BioGecko Vol 12 Issue 01 2023

ISSN NO: 2230-5807

A Comprehensive Review on Stroke and Gut Microbiota as a New Therapeutic Approach for Stroke

Sudakshina Dutta[1], Tamilanban T[2]*, Goutam Choudhary[3], Abdul Rohim Tualeka[4], Chitra V[5]

[1,2*,3,5]Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603 203, Tamil Nadu, India.
[4]Department of Occupational Health and Safety, Airlangga University, 60115, Surabaya, East Java, Indonesia.

***Corresponding Author: T. Tamilanban[2*]**
[2*]Associate Prof, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Tamil Nadu, India.
Email: [2*]tamilant@srmist.edu.in

Abstract

Stroke is a key factor to impairment and is the 2nd most common reason for mortality worldwide. A cerebrovascular blockage, either transient or lifelong ("ischemic stroke"), causes infarction of the brain in the majority of instances of cerebral stroke. Our knowledge of the basic mechanisms of ischemia injuries and the physiological processes associated with stroke has advanced significantly. A plethora of signaling pathways, both harmful and neuroprotective, are also heavily engaged in the aforementioned pathophysiology. Improving blood supply to the brain and treating the cognitive impairment caused by stroke are the main objectives of stroke treatment. A basic knowledge of the primary molecular mechanisms induced by ischemic circumstances has previously been obtained through in vitro research. One of the most often used in vivo models for stroke research is middle cerebral artery (MCA) blockage, which produces repeatable cerebral infarction in the MCA area. It permits reperfusion by removing the occluding filament without necessitating a craniectomy. Current investigations on the gut microbiome have demonstrated their impact on the pathophysiology of ischemic stroke and the effectiveness of therapy. The term "gut dysbiosis" refers to changes in the diversity, abundance, and functionality of the gut microbiome. The pathogenesis of stroke, significant advancements in the discovery of therapy targets, and current breakthroughs in gut microbe-related studies on stroke are the primary concerns of this review.

Keywords: Stroke; Gut microbiome, Stroke animal models, Brain Hemorrhage.

1. INTRODUCTION

An obstruction in the blood vessel can be characterized as a neurological disorder known as stroke. Blood clots that develop in the brain restrict arteries and cause blood vessels to rupture, which results in blood loss. When the blood vessels that serve the brain break apart during a stroke, brain cells suddenly die from an inadequate supply of oxygen. Following a stroke, depression and dementia may occur [1].

A stroke, also known as an assault on the brain, happens when a blood artery ruptures in the brain or when something cuts off the supply of blood to a portion of the brain. The brain

ISSN NO: 2230-5807

either perishes or suffers harm in both scenarios. One may argue that a stroke can result in permanent brain damage, lifelong incapacity, or even death [1].

Numerous studies show that stroke fatalities occur more frequently in women as compared to men. Typically, women account for six out of ten deaths from strokes that are ischemic or haemorrhagic. Numerous variables contribute to this increased risk. Among these is the fact that women survive longer compared to men on average and that this prolonged lifespan makes them more vulnerable to stroke. High blood pressure during pregnancy and high blood pressure brought on by some birth control medications are two additional distinct risk factors that are exclusive to women [2-4].

Usually, stroke patients suffer from two distinct types of strokes: one is ischemic, and the other is haemorrhagic [5][6]. In many ischemic stroke cases, the obstruction is caused by a blood clot that becomes stuck within any of the brain's arteries. The sole FDA-approved medication for treating ischemic stroke at this time is tissue plasminogen activator (tPA), a kind of thrombolytic that breaks down the clot. However, this medication needs to be administered to the stroke patient no later than 4.5 hours after the onset of symptoms [7][8]. When tPA is administered beyond this therapeutic window, it may cause a haemorrhagic alteration that aggravates pre-existing brain damage. If the clot fails to dissolve away on its own or if the patient does not show up at the hospital within the window required for tPA therapy, there are other options available, such as a thrombectomy, which involves removing the clot surgically. Since there is an increased chance of having another stroke soon after the first, preventative measures such as anticoagulants, blood pressure, and cholesterol-lowering drugs may also be used [6]. By using these therapies as soon as possible, the effects of any deficits that a stroke can lead to can be reduced [9].

