

# Relationships Among Gut Microbiota, Ischemic Stroke and Its Risk Factors: Based on Research Evidence

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**Abstract:** Stroke is a highly lethal disease and disabling illness while ischemic stroke accounts for the majority of stroke. It has been found that inflammation plays a key role in the initiation and progression of stroke, and atherosclerotic plaque rupture is considered to be the leading cause of ischemic stroke. Furthermore, chronic inflammatory diseases, such as obesity, type 2 diabetes mellitus (T2DM) and hypertension, are also considered as the high-risk factors for stroke. Recently, the topic on how gut microbiota affects human health has aroused great concern. The initiation and progression of ischemic stroke has been found to have close relation with gut microbiota dysbiosis. Hence, this manuscript briefly summarizes the roles of gut microbiota in ischemic stroke and its related risk factors, and the practicability of preventing and alleviating ischemic stroke by reconstructing gut microbiota.

**Keywords:** ischemic stroke, gut microbiota, inflammation, obesity, T2DM, hypertension, type 2 diabetes mellitus

## Introduction

Stroke is the most common cause of severe disability and also the second leading cause of death worldwide after coronary heart disease.<sup>1,2</sup> Stroke is generally divided into two types, namely ischemic and hemorrhagic stroke, and ischemic stroke accounts for 85%.<sup>3</sup> During the initiation and progression of ischemic stroke, inflammation is an important pathological process. Preclinical stroke study has shown that inhibiting inflammatory response can significantly reduce the injuries of brain and improve neurological prognosis.<sup>4</sup> Furthermore, atherosclerosis, obesity, diabetes, hypertension and peripheral infection are complications that most stroke patients suffer from,<sup>5</sup> and all of these diseases are risk factors for stroke, while inflammation as a pathogenic factor plays a significant role in the initiation and progression of these diseases.<sup>6,7</sup>

Gut microbiota, which has been identified by Metagenomic analysis, mainly consists of six families, namely Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Clostridium and Verrucomicrobia, most of which are anaerobic bacteria.<sup>8</sup> Among them, Firmicutes and Bacteroidetes are the predominant bacteria in healthy individuals, accounting for more than 90% of the total population.<sup>9</sup> Because the gut microbiota has close relation to inflammation and many chronic diseases, it has drawn a lot of attention these years. It is found that there exist intercommunication pathways connecting the brain and the gut in the human body, known as the gut-brain axis, including sympathetic and parasympathetic activation, hypothalamus-pituitary-adrenal axis and central immune system.<sup>10,11</sup> Changes in gut microbiota are proved to affect the physiology, behavior and cognitive function of the brain through the gut-brain axis.<sup>12-16</sup>

Gut microbiota dysbiosis can be found in gastrointestinal diseases, which are been reported to have close relation with cardiovascular and cerebrovascular diseases. Researchers conducted a retrospective study by using the Cox proportional hazard model to assess the correlation between different gastrointestinal diseases and ischemic stroke with 1,725,246

beneficiaries in average in each analysis, and concluded that several gastrointestinal diseases are associated with increased risk of ischemic stroke after adjusting the known risk factors for stroke. The most significant positive correlations included gastric diseases (risk ratio: 1.17 [95% confidence interval (CI): 1.15–1.19]), functional gastrointestinal diseases (1.16 [95% CI: 1.15–1.17]), inflammatory gastrointestinal diseases (1.13 [95% CI: 1.12–1.15]) and infectious gastrointestinal diseases (1.13 [95% CI: 1.12–1.15]).<sup>17</sup> Further, a study shows that alteration of gut microbiota by using antibiotics, ischemic stroke is significantly alleviated through the gut–brain immune axis mechanism.<sup>18</sup> Besides, it is found that ischemic stroke in turn leads to gut microbiota dysbiosis. Researchers have found that cerebral ischemia rapidly induces intestinal ischemia and produces excessive nitrates through free radical reactions, resulting in gut microbiota dysbiosis accompanied with Enterobacteriaceae proliferation. The proliferation of Enterobacteriaceae aggravates cerebral infarction by exacerbating systemic inflammation, which is an independent risk factor for the adverse prognosis of stroke patients.<sup>19</sup>

Obviously, the dysbiosis of gut microbiota and the initiation and progression of ischemic stroke has a mutually reinforcing effect. In this review, we summarize the relationships among gut microbiota dysbiosis and ischemic stroke and its relative risk factors, and discuss ways to alleviate ischemic stroke and improve its prognosis by reconstructing gut microbiota.

## Gut Microbiota and Inflammation in Ischemic Stroke

### Inflammation Induces Ischemic Stroke

It has been found that the elevated levels of inflammatory factors, such as interleukin-6, C-reactive protein and lipoprotein-associated phospholipase A2, are associated with the increased risk of stroke.<sup>20</sup> Also, soluble lectin-like oxidized low density lipoprotein receptor-1, a kind of inflammation-induced lipid receptor, attributing to atherosclerosis, has been proved be associated with high risk of stroke.<sup>21,22</sup> Atherosclerosis, as a kind of vascular inflammatory disease, is a high-risk factor for ischemic stroke, because thromboembolism caused by atherosclerotic plaque rupture is the most common cause of ischemic stroke.<sup>23</sup> The cause of thromboembolic events is due to the activation of molecules and inflammatory mediators in atherosclerotic plaques, which promote the progression of plaques from relatively benign to highly unstable stages, leading to vulnerable plaque rupture. In the pathogenesis, endothelial dysfunction and activation are key events, leading to the expression of adhesion molecules, which promote the recruitment of immune cells, especially monocyte-derived macrophages and T lymphocytes. Activated macrophages and T cells secrete proteolytic enzymes, such as matrix metalloproteinases (MMPs), which destroy the stability of plaque and eventually lead to plaque rupture.<sup>7</sup> Besides atherosclerosis, studies have shown that various inflammatory diseases are also associated with increased risk of ischemic stroke.<sup>24</sup> All in all, these above suggest that inflammation may be a major cause of ischemic stroke.

### Inflammation Affects the Progress and Prognosis of Ischemic Stroke

In many animal models and patients with stroke, rapid activation of resident cells is the feature of the cerebral inflammatory response after ischemia, primarily microglia, followed by the infiltration of circulating inflammatory cells, including granulocytes (mainly neutrophils), T cells, monocytes/macrophages, in ischemic brain regions and other regions.<sup>25–30</sup> In the mouse model of transient middle cerebral artery occlusion (MCAO), the result of the flow cytometry showed the significantly increased activated CD11b<sup>+</sup> microglia/macrophages and Ly6G<sup>+</sup> neutrophils in the early stage after ischemia, while CD3<sup>+</sup> T cells increased relatively late.<sup>31</sup>

In the acute phase of ischemic stroke (a few minutes to several hours), reactive oxygen species (ROS) and pro-inflammatory cytokines and chemokines are rapidly released from the injured tissue, leading to the brain endothelial cells activation and the up-regulation of adhesion molecules on the brain endothelial cells, which promotes the adhesion and transendothelial migration of circulating leukocytes through the blood–brain barrier (BBB) into the brain parenchyma.<sup>4,32–34</sup> In the subacute phase (several hours to several days), the infiltrated leucocytes release cytokines and chemokines, especially the excessive production of ROS and the induction/activation of MMP (mainly MMP-9), further amplify the inflammatory response in the brain. The extensive activation of resident cells in the brain and the infiltration of leucocytes in the blood will

eventually aggravate the interruption of BBB, brain edema, neuronal death and hemorrhagic transformation of ischemic stroke.<sup>33,34</sup>

