zimmermann F, et al. Gut microbiota-dependent trimethylamine n-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol*. 2018;38(9):2225–35.
28. Hayashi C, Viereck J, Hua N, Phinikaridou A, Madrigal AG, Gibson FC, et al. *Porphyromonas gingivalis* accelerates inflammatory atherosclerosis in the innominate artery of ApoE deficient mice. *Atherosclerosis*. 2011;215(1):52–9.
29. Blessing E, Campbell LA, Rosenfeld ME, Chough N, Kuo CC. *Chlamydia pneumoniae* infection accelerates hyperlipidemia induced atherosclerotic lesion development in C57BL/6J mice. *Atherosclerosis*. 2001;158(1):13–7.
30. Km V, Bg I. Induction of macrophage foam cell formation by *Chlamydia pneumoniae*. *J Infect Dis*. 1998;177(3):725–9.
31. Vlacil AK, Schuett J, Ruppert V, Soufi M, Oberoi R, Shahin K, et al. Deficiency of nucleotide-binding oligomerization domain-containing proteins (NOD) 1 and 2 reduces atherosclerosis. *Basic Res Cardiol*. 2020;115(4):47.
32. Brandsma E, Kloosterhuis NJ, Koster M, Dekker DC, Gijbels MJJ, van der Velden S, et al. A proinflammatory gut microbiota increases systemic inflammation and accelerates atherosclerosis. *Circ Res*. 2019;124(1):94–100.
33. Chan YK, Brar MS, Kirjavainen PV, Chen Y, Peng J, Li DX, et al. *Bmc Microbiol*. 2016;16:264.
34. Wang ZN, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472(7341):57–U82.
35. Din AU, Hassan A, Zhu Y, Yin TY, Gregersen H, Wang GX. Amelioration of TMAO through probiotics and its potential role in atherosclerosis. *Appl Microbiol Biotechnol*. 2019;103(23–24):9217–28.
36. Zhu WF, Gregory JC, Org E, Buffa JA, Gupta N, Wang ZN, et al. Gut microbial metabolite tmao enhances platelet hyperreactivity and thrombosis risk. *Cell*. 2016;165(1):111–24.
37. Fedotcheva N, Olenin A, Beloborodova N. Influence of microbial metabolites on the nonspecific permeability of mitochondrial membranes under conditions of acidosis and loading with calcium and iron ions. *Biomedicines*. 2021;9(5):558.
38. Tang WHW, Wang ZE, Levison BS, Koeth RA, Britt EB, Fu XM, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368(17):1575–84.
39. Yin J, Liao SX, He Y, Wang S, Xia GH, Liu FT, et al. Dysbiosis of gut microbiota with reduced trimethylamine-n-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. *J Am Heart Assoc*. 2015;4(11):e002699.
40. Zhu WF, Romano KA, Li L, Buffa JA, Sangwan N, Prakash P, et al. Gut microbes impact stroke severity via the trimethylamine N-oxide pathway. *Cell Host Microbe*. 2021;29(7):1199.
41. Nemet I, Saha PP, Gupta N, Zhu WF, Romano KA, Skye SM, et al. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell*. 2020;180(5):862.
42. Xu X, Lu WJ, Shi JY, Su YL, Liu YC, Wang L, et al. The gut microbial metabolite phenylacetylglutamate protects against cardiac injury caused by ischemia/reperfusion through activating beta 2AR. *Arch Biochem Biophys*. 2021;697:108720.
43. Hosomi N, Aoki S, Matsuo K, Deguchi K, Masugata H, Murao K, et al. Association of serum anti-periodontal pathogen antibody with ischemic stroke. *Cerebrovasc Dis*. 2012;34(5–6):385–92.
44. Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J. Systemic exposure to *Porphyromonas gingivalis* predicts incident stroke. *Atherosclerosis*. 2007;193(1):222–8.

45. Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gamma delta T cells. *Nat Med*. 2016;22(5):516–23.