Motor problems such as hemiparesis, hemiplegia, and central facial paresis are common problems after a stroke [10]. Deficits in speech and language are also common; these might include dysarthria and global or mixed aphasia [11]. Other anomalies include reduced blood circulation to certain brain regions, altered consciousness, and visual problems [12]. Each of these deficits has a significant effect on the quality of life experienced by stroke patients.

PATHOPHYSIOLOGY

Stroke is a term used to describe a sudden neurologic episode that results from a reduction in the flow of the blood to the brain. Hemorrhagic stroke is brought about by bleeding or rupture of blood vessel, as opposed to ischemic stroke, which is brought on by insufficient blood and oxygen supply to the brain (Figure 2).

Stroke victims die from ischemic occlusions in around 85% of cases; intracerebral bleeding causes the remaining 15% of deaths. Ischemic occlusion is the cause of brain embolism and thrombosis [13]. A thrombotic stroke occurs when the vascular chamber narrows and clots due to plaque accumulation. An embolic stroke is defined as a decrease in blood supply to the brain, which in turn causes severe stress and early cell death (necrosis). The consequences of necrosis include rupture of the membrane of the plasma cell, loss of neuronal function, and cellular contents seeping into the extracellular space [14–19].

Hemorrhagic strokes, which result in a high mortality rate, account for 10% to 15% of all stroke cases. Blood vessels rupture as a result of internal structural stress and harm to brain tissue. Infarction results from its detrimental effects on the vascular system [20]. An abnormal buildup of blood inside the brain is the result of blood vessels rupturing, known as Intracerebral hemorrhage (ICH). Hypertension, anomalies of the vasculature, overuse of anticoagulants, and thrombolytic drugs are the main causes of ICH. Blood can build up in the subarachnoid space of the brain due to brain injury or cerebral aneurysm, which can result in subarachnoid hemorrhage [21, 22].

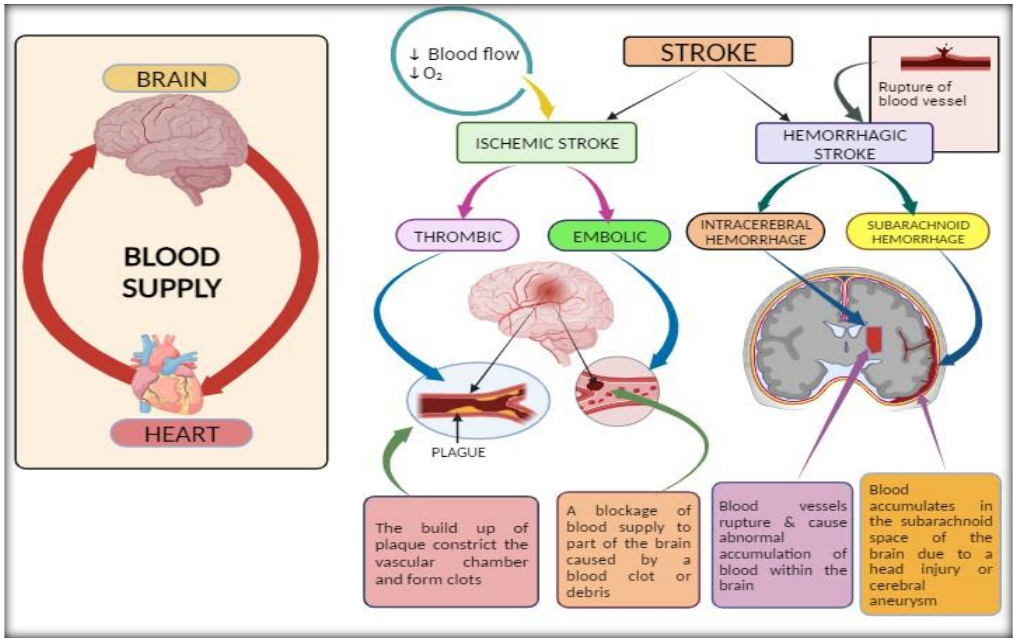

Figure 1: Pathophysiology of Stroke

SIGNALLING PATHWAYS

The complicated signaling pathways connected to stroke encompass numerous events that result in brain damage. The latest study has been centered on understanding the etiology of stroke that is ischemic, encompassing cellular excitotoxicity, oxidative stress, cell death pathways, and neural inflammation [23]. These pathways constitute an intricate signaling network that is intricately entwined. The following are a few signaling pathways connected to stroke:

JAK/STAT Pathway: This pathway is involved in inflammation and immune response and usually has a significant part in the pathogenesis of stroke [24].
AMPK Pathway: It has been demonstrated that this pathway, which is involved in energy metabolism, contributes to the pathogenesis of stroke [24].
MAPK Pathway: This pathway is involved in cell proliferation, differentiation, and apoptosis and play a major function in the pathophysiology of stroke [24].
PI3K/AKT Pathway: It has been demonstrated that this pathway, which plays a vital role in the continued existence of cells, contributes to the pathogenesis of stroke [24].
Extrinsic (or Death Receptor) Pathway: This pathway is one of the principal apoptotic pathways triggered by stroke [23].
Intrinsic (or Mitochondrial) Pathway: This pathway is another principal apoptotic pathway triggered by stroke [23].
Notch Signaling Pathway: This pathway is involved in cell fate determination and play a function in the pathophysiology of stroke and Alzheimer's disease [25].
Autophagy Pathway: This pathway has been demonstrated to be implicated in the pathogenesis of stroke and Alzheimer's disease. It is involved in the breakdown of damaged organelles [25].

The use of therapeutic strategies against ischemic stroke that target these signaling pathways is conceivable [23]. To completely comprehend the intricate signaling network

involved in stroke, however, and to create efficient treatments and interventions to reduce brain damage and enhance outcomes for stroke patients, further research is required.

RISK FACTORS

Age-associated factors increase the risk of stroke in both men and women beyond the age of 55. An individual's risk is significantly increased if they already have a medical condition such as hypertension, coronary artery disease, or hyperlipidemia. While many stroke risk factors are non-modifiable, others may be modified (Figure 2).

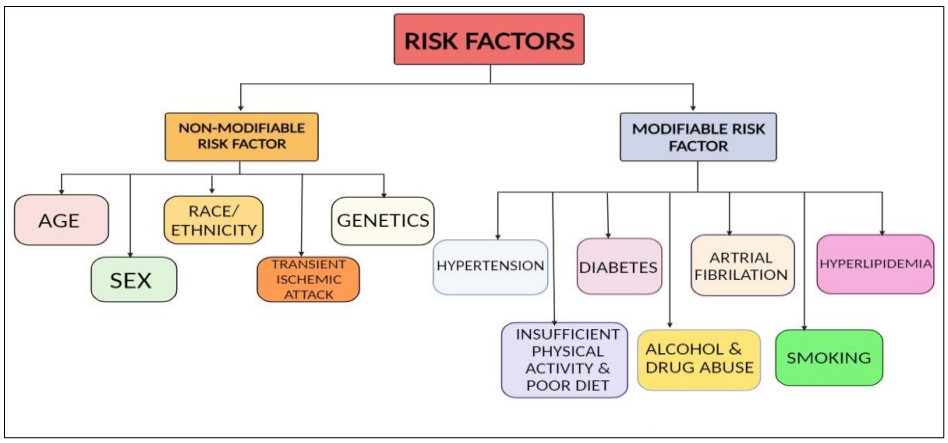

Figure 2: Modifiable and non-modifiable risk factors involved in stroke

1.1. Non-Modifiable Risk Factors

These comprise age, sex, race, transient ischemic attack (TIA), and genetic traits [26]. According to the latest research, already existing extra risk factors enhance the incidence of stroke in adults between the ages of 20 and 54 [27]. Regardless of age, women are thought to be at equal or higher risk of stroke than males [28].