Although inflammation plays a defensive role in some extent, it is generally believed that inflammatory infiltration is more harmful than beneficial in the progression of ischemic stroke. The activation of microglial cells in the brain is within a few minutes after ischemia, which exacerbates ischemic tissue damage by releasing pro-inflammatory mediators, mainly including IL-1 $\beta$  and TNF- $\alpha$ .<sup>35–37</sup> Transcription factor NF- $\kappa$ B, which plays a key role in cell survival and inflammatory response by regulating various genes, is activated in neurons, astrocytes, microglia and invasive inflammatory cells after cerebral ischemia.<sup>38</sup> Moreover, there are experimental evidences showing that inhibiting the inflammatory response after ischemic stroke effectively reduces the damage of ischemic stroke to the brain and nerves function. By the inhibition of cerebral ischemia-induced time-dependent inflammatory cell recruitment and activation, including neutrophils, T cells and monocytes/macrophages, the size of cerebral infarction and alleviates neurological impairment in experimental stroke reduces.<sup>4,39</sup> Inhibiting the activation of NF- $\kappa$ B with S-nitrosoglutathione in the rat model of experimental stroke alleviates the damage of inflammation and reduce the area of large cerebral infarction.<sup>40</sup> Further, inhibiting the activity of inflammatory mediator Mac-1 by using its antibody alleviates cells damage after transient MCAO in rats.<sup>41</sup> Besides, anti-inflammation in animal models targeting on other inflammatory mediators also shows positive results.<sup>42–44</sup>

In addition to focal brain infiltration, inflammation outside the brain, especially systemic inflammation caused by intestinal lipopolysaccharide (LPS) leakage, has adverse effects on the progression and prognosis of ischemic stroke. Some clinical studies reported that stroke patients with more severe neurological deficits have precursor infection.<sup>45,46</sup> Animal systemic inflammatory model also provides consistent result. Using bacterial endotoxin, mainly LPS, to systematically attack and imitate Gram-negative bacterial infection, significantly increases the severity of ischemic brain injury and neurological deficits in mice after focal cerebral ischemia.<sup>47</sup> LPS in the circulation can pass through the impaired BBB, activating microglia in brain, and the microglia activated by LPS impairs neuronal activity by changing neuronal computing power and promotes asynchrony.<sup>48</sup> Moreover, LPS-activated microglia promote the activation of astrocytes through the release of adenosine triphosphate (ATP) and the subsequent increase of glutamatergic synaptic transmission, which causes excitotoxicity and chemokine (mainly CCL2) secretion from damaged neurons.<sup>49</sup> CCL2 triggers chemotaxis and activation of microglia migration by stimulating CXCR3 receptors,<sup>50</sup> therefore, repeated LPS-induced microglia activation leads to synaptic stripping of inhibitory terminals in the cortex, which aggravates nerve damage.<sup>51</sup>

So far, we can see that inflammation increases the risk of ischemic stroke, even directly causes ischemic stroke by inducing atherosclerotic plaque rupture, causing vascular embolism/occlusion. In addition, local infiltration of inflammatory brain has adverse effects on the progression and prognosis of ischemic stroke. In view of a large number of articles have directly discussed the relationship between inflammation and ischemic stroke in details, here we only introduce briefly. Next, we will focus on how gut microbiota affects ischemic stroke through inflammatory pathways.

## **Gut Microbiota Dysbiosis and Ischemic Stroke Promote Each Other Through Inflammatory Pathways**

### **Gut Microbiota Dysbiosis Affects the Occurrence and Progression of Ischemic Stroke**

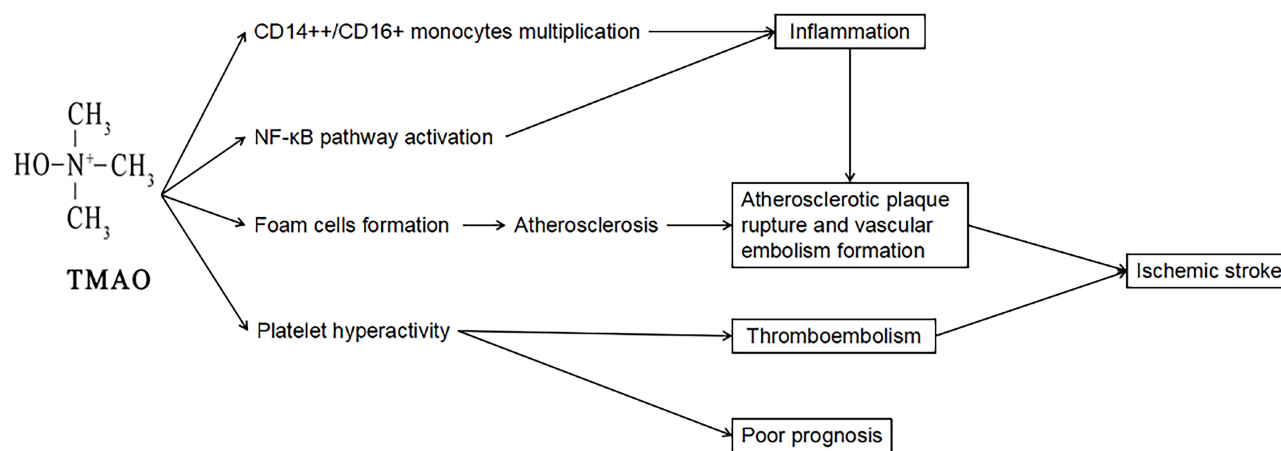
The digestive tract is considered to be the main organ of immune response, rich in immune cells, responsible for more than 70% of the entire immune system activities.<sup>52</sup> Many evidences show that intestinal inflammation and immune response play an important role in the pathophysiology of stroke, which can become an important therapeutic target for the treatment of stroke.<sup>53</sup> Studies have found that gut microbiota dysbiosis is a risk factor for stroke and affects the prognosis after stroke. Gut microbiota dysbiosis has been observed in several acute ischemic stroke (AIS) animal models, indicating that gut microbiota regulates neuroinflammation and affects brain recovery.<sup>18</sup> It has been proved that in the first few hours/days after AIS, peripheral adaptive immune cells are activated and recruited into the brain,<sup>54</sup> and these cells can regulate gut microbiota and be regulated by gut microbiota.<sup>55</sup>

Gut microbiota dysbiosis induces ischemic stroke, mainly because the imbalance of gut microbiota increases the abundance of opportunistic pathogens in the gut. It has been found that the opportunistic pathogens enriched in patients