46. Sun J, Wang FY, Ling ZX, Yu XC, Chen WQ, Li HX, et al. Clostridium butyricum attenuates cerebral ischemia/reperfusion injury in diabetic mice via modulation of gut microbiota. *Brain Res*. 2016;1642:180–8.
47. Wu WF, Sun YH, Luo N, Cheng C, Jiang CT, Yu QP, et al. Integrated 16S rRNA gene sequencing and LC-MS analysis revealed the interplay between gut microbiota and plasma metabolites in rats with ischemic stroke. *J Mol Neurosci*. 2021;71(10):2095–106.
48. Xu KY, Gao XX, Xia GH, Chen MX, Zeng NY, Wang S, et al. Rapid gut dysbiosis induced by stroke exacerbates brain infarction in turn. *Gut*. 2021;70(8):1486–94.
49. Wang HD, Song W, Wu QH, Gao XX, Li J, Tan CH, et al. Fecal transplantation from db/db mice treated with sodium butyrate attenuates ischemic stroke injury. *Microbiol Spectr*. 2021;9(2):e0004221.
50. Jeon J, Lourenco J, Kaiser EE, Waters ES, Scheulin KM, Fang X, et al. Dynamic changes in the gut microbiome at the acute stage of ischemic stroke in a pig model. *Front Neurosci*. 2020;14:587986.
51. Lee S. Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intest Res*. 2015;13(1):11–8.
52. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009;9(11):799–809.
53. Takuya S. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci*. 2013;70(4):631–59.
54. Ye DY, Hu YT, Zhu N, Gu WZ, Long G, Tao EF, et al. Exploratory investigation of intestinal structure and function after stroke in mice. *Mediators Inflamm*. 2021;2021:1315797.
55. Xia ZY, Luo C, Liu BW, Bian XQ, Li Y, Pang AM, et al. Shengui Sansheng Pulvis maintains blood-brain barrier integrity by vasoactive intestinal peptide after ischemic stroke. *Phytomedicine*. 2020;67:153158.
56. Wu J, Fan CL, Ma LJ, Liu T, Wang C, Song JX, et al. Distinctive expression signatures of serum microRNAs in ischaemic stroke and transient ischaemic attack patients. *Thromb Haemost*. 2017;117(5):992–1001.
57. Nakata K, Sugi Y, Narabayashi H, Kobayakawa T, Nakanishi Y, Tsuda M, et al. Commensal microbiota-induced microRNA modulates intestinal epithelial permeability through the small GTPase ARF4. *J Biol Chem*. 2017;292(37):15426–33.
58. Kurita N, Yamashiro K, Kuroki T, Tanaka R, Urabe T, Ueno Y, et al. Metabolic endotoxemia promotes neuroinflammation after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2020;40(12):2505–20.
59. Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, et al. Microbiota dysbiosis controls the neuroinflammatory response after stroke. *J Neurosci*. 2016;36(28):7428–40.
60. Hyakkoku K, Hamanaka J, Tsuruma K, Shimazawa M, Tanaka H, Uematsu S, et al. Toll-like receptor 4 (TLR4), but not TLR3 or TLR9, knock-out mice have neuroprotective effects against focal cerebral ischemia. *Neuroscience*. 2010;171(1):258–67.
61. Ra J, Pj S, Giles B, M SE. Microbial disruption in the gut promotes cerebral endothelial dysfunction. *Physiol Rep*. 2021;9(21):e15100.
62. Yamashiro K, Kurita N, Tanaka R, Urabe T, Hattori N. Metabolic endotoxemia promotes neuroinflammation after focal cerebral ischemia. *Int J Stroke*. 2020;15(1):612.
63. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. 2010;11(5):373–84.
64. Guo YJ, Li HL, Liu M, Li CG, Chen YQ, Jiang C, et al. Impaired intestinal barrier function in a mouse model of hyperuricemia. *Mol Med Rep*. 2019;20(4):3292–300.