A transient ischemic attack, often known as a minor stroke, has the same fundamental mechanisms as a actual form of stroke. In TIA, part of the brain's blood circulation is briefly stopped. Before the actual incident, it serves as an alert, allowing patients to modify their lifestyle and begin taking medicine to reduce the possibility of stroke [29, 30].

Genetics affects both controllable and non-controllable stroke risk factors. Hereditary risk is influenced by age, sex, and race [31, 32], but other inherited factors can potentially increase the likelihood of stroke. First off, having a family history of stroke boosts a person's risk of developing this neurological condition. Second, as in cerebral autosomal dominant arteriopathy, a rare single gene mutation may play a role in a pathogenesis where the primary clinical manifestation is stroke. Thirdly, stroke is one of the many side consequences of several illnesses caused by genetic mutations, such as sickle cell anemia. Fourth, a greater risk of stroke has been associated with many common genetic variants, including the genetic polymorphism in 9p21 [33].

1.2. Modifiable Risk Factors

Hypertension: It is among the primary contraindication for stroke. One research found that 54% of patients with stroke had a previous diagnosis of hypertension and a blood pressure (BP) value of about 160/90 mmHg, either of which can be taken into account as significant risk factors for stroke [34, 35].

ISSN NO: 2230-5807

Diabetes: It increases the probability of an ischemic stroke and raises death rates by 20%. Furthermore, after a stroke, people with diabetes had a poorer prognosis than those without the disease, with higher rates of severe damage and a less rapid rate of healing [36, 37].

Atrial fibrillation (AF): Depending on the person's age, AF increases the chance of stroke by anywhere between two to five times, thus becoming a significant contributor to risk. Compared to strokes unrelated to AF, it accounts for 15% of all strokes, is more lethal, and causes more severe disability [38].

Hyperlipidemia: Despite the convoluted nature of its relationship with stroke, it is a substantial contributor to coronary heart disease. While overall cholesterol raises the chance of stroke, high-density lipoprotein (HDL) reduces the rate of stroke [39–41].

Alcohol and drug abuse: There is a nonlinear association between the two variables, indicating that daily alcohol use raises the possibility of stroke. When consumption of alcohol is modest to moderate (two typical drinks each day for men and one for women), the chance of stroke is reduced; when input is high, the risk increases. On the other hand, even moderate alcohol use raises one's likelihood of stroke caused by haemorrhaging [42–44].

Smoking: There is a definite correlation between smoking and a higher risk of stroke. In comparison with non-smokers, the average smoker has a two times greater chance of stroke. Fifteen percent of deaths connected to stroke are caused by smoking. Research indicates that while prolonged contact with smoke as a passive smoker raises a person's risk of stroke by 30%, quitting smoking decreases the danger when compared to others [45–54].

ANIMAL MODELS OF STROKE

There are various animal models used for screening of Stroke which are listed below (depicted in Table 1).

Table 1: List of Animal models of Stroke

Stroke models	Procedure	Advantages
1. The intraluminal suture MCAo (Middle Cerebral Artery Occlusion) model	The MCAo method, which is minimally invasive, entails blocking the carotid artery with a suture until the MCA is no longer receiving blood from it.. This method is used to cause infarction for durations of 60 or 90 min or forever, and it has an 88–100% success rate in rats and mice [55]. The Sprague-Dawley rat, whose infarct area is not much, is the most often utilized animal for exploring pre-clinical stroke [56]. SV129 and C57BL/6 mice are frequently used to inflict MCA infarction [57].	• Mimics human ischemic stroke, • Exhibits a penumbra, • Highly reproducible, • No craniectomy
2. Craniectomy model	This model employs surgery to cause arterial occlusion. By electro-coagulating mice and generating a permanent injury or a microaneurysm that persists until the blood supply is cut off, a neurological deficiency can be induced using this method. Alternately, three-vessel obstruction is utilized, which reduces blood supply and causes tissue injury. Whether the occlusion is temporary or permanent affects the infarct volume [58-60].	• Elevated rates for long-lasting survival
3. Photo-thrombosis model	The basis for this hypothesis is the photo-oxidation of the vasculature, which causes lesions to occur in the cortex and striatum. In this procedure, a	• Renders it possible for an ischemic lesion