with stroke and transient ischemic attack, including *Enterobacter*, *Odoribacter*, *Megasphaera*, *Oscillibacter*, *Akkermansia* and *Desulfovibrio*.<sup>52,56,57</sup> The increased levels of opportunistic pathogens in the gut are associated with increased risk of developing AIS,<sup>58</sup> because more endotoxin is produced and released by the increased Gram-negative bacteria, which directly impairs the intestinal barrier.<sup>59</sup> With the dysfunction/damage of intestinal barrier, some metabolites related to microorganisms enter the circulation from intestinal tract. As a response, human body produces a lot of cytokines and other media, which effectively trigger inflammatory reactions.<sup>60</sup> Intestinal LSP leakage and the increased intestinal permeability induce macrophages to infiltrate the region, produce and activate inflammatory cytokines, leading to local inflammation.<sup>61</sup> Moreover, the inflammation caused by intestinal permeability damage is not just limited to the intestine, because pro-inflammatory cytokines can finally reach the brain through the blood.<sup>62</sup> Meanwhile, LPS, as an important component of Gram-negative bacteria, is a ligand of toll-like receptor 4 (TLR-4) that can form a complex with CD14 and bind to TLR-4 located on the surface of innate immune cells, activating local and distant inflammatory cascades.<sup>59,63,64</sup> The persistence of inflammatory responses caused by microbiota dysbiosis leads to subacute or chronic inflammation, which as a result, leads to the development of relative diseases, such as inflammatory bowel disease, diabetes and cardiovascular disease (CVD).<sup>65</sup> The inflammatory bowel disease and Crohn's disease are proved to be associated with changes in gut microbiota and increased production of intestinal IL-17,<sup>66,67</sup> and also have been identified as risk factors for stroke.<sup>68,69</sup> Moreover, studies show that gut microbiota dysbiosis leads to progression of atherosclerosis.<sup>70,71</sup> Compared with the conventionally raised controls, absence of microbiota could lead to the formation of atherosclerotic lesions, suggesting that gut microbiota dysbiosis, especially the reduction or deletion of certain categories, directly induces atherosclerosis.<sup>70</sup> Under this condition, inflammation can induce the rupture of atherosclerotic plaque, causing vascular embolism and ischemic stroke. In addition to induce ischemic stroke, gut microbiota contributes to the progression of ischemic stroke by regulating  $\gamma\delta$  T lymphocytes,<sup>53</sup> which are a major lymphocyte group with innate immune function, located on the epithelial surface including the intestine,<sup>72</sup> and these lymphocytes excrete IL-17 and produce chemotactic signals for peripheral myeloid cells (such as neutrophils and monocytes), which exacerbate ischemic brain damage.<sup>73,74</sup>

## TMAO and Ischemic Stroke (Figure 1)

Trimethylamine-n-oxide (TMAO) is a by-product of gut microbiota closely related to stroke.<sup>75</sup> TMAO is formed by the trimethylamine (TMA) that enters the liver through portal circulation, oxidized by flavin monooxygenase.<sup>76–78</sup> Microbial community plays a key role in the formation of TMA, because antibacterial research shows that TMAO will not be formed without microbial community.<sup>79</sup> TMAO is a high risk factor and prognostic marker for stroke and CVD, because the increased generation of TMAO promotes atherosclerosis, platelet activation, and inflammation.<sup>80,81</sup> Many studies have emphasized that TMAO participates in the development of CVD in various patient cohorts,<sup>82–84</sup> and the association



**Figure 1** A summary on how TMAO contributes to occurrence of ischemic stroke.

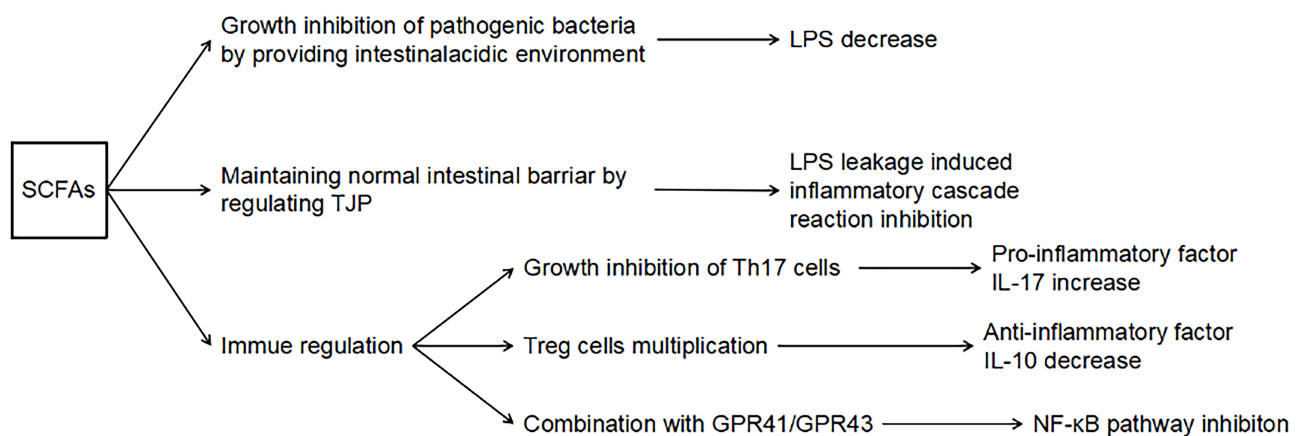
between TMAO levels and adverse clinical outcomes also has been found in many independent cohorts.<sup>77,85,86</sup> Microbial transplantation study has shown that TMAO is a risk factor for atherosclerosis and thrombosis.<sup>87</sup> When TMAO exists at a high serum level, it is related to harmful effects such as endothelial dysfunction, which promotes vascular inflammation, atherosclerosis and other risk factors for CVD,<sup>88–90</sup> and one of the main reasons is that TMAO promotes atherosclerosis by increasing the expression of scavenger receptors in macrophages and the formation of foam cells in the arterial wall.<sup>77</sup> Subsequent experiment shows that the formation of macrophage foam cells and atherosclerosis are suppressed, when the formation of TMA and TMAO by microorganisms are suppressed by small molecule inhibitor of microbial choline TMA lyase.<sup>91</sup>

TMAO is involved in inflammatory response and thromboembolism. Researchers found the positive correlation among TMAO level, TNF- $\alpha$  in human<sup>92</sup> and the number of CD14<sup>++</sup>/CD16<sup>+</sup> monocytes, a kind of pro-inflammatory intermediate.<sup>87</sup> Moreover, a study using cultured human aortic endothelial cells and vascular smooth muscle cells reported that TMAO induces inflammation by up-regulating the expression of various inflammatory cytokines and adhesion molecules through NF- $\kappa$ B signaling pathway.<sup>93,94</sup> In this case, atherosclerotic plaque rupture resulting in vascular embolism caused by inflammation can induce ischemic stroke. In addition, it is observed that regardless of the traditional risk factors, TMAO is directly related to the adverse treatment results of patients with ischemic brain injury.<sup>75,95</sup> The association between TMAO and poor prognosis in patients with ischemic brain injury is mainly because of the formation of thrombosis. TMAO is reported to increase the risk of thrombosis, and high-level TMAO promotes endothelial dysfunction, induces platelet activation and increases platelet reactivity.<sup>96</sup> The activation and aggregation of platelet and the subsequent formation of occlusive intra-arterial thrombosis are critical processes in atherosclerotic thrombosis and the increase of platelet reactivity is related to the degree of end-organ injury and poor prognosis.<sup>97,98</sup>

## The Reduction of SCFAs Caused by Gut Microbiota Dysbiosis Affects Ischemic Stroke

Short-chain fatty acids (SCFAs), mainly include acetate, propionate and butyrate, which are produced by gut microbiota fermenting and degrading the insoluble carbohydrates in cecum and colon.<sup>99,100</sup> Ischemic stroke will lead to microbiota dysbiosis and intestinal barrier damage,<sup>101</sup> resulting LPS leakage and systemic inflammatory response, which are important pathological processes of ischemic stroke. It is found that AIS induces the long-term increase of LPS and proinflammatory cytokines in circulation of cynomolgus monkeys, which is related to the increased relative abundance of Bacteroidetes, meanwhile, the gut microbiota dysbiosis and mucosal injury resulting from AIS persist for almost 12 months.<sup>102</sup>

As beneficial substances, SCFAs can arrest the progression of ischemic stroke (Figure 2). First, SCFAs provide colon an acidic environment so that the growth of pathogenic bacteria is inhibited and the balance of water and electrolyte is maintained, preventing gut dysfunction.<sup>103–106</sup> Second, SCFAs, as the main kind of energy source of colonic