65. Joshua C, Rodney R, Rajkumar V, RVV, Fudong L, Anjali C, et al. Ischemic stroke induces gut permeability and enhances bacterial translocation leading to sepsis in aged mice. *Aging*. 2016;8(5):1049–63.
66. Oyama N, Winek K, Backer-Koduah P, Zhang T, Dames C, Wench M, et al. Exploratory investigation of intestinal function and bacterial translocation after focal cerebral ischemia in the mouse. *Front Neurol*. 2018;9:937.
67. Vila N, Castillo J, Davalos A, Chamorro A. Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke*. 2000;31(10):2325–9.
68. Zhao XR, Wang H, Sun GH, Zhang J, Edwards NJ, Aronowski J. Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. *J Neurosci*. 2015;35(32):11281–91.
69. Tian DS, Li CY, Qin C, Murugan M, Wu LJ, Liu JL. Deficiency in the voltage-gated proton channel Hv1 increases M2 polarization of microglia and attenuates brain damage from photothrombotic ischemic stroke. *J Neurochem*. 2016;139(1):96–105.
70. Al Mamun A, Chauhan A, Qi SH, Ngwa C, Xu Y, Sharmeen R, et al. Microglial IRF5-IRF4 regulatory axis regulates neuroinflammation after cerebral ischemia and impacts stroke outcomes. *Proc Natl Acad Sci USA*. 2020;117(3):1742–52.
71. Bowman CC, Rasley A, Tranguch SL, Marriott I. Cultured astrocytes express toll-like receptors for bacterial products. *Glia*. 2003;43(3):281–91.
72. Liu QK, Johnson EM, Lam RK, Wang Q, Ye HB, Wilson EN, et al. Peripheral TREM1 responses to brain and intestinal immunogens amplify stroke severity. *Nat Immunol*. 2019;20(8):1023.
73. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol*. 2011;11(3):201–12.
74. Magnus T, Wiendl H, Kleinschnitz C. Immune mechanisms of stroke. *Curr Opin Neurol*. 2012;25(3):334–40.
75. Rubartelli A. DAMP-mediated activation of NLRP3-inflammasome in brain sterile inflammation: the fine line between healing and neurodegeneration. *Front Immunol*. 2014;5:1–2.
76. Neumann J, Riek-Burchardt M, Herz J, Doepfner TR, König R, Hutten H, et al. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. *Acta Neuropathol*. 2015;129(2):259–77.
77. Gibot S, Massin F, Alauzet C, Monternont C, Lozniewski A, Bollaert PE, et al. Effects of the TREM-1 pathway modulation during mesenteric ischemia-reperfusion in rats. *Crit Care Med*. 2008;36(2):504–10.
78. Shi N, Li N, Duan XW, Niu HT. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res*. 2017;4:14.
79. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Dev Brain Res*. 1999;117(2):145–52.
80. Matcovitch-Natan O, Winter DR, Giladi A, Aguilar SV, Spinrad A, Sarrazin S, et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science*. 2016;353(6301):aad8670.
81. Pronovost GN, Hsiao EY. Perinatal interactions between the microbiome, immunity, and neurodevelopment. *Immunity*. 2019;50(1):18–36.
82. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*. 2005;8(6):752–8.
83. Masuda T, Croom D, Hida H, Kirov SA. Capillary blood flow around microglial somata determines dynamics of microglial processes in ischemic conditions. *Glia*. 2011;59(11):1744–53.
84. Ju FR, Ran YL, Zhu LR, Cheng XF, Gao H, Xi XX, et al. Increased BBB permeability enhances activation of microglia and exacerbates loss of dendritic spines after transient global cerebral ischemia. *Front Cell Neurosci*. 2018;12:236.
85. Yu XB, Zhou GY, Shao B, Zhou H, Xu CR, Yan F, et al. Gut microbiota dysbiosis induced by intracerebral hemorrhage aggravates neuroinflammation in mice. *Front Microbiol*. 2021;12:647304.
86. Singh V, Sadler R, Heindl S, Llovera G, Roth S, Benakis C, et al. The gut microbiome primes a cerebroprotective immune response after stroke. *J Cereb Blood Flow Metab*. 2018;38(8):1293–8.