	photoactive dye is used to irradiate the skull, resulting in endothelial damage, intraparenchymal vessel aggregation, and platelet activation in the region of injury. In rats, it is given intravenously, while mice receive it intraperitoneally [61].	to be precisely located • Highly reproducible, • Not that invasive
4. Endothelin-1 model	Endothelin-1 (ET-1): The endothelium and smooth muscle cells both produce this small peptide molecule. It is a paracrine factor that, by way of cell-specific receptors, limits the vascular system. An ischemic lesion is caused by direct stereotaxic injection of ET-1 into the uncovered MCA located within the intracerebral or cortical area. [62]. Following an administration of ET-1, cerebral blood flow was seen to decrease by 70–90% followed by reperfusion [63]. This procedure can be used on both deep and superficial layers of the brain, it is minimally invasive, and it has a low mortality rate. Reproducibility depends on the ET-1 concentration being adjusted to control the area of injury size [63]. It is suitable for long-term lesion research.	• Not that invasive • Ischemia lesion generation in cortical areas • Death rate is low
5. The embolic stroke model:	It contains thromboembolic, macrosphere, and microsphere models. In the microsphere model, multifocal infarcts are created by introducing spheres with a diameter of 20 to 50 μm into the circulatory system with the help of a microcatheter [64]. To create reproducible lesions in the MCA, macrospheres of 100–400 m in diameter are injected into the intracerebral artery (ICA) [65]. To form clots in the ICA or MCA, thrombin is directly administered in the thromboembolic model. The size of the clot that forms determine the infarct's volume [66].	• Replicates the mechanisms behind stroke in humans

2. GUT MICROBES AND STROKE

2.1. Gut Microbiota

The gastrointestinal (GI) tract of humans is home to trillions of bacteria, fungi, viruses, and protozoa [67, 68]. The term "gut microbiota" normally refers to the microorganisms found in the GI tract; when linked with their genetic materials and functional traits, this term is used to describe the gut microbiome [69–71]. The gut-brain axis, or GBA, is regulated by gut microbes, which also control neurological, metabolic, immunological, and digestive activities and maintain body homeostasis [72–76]. The microbes in the gut not only preserve the integrity of the intestinal epithelium barrier but also promote mucin and other metabolic product formation such as bile acids, ethanol, acetaldehyde, acetate, and other SCFAs [77]. Approximately 51% or 48% of gut bacteria are members of the Bacteroidetes or Firmicutes phyla, respectively. The term gut dysbiosis, which is often referred to as gut microbial dysbiosis, describes pathogenic modifications in the variety and composition of microbes in the gut that lead to altered states of the gut-immune and neuroimmune systems [78, 79]. The

disruption of GBA signaling that develops from gut dysbiosis typically contributes to higher intestinal barrier malfunction and local inflammation [80–82], which leads to pathophysiological effects [83, 84].

2.2. Gut–Brain Axis

The central nervous system's (CNS) and gastrointestinal tract's (GI) bidirectional communication is termed as gut-brain axis (GBA) [69,85]. As parts of the intricate system known as the gut-brain axis, the enteric nervous system (ENS), gut microbes, and vagus nerve all interact with the GIT and the central nervous system. The ENS, commonly defined as the "second brain," regulates local immune responses and gastrointestinal motility through the myenteric and submucosal plexuses. The ENS consists of a network of neurons and glial cells that operate independently of the CNS. Intestinal motility, secretion, inflammation, and the transmission of sensory signals are all regulated by the vagus nerve. It forms a bridge between gastrointestinal tract (GIT) and brainstem. The GIT is inhabited by a colony of bacteria called the gut microbiota, which communicates with the central nervous system through neurotransmitters and immunological control (Fig. 3). In general, these mechanisms keep the gut's balance intact and communicate the state of the gut to the central nervous system [72]. The GI tract's sensory, motor, and secretory functions are influenced by top-to-bottom routes from the brain to the gut, whereas bottom-to-top signals influence cognitive and neurobehavioral functioning [72], [86–88].