**Figure 2** A summary on how SCFAs arrest the inflammation to alleviate ischemic stroke.

epithelium,<sup>99</sup> playing an important role in cell growth and differentiation,<sup>107,108</sup> are important substances for maintaining intestinal epithelial barrier function.<sup>109</sup> In colon-derived SCFAs, butyrate is the most important regulator of tight junction protein (TJP), and has been proved to enhance intestinal barrier function by increasing the expression of claudin-1 and Zonula Occludens-1 (ZO-1) and the redistribution of occludin.<sup>110</sup> Meanwhile, butyrate can reverse the abnormal expression of ZO-1 so that repairs the dysfunctional intestinal barrier and reduces LPS translocation, thereby inhibiting macrophage activation, proinflammatory cytokine production and neutrophil infiltration.<sup>111</sup> Last but not least, SCFAs can directly regulate inflammation. Studies have shown that SCFAs suppress the production of pro-inflammatory factors, facilitate the repair of mucosal inflammation, and inhibit the inflammatory response in the colon.<sup>112,113</sup> The anti-inflammatory effect of SCFAs is partly due to the inhibition of NF- $\kappa$ B activation by binding to G protein-coupled receptors 41 and 43 (GPR41 and GPR43) of host immune cells.<sup>114–117</sup> Between them, GPR43 plays a more important role in mediating anti-inflammatory stimulation induced by acetic acid.<sup>118</sup> In addition, studies have shown that the activation of Th17 and Treg cells is regulated by SCFAs. SCFAs reduce the secretion of pro-inflammatory factor IL-17 by inducing the decrease of Th17, while they increase the level of Treg cells in intestinal tract.<sup>119,120</sup> Treg cells are key brain protection regulators in acute cerebral ischemia, secreting anti-inflammatory factor IL-10 and inhibiting the neurotoxicity of pro-inflammatory factors TNF- $\alpha$  and IFN- $\gamma$ .<sup>121</sup> By these ways, SCFAs inhibit the progress of ischemic stroke and alleviate inflammatory injury.

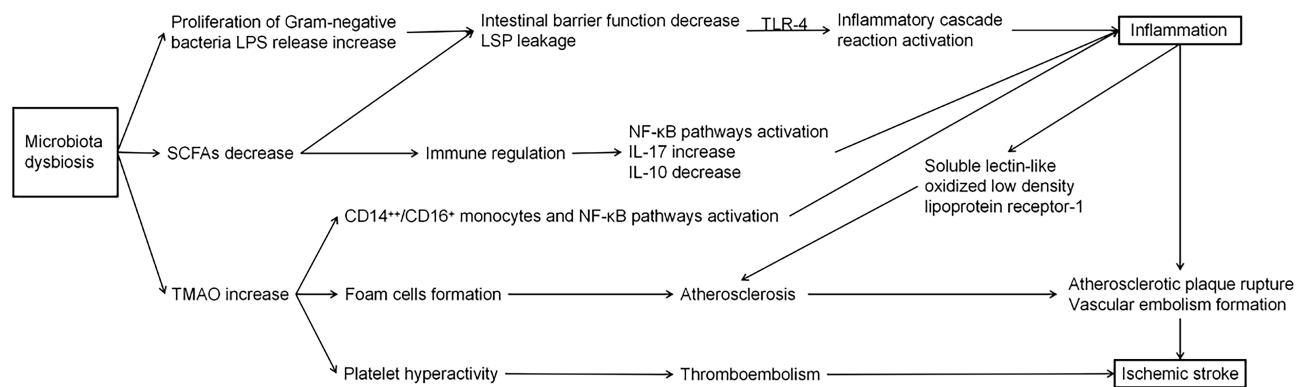
Since SCFAs have many benefits for maintaining normal intestinal function, the decrease of SCFAs caused by gut microbiota dysbiosis significantly affects the development of ischemic stroke. A fecal microbiota transplantation experiment showed that increasing SCFAs in elderly stroke mice improves the prognosis. In the experiment, the fecal transplantation tube feeding (FTG) method from young mice was used to reconstruct the microbial community in elderly mice after stroke. Younger fecal grafts contain higher levels of SCFAs and associated bacterial strains, including *Bifidobacterium longum*, *Clostridium symbioticum*, *Clostridium pratense* and *Lactobacillus fermentum*. The result showed that SCFAs producers increased SCFAs concentrations in intestine, brain and plasma of aged stroke mice, alleviating neurological deficits and inflammation after stroke and improving functional and behavioral outcomes. Basing on this result, the researchers suggested that the decrease of SCFAs from bacteria in the elderly microbial group seemed to be partly responsible for the enhancement of immune status and poor prognosis after stroke at least.<sup>122</sup> Another prospective study on the spectrum of gut microbiota and feces SCFAs in Chinese AIS patients reported that gut microbiota in AIS patients (especially those with increased stroke severity) was significant imbalance, accompanied by the lack of SCFAs. In AIS patients with higher severity, the abundance of SCFAs-producing bacteria was lower, while the abundance of *Lactobacillus*, *Akmania*, *Enterobacteriaceae* and *Porphyromonas* was higher. Moreover, the decreased content of SCFAs, particularly acetate, was also significantly correlated with the increase in the risk of 90-day adverse functional results.<sup>123</sup>

In summary, we know that microbiota dysbiosis can lead to the proliferation of pathogenic bacteria, SCFAs decrease and TMAO increase, resulting in atherosclerosis, systemic inflammation, and platelet hyperresponsiveness, which directly contributes to the occurrence and development of ischemic stroke (Figure 3). In turn, ischemic stroke induces gut microbiota dysbiosis and impairs intestinal barrier function, leading to systemic inflammation, which aggravates the pathological process and symptoms of ischemic stroke and adversely affects its prognosis.

## Gut Microbiota Dysbiosis and the Risk Factors for Ischemic Stroke

### Gut Microbiota Dysbiosis Induces Obesity

Obesity is characterized by excessive adipose tissue, which occurs when there is an imbalance between energy intake and consumption.<sup>124</sup> Evidences show that gut microbiota help to collect energy and increase host fat storage.<sup>125,126</sup> The total fat of sterile mice is shown to be 40% less than that of normal mice, although their calorie intake was 29% more than that of normal-feed littermates.<sup>126</sup> It is generally believed that changes in gut microbiota is one of the main causes of obesity.<sup>127–129</sup> When compared with lean subjects, obese subjects show significantly lower diversity of gut microbiota, lower proportion of Bacteroidetes, and higher abundance of Firmicutes. Moreover, after dietary treatment, the relative abundance of Bacteroides increased, while that of Firmicutes decreased.<sup>130</sup> Animal model also support this view. The