87. Feng YK, He XF, Luo SJ, Chen XF, Long SM, Liang FY, et al. Chronic colitis induces meninges traffic of gut-derived T cells, unbalances M1 and M2 microglia/macrophage and increases ischemic brain injury in mice. *Brain Res*. 2019;1707:8–17.
88. Schulte-Herbruggen O, Quarcoo D, Meisel A, Meisel C. Differential affection of intestinal immune cell populations after cerebral ischemia in mice. *NeuroImmunoModulation*. 2009;16(3):213–8.
89. Meisel C, Schwab JM, Prass K, Meisel A, Dirnag U. Central nervous system injury-induced immune deficiency syndrome. *Nat Rev Neurosci*. 2005;6(10):775–86.
90. Wen SW, Shim R, Ho L, Wanrooy BJ, Srikhanta YN, Kumar KP, et al. Advanced age promotes colonic dysfunction and gut-derived lung infection after stroke. *Aging Cell*. 2019;18(5):e12980.

91. Tascilar N, Irkorucu O, Tascilar O, Comert F, Eroglu O, Bahadir B, et al. Bacterial translocation in experimental stroke: what happens to the gut barrier? *Bratisl Med J-Bratisl Lek Listy*. 2010;111(4):194–9.
92. Caso JR, Hurtado O, Pereira MP, Garcia-Bueno B, Menchen L, Alou L, et al. Colonic bacterial translocation as a possible factor in stress-worsening experimental stroke outcome. *Am J Physiol-Regul Integr Comp Physiol*. 2009;296(4):R979–85.
93. Caso JR, Pradillo JM, Hurtado O, Leza JC, Moro MA, Lizasoain I. Toll-like receptor 4 is involved in subacute stress-induced neuroinflammation and in the worsening of experimental stroke. *Stroke*. 2008;39(4):1314–20.
94. CJ R, Mm A, Pedro L, Ignacio L, C LJ. Involvement of IL-1beta in acute stress-induced worsening of cerebral ischaemia in rats. *Eur neuropsychopharmacol*. 2007;17(9):600–7.
95. Pooja B, Shehar B, Sameet K, Priyanka S, Ahmed A, Pariya D, et al. Gastroesophageal reflux disease in the young population and its correlation with anxiety and depression. *Cureus*. 2021;13(5):e15289.
96. Losa M, Manz SM, Schindler V, Savarino E, Pohl D. Increased visceral sensitivity, elevated anxiety, and depression levels in patients with functional esophageal disorders and non-erosive reflux disease. *Neurogastroenterol Motil*. 2021;33(9):e14177.
97. Liu PH, Li GZ, Zhang AX, Yang CX, Liu ZF, Sun N, et al. Brain structural and functional alterations in MDD patient with gastrointestinal symptoms: a resting-state MRI study. *J Affect Disord*. 2020;273:95–105.
98. Koloski N, Holtmann G, Talley NJ. Is there a causal link between psychological disorders and functional gastrointestinal disorders? *Expert Rev Gastroenterol Hepatol*. 2020;14(11):1047–59.
99. Jiang WX, Gong L, Liu F, Ren YK, Mu J. Alteration of gut microbiome and correlated lipid metabolism in post-stroke depression. *Front Cell Infect Microbiol*. 2021;11:663967.
100. Kang Y, Yang YT, Wang JH, Ma Y, Cheng H, Wan D. Correlation between intestinal flora and serum inflammatory factors in post-stroke depression in ischemic stroke. *J Coll Physicians Surg Pak*. 2021;31(10):1224–7.
101. Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. *Gut*. 2018;67(8):1555.
102. Yin QQ, Du T, Yang CL, Li XL, Zhao ZY, Liu RT, et al. Gadd45b is a novel mediator of depression-like behaviors and neuroinflammation after cerebral ischemia. *Biochem Biophys Res Commun*. 2021;554:107–13.