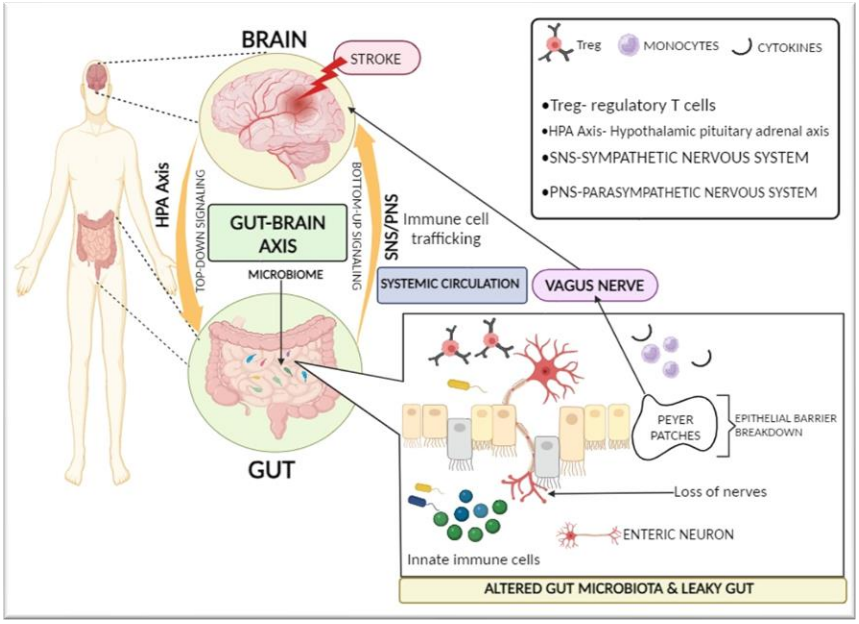

Figure 3: Impact of stroke on the brain-gut axis

2.3. The Gut Microbiota's Role in Stroke

The cause of stroke is significantly influenced by the gut microbiota, as recent investigations have demonstrated, through its regulatory effect on immune function. Dysbiosis, or an inappropriate balance in the diversity of the gut microbiota, has been associated with a surge in oxidative stress and inflammation. These two elements play a role over initiation and severity of stroke. Studies on animals have shown that changes to the gut microbiota may influence how a stroke develops; mice that were devoid of germs showed improved neuronal activity and

smaller infarct sizes. Addressing dysbiosis may be a beneficial strategy for stroke treatment as research on stroke patients has revealed shift in the diversity of the gut microbiota [89].

2.4. Stroke's Impact on Gut Function

The gut health may be affected by stroke in several ways. Dysphagia, or trouble engulfing, is a frequent side effect of stroke that can result in pneumonia and other unfavourable consequences [90]. Increased plasma LPS levels cause metabolic endotoxemia, which increases intestinal permeability [91]. A disruption of the blood-brain barrier (BBB) and neuroinflammation are caused by metabolic endotoxemia [79,94], which also stimulates the innate immune cells and causes chronic systemic inflammation [92,93]. Furthermore, post-stroke issues like infections and malnutrition may be exacerbated by alterations in gut motility and the composition of the microbiota brought on by a stroke [95]. Addressing the gut-brain axis may present a viable treatment strategy to lower the death and disability rates linked to stroke because it is thought to be crucial for the pathogenesis of the condition [96].

2.5. Stroke treatment strategies addressing the gut-brain axis

Researchers have investigated several therapeutic approaches to target gut-brain axis changes, including probiotics, synbiotics, fecal microbiota transplantation (FMT), and vagus nerve modulation.

Prebiotics, Probiotics, and Synbiotics

While probiotics are live microbes that provide health advantages when ingested in suitable levels, prebiotics are indigestible food substances that aid in the formation of healthy bacteria in the gut. Probiotics and prebiotics together form synbiotics, which support the development of good bacteria in the gut [97]. Probiotics comprising strains of Bifidobacterium and Lactobacilli have been shown in clinical research to enhance neurological performance in stroke patients. Similarly, synbiotics containing Bifidobacterium and oligofructose have been shown to improve cognitive function in stroke patients [98].