**Figure 3** Pathogenesis of ischemic stroke caused by gut microbiota dysbiosis.

number of *Bacteroides* in abnormal leptin gene homozygous mice (ob/ob) decreased by 50% compared with that in wild-type (+/+) or hybrid (ob/+) mice, and the number of Firmicutes increased proportionally ( $p < 0.05$ ).<sup>130</sup> Using bird-gun metagenomic sequencing technology to detect the cecum microbial DNA of mice with different degrees of obesity (ob/ob, ob/+, +/+), the result shows that compared with the lean mice, the ratio of Firmicutes to Bacteroidetes in obese mice increased.<sup>126</sup> However, there are different results about the changes of *Bacteroides* and Firmicutes. A study reported that when compared with lean people, the proportion of *Bacteroides* in obese people is lower, the proportion of actinomycetes is higher, but there is no difference in the proportion of Firmicutes.<sup>131</sup> Evaluating the gene expression profiles of gut microbiota in different subjects by using real-time PCR found the remarkably lower concentrations of *Bacteroides* in lean ( $p < 0.01$ ) or anorexia ( $p < 0.05$ ) subjects than those in obese subjects, while the concentrations of Firmicutes in three groups were quite similar.<sup>132</sup> Other studies even found that the proportion of *Bacteroides* in overweight and obese subjects increased significantly.<sup>133,134</sup> Besides *Bacteroides* and Firmicutes, other strains may also be involved in the occurrence of obesity, but opinions are still inconsistent. A prospective study found infants between three weeks and one year old with low levels of *Staphylococcus* and high levels of *Bacteroides fragilis* were correlated with high risk of developing obesity in future life.<sup>135</sup> Moreover, a self-controlled study show that the level of *Bifidobacterium* of children, who become obese at the age of 10, is much lower they were in 3 months old ( $p = 0.087$ ).<sup>136</sup> Also, there is analysis showing that the composition of bacteria cultured in the obese group was remarkably lower than the normal weight group, mainly caused by *Clostridium perfringens* ( $p = 0.001$ ) and *Bacteroides* ( $p = 0.012$ ).<sup>137</sup> But interestingly, it has been reported that the levels of *Clostridium* and *Bifidobacterium* in obese adolescents decreased significantly after receiving obesity treatment plans.<sup>138</sup> The study of *Methanoberebacter smithii* (*M. smithii*) also yielded two opposite results. Studies found that compared with lean subjects, the proportion of *M. smithii* in obese subjects was low and the number was significantly decrease.<sup>134,139</sup> However, Zhang et al<sup>140</sup> found that *M. smithii* was more abundant in obese individuals than in lean individuals, which is consistent with Armougom's finding.<sup>132</sup>

LPS leakage mediated inflammation caused by microbiota dysbiosis involves in the occurrence of obesity. It was found that there was a large amount of LPS in the blood circulation of mice fed with high-fat diet, causing endotoxemia, but when using antibiotics, no increase in LPS was found.<sup>141</sup> On the one hand, LPS through combining with CD14, activate the TLR inflammation pathway.<sup>64</sup> Infusion of LPS in mice fed with normal diet causes metabolic disorder that are similar to high-fat diet feeding mice, while the metabolic disorder caused by high-fat diet was alleviated in CD14 deficient mice. Further, it is observed that macrophage infiltration in adipose tissue increases in both wild-type mice fed with high-fat diet and mice injected with LPS, but no such phenomenon was observed in CD14 deficient mice.<sup>142</sup> On the other hand, LPS triggers the expression of prostaglandin-endoperoxidase 2/ cyclooxygenase 2 (Ptgs2/COX2) mRNA, which leads to the increase of COX2 level and activity.<sup>143</sup> COX2 is a pro-inflammatory enzyme that catalyzes the conversion of arachidonic acid to prostaglandin, while prostaglandin dilated vessels leading to the increase of lymphocyte migration and TLR4-dependent inflammation heavier, which is related to leptin resistance.<sup>144,145</sup> These supported that microbiota dysbiosis through LPS mediated inflammation pathway affects obesity. In addition to LPS, the SCFAs

level of obese individuals and lean individuals are also different. A study found that there exists a 20% higher average levels of total SCFAs in obese individuals than those of lean individuals ( $p = 0.024$ ) by fecal samples detection.<sup>134</sup> A molecular analysis experiment for Indian individuals also shown that SCFAs are significantly overproduced in obese subjects through microbial glycolysis.<sup>146</sup> As a result, excessive SCFAs provide the host with additional energy substrate, and then contribute to the formation of lipid or glucose, leading to obesity.<sup>147</sup> More directly, changes in gut microbiota composition in obesity reduce fasting-induced adipokines, a kind of circulation lipoprotein lipase inhibitor, which increase white fat storage.<sup>148</sup>

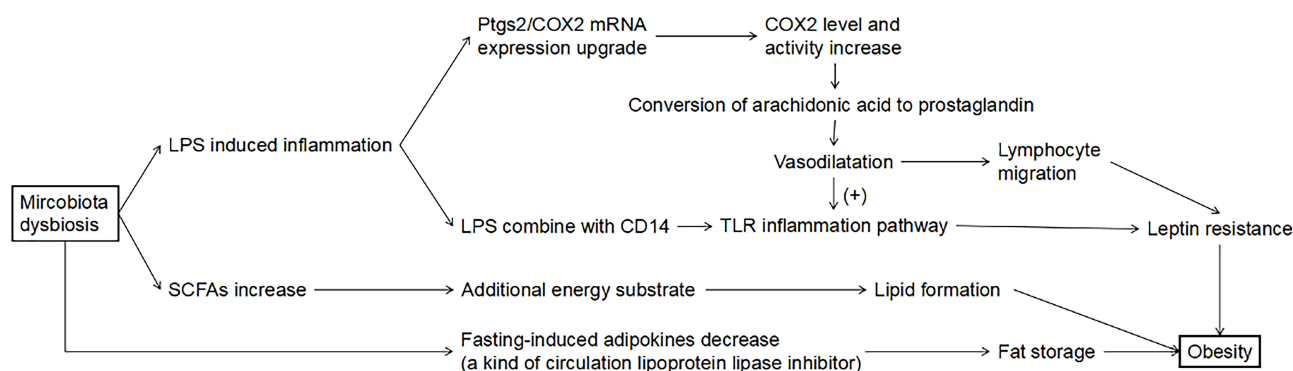
### Obesity Increases the Risk of Developing Ischemic Stroke

Obesity, a complex disease concerned with genetic and environmental factors, is often correlated with the development of several chronic complications, such as hyperglycemia, hypertriglyceridemia, dyslipidemia and hypertension,<sup>149</sup> and these obesity complications are also risk factors for ischemic stroke. In terms of obesity itself, studies have shown that central obesity increases the risk of ischemic stroke and other cardiovascular phenotypes.<sup>150,151</sup> Compared with people of normal weight, obese people have about twice the risk of stroke.<sup>152</sup> At present, the definition of obesity is mainly based on waist–hip ratio (WHR) and BMI, both show a strong correlation with the risk of ischemic stroke. Obesity ( $BMI > 30 \text{ kg/m}^2$ ) was correlated with increased stroke risk (odds ratio, 1.57, 95% CI = 1.28–1.94).<sup>153</sup> WHR showed a stronger correlation than BMI.<sup>154</sup> For every 10% increase in WHR, the risk of ischemic stroke in large arteries increases by 75% (95% CI = 44% – 113%), and the risk of ischemic stroke in small vessels increases by 57% (CI = 29% – 91%).<sup>155</sup> In addition, obesity increases the risk of ischemic stroke by inducing complications such as type 2 diabetes mellitus (T2DM) and hypertension. About a tenth of the observed WHR effect was SBP-mediated ischemic stroke (12%; CI = 4% – 20%).<sup>155</sup> Case-control study also reported that obesity is a risk factor for ischemic stroke in young adults, and that this correlation may be partially mediated by hypertension, diabetes and/or other variables correlated with these diseases.<sup>153</sup>

From these above, we can know that gut microbiota disturbance is involved in the occurrence of obesity through a variety of ways, increasing the risk of ischemic stroke (Figure 4). However, there is still controversy about the content change of specific strains in obesity. Even so, when compared with normal individuals, the content of these bacteria in obese patients is not normal and the common features, such as inflammation and SCFAs increase, are found in obese patients, which suggesting that gut microbiota dysbiosis do affect the occurrence of obesity, but the causes of different results need further study.