103. Mu YY, Wang ZC, Zhou JX, Tan CX, Wang HJ. Correlations of post-stroke depression with inflammatory response factors. *Iran J Public Health*. 2018;47(7):988–93.
104. Guo JL, Wang JJ, Sun W, Liu XF. The advances of post-stroke depression. *J Neurol*. 2021;30:1–4.
105. Yi L, Qilu G, Junmei Z, Tianyu G, Xiongpeng W, Jiaming L, et al. Structural change of gut microbiota in patients with post-stroke comorbid cognitive impairment and depression and its correlation with clinical features. *J Alzheimer's dis*. 2020;77(4):1595–608.
106. O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*. 2009;14(5):511–22.
107. Cao P, Chen CM, Liu A, Shan QH, Zhu X, Jia CH, et al. Early-life inflammation promotes depressive symptoms in adolescence via microglial engulfment of dendritic spines. *Neuron*. 2021;109(16):2573.
108. Garate I, Garcia-Bueno B, Madrigal JLM, Bravo L, Berrocoso E, Caso JR, et al. Origin and consequences of brain Toll-like receptor 4 pathway stimulation in an experimental model of depression. *J Neuroinflammation*. 2011;8:1–4.
109. Yan TX, Wang NZ, Liu B, Wu B, Xiao F, He BS, et al. Schisandra chinensis ameliorates depressive-like behaviors by regulating microbiota-gut-brain axis via its anti-inflammation activity. *Phytother Res*. 2021;35(1):289–96.
110. Shao SY, Jia R, Zhao L, Zhang YR, Guan YF, Wen HT, et al. Xiao-Chai-Hu-Tang ameliorates tumor growth in cancer comorbid depressive symptoms via modulating gut microbiota-mediated TLR4/MyD88/NF-kappa B signaling pathway. *Phytomedicine*. 2021;88:153606.
111. Yan TX, Nian TT, Liao ZZ, Xiao F, Wu B, Bi KS, et al. Antidepressant effects of a polysaccharide from okra (*Abelmoschus esculentus* (L) Moench) by anti-inflammation and rebalancing the gut microbiota. *Int J Biol Macromol*. 2020;144:427–40.
112. Cj H, Pe W, Bw J, Nc P, Mg T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*. 1987;28(10):1221–7.
113. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*. 2003;62(1):67–72.
114. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol*. 2019;16(8):461–78.
115. Claude B, Jean-Pierre C, Josef B. Short chain fatty acids in plasma and brain: Quantitative determination by gas chromatography. *Clin Chim Acta*. 1979;92(2):153–9.
116. Ow H. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am J Physiol*. 1973;224(6):1450–3.
117. Xia GH, Zhang MS, Wu QH, Wang HD, Zhou HW, He Y, et al. Dysbiosis of gut microbiota is an independent risk factor of stroke-associated pneumonia: a Chinese Pilot Study. *Front Cell Infect Microbiol*. 2021;11:71545.
118. Chen RZ, Xu Y, Wu P, Zhou H, Lasanajak Y, Fang YY, et al. Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota. *Pharmacol Res*. 2019;148:104403.
119. Lee J, d'Aigle J, Atadja L, Quaicov V, Honarpishev P, Ganesh BP, et al. Gut microbiota-derived short-chain fatty acids promote poststroke recovery in aged mice. *Circ Res*. 2020;127(4):453–65.
120. Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurberier KR, et al. Short-chain fatty acids improve poststroke recovery via immunological mechanisms. *J Neurosci*. 2020;40(5):1162–73.
121. Ziemka-Nalecz M, Jaworska J, Sypecka J, Polowy R, Filipkowski RK, Zalewska T. Sodium butyrate, a histone deacetylase inhibitor, exhibits neuroprotective/neurogenic effects in a rat model of neonatal hypoxia-ischemia. *Mol Neurobiol*. 2017;54(7):5300–18.