### Microbiota Dysbiosis Leads to T2DM

T2DM is distinguished by insulin resistance (IR) and hyperglycemia. It has been proved that microbiota dysbiosis are involved in the initiation and progression of T2DM.<sup>156</sup> In T2DM rat models, researchers found that the abundance of Bacteroides decreased, while the abundance of Firmicutes increased, which means the ratio of Firmicutes/Bacteroides increased.<sup>157,158</sup> Also, it has proved that the enhanced relative abundance of Firmicutes and Actinobacteria was associated with fasting blood glucose, while the decreased relative abundance of Bacteroides and Proteus was associated



**Figure 4** Pathogenesis of obesity caused by gut microbiota dysbiosis.

with fasting blood glucose.<sup>159</sup> In the feces of patients with diabetes, lower *Bacillus* content and higher *Enterococcus* content were also found when compared with those of normal people.<sup>160,161</sup> Further, it is reported that T2DM is related to the decreased abundance of butyrate-producing bacteria and the increased abundance of *Lactobacillus*.<sup>156,162,163</sup> Consistently, the decreased percentage of butyrate-producing bacteria in T2DM patients, including *Roseburia* and *Faecalibacterium prausnitzii*, has been found when compared with healthy subjects.<sup>164,165</sup> Decreasing the level of butyrate-producing bacteria in patients with metabolic syndrome by using vancomycin results in the reduced of insulin sensitivity,<sup>166</sup> which has a negative effect on T2DM.

As an inflammation-related disease, diabetes is affected by intestinal disorder-induced inflammation. Intestinal disorders cause increased abundance of opportunistic pathogens, including Gram-negative bacteria, resulting in increased release of LPS, which follows that LPS directly impairs the intestinal barrier and triggers the release of pro-inflammatory cytokines, inducing inflammatory response through TLR signaling pathway.<sup>59</sup> Inflammation destroys the structure and function of endothelial cells, leading to the imbalance of insulin transport, thereby inducing IR.<sup>167,168</sup> In addition, the activation of mitogen-activated protein kinase (MAPK) signaling pathway inhibits the activation of insulin receptor substrate, inducing the death of  $\beta$  cell and IR.<sup>169</sup> Besides LPS leakage, diabetic patients showed low SCFAs content and the proportion of SCFAs-producing bacteria,<sup>170</sup> while the decrease of SCFAs attributes to the reduce amount of islet cells and the decreased sensitivity of insulin, which leads to IR.<sup>171</sup> It has been mentioned above that SCFAs maintain the normal function of intestinal barrier and regulate Th17 and Treg cells.<sup>119,120</sup> The decrease of SCFAs makes the intestinal barrier lack of protection, and reduces the intestinal anti-inflammatory ability, which promote the occurrence and development of IR. Experiments have shown that increasing Treg cells reduces the infiltration of macrophages in adipose tissue, which is important for alleviating IR.<sup>172</sup> In addition, the damage to the diversity and amount of SCFAs-producing bacteria in the gut increases the glycated hemoglobin (HbA1c) level in T2DM patients,<sup>173</sup> a kind of protein that represents the average plasma glucose levels over the past 2 to 3 months,<sup>174</sup> suggesting that the poor glycemic control.

It is worth noting that T2DM, as a complication of obesity, is also affected by obesity to some extent.<sup>175,176</sup> Obesity individuals are usually characterized by IR, which is related to low-grade subclinical inflammation leading to hyperglycemia and is conducive to the occurrence of T2DM.<sup>177</sup> Studies have shown that when compared with healthy people, obese people with IR are distinguished by changed composition of gut microbiota, especially the increased ratio of Firmicutes/Bacteroidetes.<sup>178,179</sup> Moreover, it is reported that the percentage of *Akkermansia muciniphila* decreases in obesity, and reconstruct this kind of bacterial improves insulin function and glucose tolerance.<sup>180</sup> Animal study also has some findings. Sterile mice transplanted with gut microbiota of obese mice (ob/ob) increased obesity related to IR.<sup>126</sup> Transplanting fecal microbiota from lean individuals to IR patients with metabolic syndrome showed that insulin sensitivity of IR patients was improved, related to the increase number of butyrate-producing bacteria.<sup>181</sup> IR in obesity induced by high-fat diet is also largely affected by gut microbiota. Obesity caused by high-fat diets increases the proportion of LPS-containing microbial populations and plasma LPS levels, leading to endotoxemia-induced IR.<sup>142,182</sup> An experiment proved that the loss of Toll-4 receptors prevents IR induced by high-fat diet,<sup>183</sup> showing that inflammation, obesity and IR exist certain relation. Another study found that when treating diet-induced obese mice with norfloxacin and ampicillin, blood glucose was controlled accompanied by the decreased level of TNF- $\alpha$  in jejunum,<sup>184</sup> indicating that the regulation of antibiotics on gut microbiota reduces inflammation and increase glucose tolerance in obese mice. In addition, the stimulation of extracellular signal-regulated kinases 1 and 2, I $\kappa$ B kinase complex and c-Jun N-terminal kinases reduces the tyrosine phosphorylation of insulin receptor substrate protein in inflammatory regions of obese individuals, resulting in weakening insulin signaling.<sup>185</sup> These examples show that there is a causal relationship between microbial groups, obesity and IR.

### Diabetes is a Major Risk Factor for Ischemic Stroke

Animal and clinical studies have shown that diabetes is an important independent risk factor for stroke.<sup>186–189</sup> Compared with non-diabetic patients, diabetic patients are more prone to stroke. Studies estimate that about 20–33% of hospitalized patients with acute stroke have diabetes.<sup>190–192</sup> Compared with hemorrhagic stroke, ischemic stroke is more associated with diabetes.<sup>193,194</sup> Moreover, the size of cerebral infarction is larger, and mortality and disability rate are higher in

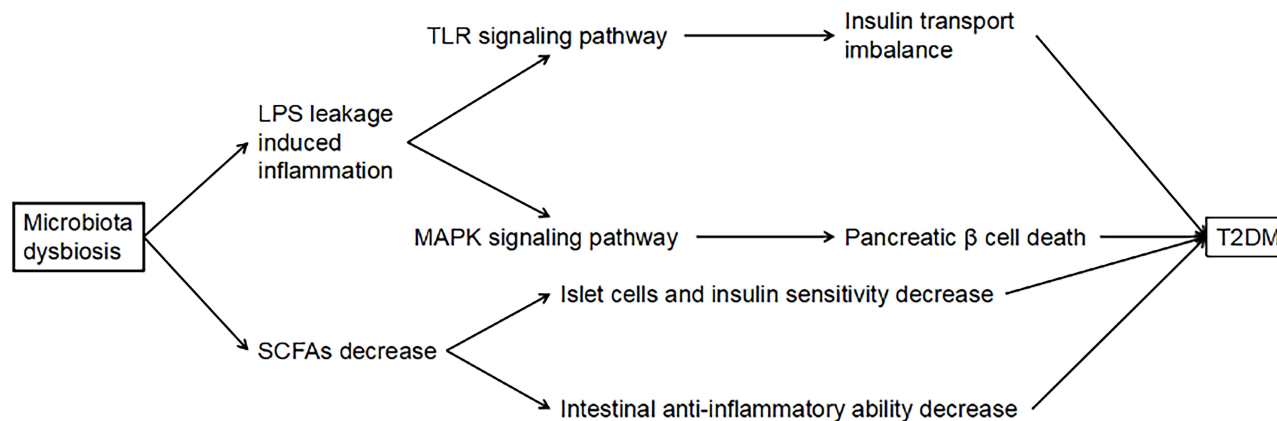
patients with hyperglycemia or diabetes after stroke.<sup>195,196</sup> In addition, study finds that the level of HbA1c can predict the risk of stroke,<sup>197</sup> and there is a correlation between elevated HbA1c and poor clinical outcomes after stroke.<sup>198</sup>

Diabetes is distinguished by IR and hyperglycemia, both of which leads to increased expression and secretion of plasminogen activator inhibitor-1 (PAI-1), while PAI-1 inhibits fibrinolysis by inhibiting plasminogen activator, causing coagulation-fibrinolysis imbalance.<sup>199</sup> Besides, through IR and hyperglycemia, diabetes also leads to vascular endothelial dysfunction and platelet hyperresponsiveness, thereby, diabetic patients are usually in prethrombotic state,<sup>200</sup> which increases the risk of ischemic stroke caused by thromboembolism. In addition to prethrombotic state, hyperglycemia leads to revascularization damage, reperfusion reduce, reperfusion injury increase and direct tissue damage.<sup>201</sup> Animal studies reported that hyperglycemia expands the volume of cerebral infarction and attributes to hemorrhagic transformation of infarction region, resulting in worse prognosis.<sup>202,203</sup> The negative prognosis is largely due to the adverse effects of hyperglycemia on multiple components involved in the development of stroke. First, hyperglycemia inhibits immune response and tissue repair during reperfusion by inhibiting microglia activation and proliferation whose activation has been shown to regulate the proliferation of brain endothelial cells.<sup>204,205</sup> Second, hyperglycemia prolonged the duration of astrocytes activation.<sup>205</sup> The activated astrocytes promote neuronal survival and plasticity following ischemia by releasing nutrients and extracellular matrix molecules.<sup>206–208</sup> Also, the persistent activation of astrocytes form glial scar tissue around the infarct cerebral tissue, which provides a physical barrier for axon growth, thereby inhibiting the recovery of neurological function after stroke.<sup>209,210</sup> Third, hyperglycemia induces an abnormal increase in the number of neutrophils in the cerebral membrane after ischemia.<sup>205</sup> Neutrophils lead to increased production of free radicals, increased neuroinflammation, destruction of BBB and hemorrhagic transformation after stroke.<sup>211</sup> Most importantly, hyperglycemia directly disrupting the BBB component, including inhibition of TJP and damage to basement membrane, explains why the transition from ischemic stroke to hemorrhagic stroke increases in animal models of stroke and clinical stroke patients.<sup>212,213</sup>

Thus, we can see that intestinal disorders induces hyperglycemia (Figure 5), thereby increasing the risk of ischemic stroke and aggravating ischemic stroke damage. Moreover, diabetes as a complication of obesity, obesity indirectly affects the occurrence and development of ischemic stroke by affecting the progress of diabetes.

## Microbiota Dysbiosis Affects Hypertension

When compared the gut microbiota composition of Wistar-Kyoto rats (WKY) with spontaneously hypertensive rats (SHR) and chronic angiotensin II perfusion hypertensive rats (Ang II), the abundance, diversity and uniformity of gut microbiota in SHR and Ang II rats were lower, with the significant increase of Firmicutes to Bacteroidetes ratio and lactobacillus but the significant reduce of acetate-producing and butyrate-producing bacteria, and the decreased abundance and diversity of gut microbiota were also found in hypertensive patients. Besides, this experiment also found that the use of broad-spectrum antibiotic minocycline lowers the blood pressure of Ang II rats and reverses the gut microbiota



**Figure 5** Pathogenesis of T2DM directly caused by gut microbiota dysbiosis.

dysbiosis mentioned above.<sup>214</sup> Further, rats experiment showed that the blood pressure decreased in SHR when the gut microbiota in normal blood pressure rats was transplanted to them by oral gavage. In turn, the blood pressure of normal blood pressure rats increased when the gut microbiota in stroke prone SHR was transplanted to them by oral gavage accompanied by a significant increased abundance of Firmicutes and a significant decreased abundance of Bacteroidetes.<sup>215</sup> Other animal studies showed that the gut microbiota in the hypertensive animal model group was significantly different from that in the control group, and these differences include low abundance of SCFAs-producing bacteria, high abundance of lactic acid-producing bacteria, low abundance of Bacteroides, and high abundance of Proteobacteria and Cyanobacteria.<sup>214,216</sup>

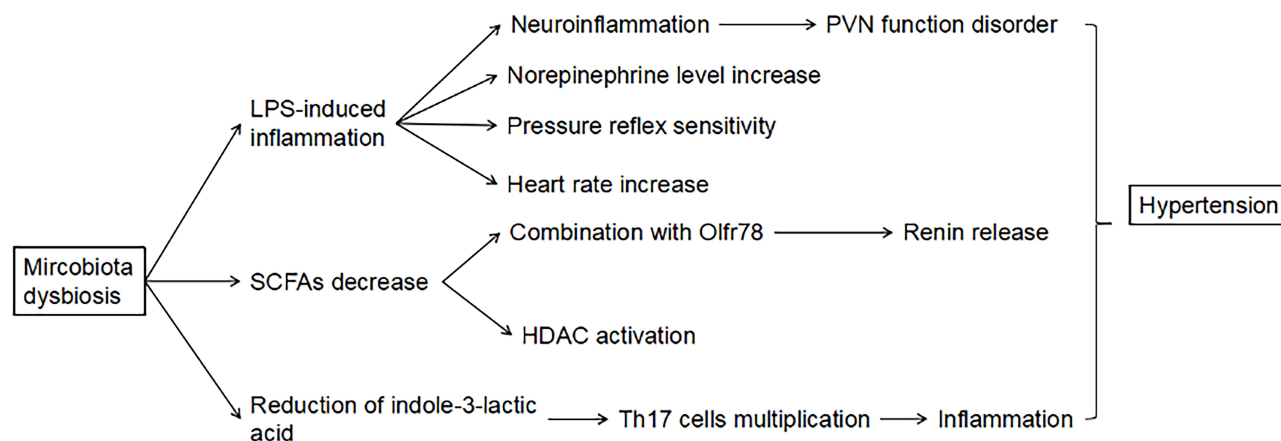
As a disease related to chronic inflammation,<sup>217</sup> hypertension can be affected by inflammation mediated by intestinal LPS leakage. An experiment showed that LPS application to rats increases heart rate and norepinephrine level, reduces pressure reflex sensitivity, and increases neuroinflammation, such as TLR and TNF- $\alpha$  expression in PVN, a region playing a key role in blood pressure regulation.<sup>218</sup> In addition to the effect of LPS, SCFAs can combine with orphan G protein-coupled receptors 41 (Gpr41) and olfactory receptor 78 (Olf78) to control the rise and fall of blood pressure. Between them, Olf78 is affected by propionic acid and acetate, inducing the release of renin and increasing blood pressure, while Gpr41 is affected by propionate, reducing blood pressure.<sup>219,220</sup> Generally, in healthy gut, the effect of SCFAs is more likely to lower blood pressure.<sup>221–223</sup> Besides, as one of SCFAs, butyrate inhibits histone deacetylase (HDAC) to exert anti-inflammatory effect, which is also effective in lowering blood pressure.<sup>224,225</sup> Studies have shown that the activation of HDAC is associated with hypertension in SHR, and using butyrate reduces blood pressure and renal inflammation by inhibiting HDAC in mice.<sup>226,227</sup> Also, in the intervention tests of patients with metabolic syndrome, the effect of butyrate on lowering blood pressure was observed.<sup>228,229</sup> Therefore, the decrease of SCFAs-producing bacteria could be one of the reasons of developing hypertension.<sup>214</sup>