122. Zhenhua Z, Ningbo X, Nathanael M, Wei MD, Yan D, Hui L, et al. Sodium butyrate attenuated neuronal apoptosis via GPR41/Gβγ/PI3K/Akt pathway after MCAO in rats. *J Cereb Blood Flow Metabol*. 2020;67:267–81.
123. Patnala R, Varumugam T, Gupta N, Dheen ST. HDAC inhibitor sodium butyrate-mediated epigenetic regulation enhances neuroprotective function of microglia during ischemic stroke. *Mol Neurobiol*. 2017;54(8):6391–411.
124. Wang HB, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR BAR. *Mol Cell*. 1999;3(5):543–53.
125. Yanguas-Casas N, Barreda-Manso MA, Nieto-Sampedro M, Romero-Ramirez L. TUDCA: An Agonist of the Bile Acid Receptor GPBAR1/TGR5 with anti-inflammatory effects in microglial cells. *J Cell Physiol*. 2017;232(8):2231–45.
126. Koch A, Bonus M, Gohlke H, Klocker N. Isoform-specific Inhibition of N-methyl-D-aspartate Receptors by Bile Salts. *Scientific Rep*. 2019;9:78.
127. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, Latour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA*. 2001;98(6):3369–74.
128. Rodrigues TMP, Spellman SR, Sola T, Grande AW, Linehan-Stieers C, Low TC, et al. Neuroprotection by a bile acid in an acute stroke model in the rat. *J Cereb Blood Flow Metab*. 2002;22(4):463–71.
129. Bian KY, Jin HF, Sun W, Sun YJ. DCA can improve the ACL-induced neurological impairment through negative regulation of Nrf2 signaling pathway. *Eur Rev Med Pharmacol Sci*. 2019;23(1):343–51.
130. Wei GZ, Martin KA, Xing PY, Agrawal R, Whiley L, Wood TK, et al. Tryptophan-metabolizing gut microbes regulate adult neurogenesis via the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA*. 2021;118:27.
131. Mohle L, Mattei D, Heimesaat MM, Bereswill S, Fischer A, Alutis M, et al. Ly6C(hi) monocytes provide a link between antibiotic-induced changes in gut microbiota and adult hippocampal neurogenesis. *Cell Rep*. 2016;15(9):1945–56.
132. Yang LL, Millischer V, Rodin S, MacFabe DF, Villaescusa JC, Lavebratt C. Enteric short-chain fatty acids promote proliferation of human neural progenitor cells. *J Neurochem*. 2020;154(6):635–46.
133. Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev*. 2017;35:36–45.
134. Wang QW, Qi YD, Shen WY, Xu JL, Wang L, Chen SJ, et al. The Aged Intestine: Performance and Rejuvenation. *Ageing Dis*. 2021;12(7):1693–712.

135. Miriam A, Mern DA, Bert HTT. A systematic review of the factors influencing microbial colonization of the preterm infant gut. *Gut microbes*. 2021;13(1):31–3.
136. El-Hakim Y, Mani KK, Eldouh A, Pandey S, Grimaldo MT, Dabney A, et al. Sex differences in stroke outcome correspond to rapid and severe changes in gut permeability in adult Sprague-Dawley rats. *Biol Sex Differences*. 2021;12:1.
137. Kexin D, Congmin W, Ping L, Yuan L, Xi M. Effects of Dietary Mycotoxins on Gut Microbiome. *Protein Peptide Lett*. 2017;24:5.
138. Eang HC, Wang OTP, Dei CP, Dang SP. Forgotten fungi-the gut mycobiome in human health and disease. *FEMS Microbiol Rev*. 2017;41:4.
139. Samtlebe M, Denis S, Chalancon S, Atamer Z, Wagner N, Neve H, et al. Bacteriophages as modulator for the human gut microbiota: Release from dairy food systems and survival in a dynamic human gastrointestinal model. *LWT*. 2018;91:e45.
140. Sofia D, Laura A-F, Jang BJ. Phages to shape the gut microbiota? *Curr Opin Biotechnol*. 2021;68:89.

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