In addition, gut microbiota dysbiosis attributes to high-salt diet induced hypertension. The positive correlation between high-salt diet and hypertension has been supported by many epidemiological studies around the world.<sup>230</sup> Among which, a study showed that high-salt diet affected the gut microbiota of mice, significantly reducing the abundance of *Lactobacillus* and their tryptophan metabolite indole-3-lactic acid, and increasing the number of Th17 cells in the lamina propria of the small intestine, spleen and spinal cord, which promote the inflammation. In turn, supplementary *Lactobacillus* in mice fed with high-salt diet increases the content of indole-3-lactic acid, reducing the blood pressure and the number of Th17 in small intestine colon and spleen. Similar phenomenon is also observed in human.<sup>231</sup> Another study showed that high-salt diet breaks the immune balance of the gut, which was manifested as increasing Th17 cells and their reactivity in the intestinal lamina propria of mice, reducing the IL-10 secretion level of Treg cells and their inhibitory effect on T cell proliferation, worsening the inflammation.<sup>232</sup>

### Hypertension is a High-Risk Factor for Ischemic Stroke

Stroke is a disease affected by multiple factors, and hypertension is the primary risk factor for stroke.<sup>233</sup> The incidence of stroke was proportional to systolic blood pressure and diastolic blood pressure, and the relative risk of stroke increased 3.1 times in males and 2.9 times in females.<sup>234,235</sup> In women with preeclampsia (a common pregnancy-induced hypertension), it is found to be 4–5 times higher the long-term risk for stroke.<sup>236–238</sup> Further, reducing the blood pressure of hypertensive patients is found to be a very effective in preventing ischemic stroke, because for every 10 mmHg decrease in systolic blood pressure and 5 mmHg decrease in diastolic blood pressure, the stroke risk can be reduced by 41% (95% CI: 33%–48%).<sup>239</sup> Hypertension also worsens stroke results. Compared with patients with normal blood pressure, patients with hypertension in the past had a smaller amount of recoverable tissue and larger infarction.<sup>240–243</sup> There are researchers who have reported that hypertension affects the intestinal barrier and increase intestinal permeability,<sup>244</sup> which contribute to the systemic inflammatory response mediated by LPS leakage. Other researchers have found that high-salt diet induces the multiplication of Th17 lymphocytes in the small intestine of mice, and IL-17 in the plasma is increased. IL-17 in the circulatory system reduces NO by inhibiting the phosphorylation of NO synthase in endothelial cells through Rho kinase pathway, promoting neurovascular injury and cognitive dysfunction.<sup>245</sup>

Therefore, we can know that gut microbiota dysbiosis, especially the increase in the ratio of Firmicutes to Bacteroides and the decrease of *Lactobacillus*, are major features of hypertension. Hypertension induced by gut microbiota dysbiosis through inflammation, immune and anabolism, directly increases the risk of ischemic stroke and deteriorates the outcome of ischemic stroke (Figure 6).



**Figure 6** Pathogenesis of hypertension caused by gut microbiota dysbiosis.

## Discussion

From the above expound, we can know that gut microbiota dysbiosis directly leads to ischemic stroke by inducing inflammation, atherosclerosis and thromboembolism, through increasing TMAO, reducing SCFAs and LPS leakage, and has a negative impact on the outcome of ischemic stroke. Moreover, gut microbiota dysbiosis is also involved in the occurrence and development of high-risk factors for ischemic stroke such as obesity, T2DM and hypertension, which increases the risk of developing ischemic stroke. Also, it is shown that the ratio of Firmicutes to Bacteroides increase and the LPS-induced inflammation are the common feature of obesity, T2DM and hypertension caused by gut microbiota dysbiosis.

In view of the above facts, therapeutic intervention measures for ischemic stroke target on regulating gut microbiota are feasible.<sup>246</sup> By regulating gut microbiota, alleviates inflammation, atherosclerosis and thrombosis, to prevent the occurrence of ischemic stroke. Also, by intervening and regulating gut microbiota, alleviates obesity, diabetes and hypertension to reduce the risk of developing ischemic stroke. Even after ischemic stroke, gut microbiota can be adjusted to promote recovery and improve prognosis. A mice experiment published in Nature Medicine found that the volume of cerebral infarction in the antibiotic treatment group was 60% less than that in the control group after experimental ischemic stroke. The use of antibiotic reduces diversity of gut microbiota, mainly including the retraction of Clostridiaceae (a kind of Firmicutes) and S24-7 spp (a kind of Bacteroidetes), and the increase of Proteobacteria. Moreover, transplanting the gut microbiota of the antibiotic treatment group into the control group significantly reduces the volume of cerebral infarction after ischemic stroke.<sup>18</sup> Also, an experiment reported that improving intestinal barrier function is helpful to the recovery of BBB function after ischemic stroke.<sup>101</sup>

In addition, the intestinal regulatory therapy for high-risk factors for ischemic stroke has shown a significant effect. Supplementing probiotics as a convenient method has shown high efficacy in alleviating obesity, hyperglycemia and hypertension. In adults, probiotic supplement including strains of Lactobacillus and Bifidobacterium, alone or in combination, as well as Pediococcus pentosaceus resulted in significant reductions in body weight, waist circumference, BMI and fat mass.<sup>247–252</sup> Probiotic therapy of Lactobacillus paracasei reduced and reversed LPS-related pathological effects in rodents, especially alleviated T2DM-related  $\beta$ -cell dysfunction.<sup>253</sup> Intervention with Lactobacillus casei in diabetic mice for 15 days found a sharp decline in blood glucose levels.<sup>8</sup> Supplementing Lactobacillus rhamnosus GG for 2 months to streptozotocin-induced diabetic rats improves the glucose tolerance and decreases the level of HbA1c significantly.<sup>254</sup> Also, adding Lactobacillus to high-salt diet mice increases the content of indole-3-lactic acid, lowers the blood pressure and reduces the amount Th17 in intestine, colon and spleen.<sup>231</sup> As a more thorough method, fecal microbial transplantation also shows positive effect. Transplantation of fecal microbiota from obese, steatosis or T2DM patients partially replicate the donors' metabolic phenotype in mice recipients.<sup>126,179,255,256</sup> Also, the transplantation of fecal microbiota from lean individuals to IR patients with metabolic syndrome improved insulin sensitivity.<sup>181</sup> Moreover, diabetic patients receiving fecal microbial transplantation showed stable blood glucose.<sup>257</sup> All these above supports that preventing and treating ischemic stroke by regulating gut microbiota through supplementing probiotics or FMT could be a practicable way.

## Conclusion

In this review, we have discussed the relationships among gut microbiota, ischemic stroke and the high-risk factors for ischemic stroke, pointing that regulating gut microbiota could be a feasible way to prevent and treat ischemic stroke. By reconstructing gut microbiota, we can alleviate the high-risk factors for ischemic stroke, such as obesity, diabetes and hypertension, and inhibit the inflammation and vascular embolization, lowering the risk of developing ischemic stroke to a great extent. Moreover, reconstructing gut microbiota after ischemic stroke can also arrest the progression of the disease and improve the prognosis. However, the consist of gut microbiota is so complex that we cannot totally figure out what exactly to do to prevent the gut microbiota dysbiosis and reform the gut microbiota thoroughly, and these aspects need further exploring. For now, supplementing probiotics or FMT can be a good way for some extent.

## Consent for Publication

All authors have read and approved the manuscript.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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