Transplantation of Faecal Microbiota

Faeces from a healthy donor are transferred to a patient with dysbiosis in a process known as Faecal Microbiota Transplantation (FMT) [99]. FMT is being investigated as a potential stroke therapy method because it has shown effectiveness in the management of recurrent *Clostridioides difficile* infection [100].

Vagus Nerve Modulation

The vagus nerve has found to be a possible stroke therapy, as it is an essential part of the gut-brain axis. Past research has demonstrated that vagus nerve induction improves the outcomes of stroke in rats [101]. One of the novel therapeutics being studied by researchers to target the gut-brain axis in stroke is the use of exosomes produced from stem cells that modify the gut microbiota and improve effects [102]. Additional possible therapies include developing drugs specific to the microbiome and focusing on the metabolites of gut microbes [103]. Although these therapies have the potential to treat stroke-related problems, further study is required to discover the best strategy and window of opportunity for their application in stroke patients.

3. CONCLUSION

Stroke is one of the prominent reasons of mortality, leading to significant economic consequences, and a major global a contributing factor in impairment. It is therefore a global health priority to improve post-stroke treatment and offer more potent therapeutic interventions. Stroke technically isn't a single neurological condition; rather, it's the symptom of an underlying systemic issue like atherosclerosis, inflammation, or infection. These conditions can also create infarcts in other organs and appear as other conditions, like a heart attack. The analysis of specific aspects of stroke that are more or less similar to human stroke can be done with the use of experimental models of ischemic stroke. However, given the intricate pathophysiology of ischemic stroke, further thorough investigation of the involved signaling pathways is required. The relationship between gut dysbiosis and the pathogenesis and prognosis of stroke has been briefly covered in this review.

4. REFERENCES

1. Goldstein L.B., Adams R., Becker K., Furberg C.D., Gorelick P.B., Hademenos G., Hill M., Howard G., Howard V.J., Jacobs B., Levine S.R., Mosca L., Sacco R.L., Sherman D.G., Wolf P.A., Zoppo G.J.D., 2001.Primary prevention of ischemic stroke: A statement for healthcare professionals from the Stroke Council of the American Heart Association. Stroke. 32(1):280-99
2. Jackson C.A., Mishra G.D., 2013. Depression and risk of stroke in mid aged women: a prospective longitudinal study. Stroke. 44(6):1555-60.
3. Leffert, L.R., Clancy C.R., Bateman B.T., Bryant A.S., Kuklina E.V., 2015. Hypertensive disorders and pregnancy-related stroke: frequency, trends, risk factors, and outcomes. Obstet Gynecol. 125(1):124-131.
4. Sacco S., Merki-Feld G.S., Ægidius K.L., Bitzer J., Canonico M., Kurth T., Lampl C., MacGregor E.A., MaassenVanDenBrink A., Mitsikostas D.D., Nappi R.E., Ntaios G., Sandset P.M., Martelletti P., 2017. Hormonal contraceptives and risk of ischemic stroke in women with migraine: a consensus statement from the European Headache Federation (EHF) and the European Society of Contraception and Reproductive Health (ESC). J Headache Pain. 30;18(1):108.
5. Barthels D., Das H., 2020. Current advances in ischemic stroke research and therapies. Biochim Biophys Acta Mol Basis Dis. 1;1866(4):165260.
6. Stroke Facts | cdc.gov. 2017 2017-09-06T08:10:23Z; Available from: https://www.cdc.gov/stroke/facts.htm.
7. Shakir R., 2018. The struggle for stroke reclassification. Nat Rev Neurol. 14(8):447-448.
8. Fukuta T., Asai T., Yanagida Y., Namba M., Koide H., Shimizu K., Oku N., 2017. Combination therapy with liposomal neuroprotectants and tissue plasminogen activator for treatment of ischemic stroke. FASEB J. 31(5):1879-1890.
9. Chapman, S.N., Mehndiratta P., Johansen M.C., McMurry T.L., Johnston K.C., Southerland A.M, 2014. Current perspectives on the use of intravenous recombinant tissue plasminogen activator (tPA) for treatment of acute ischemic stroke. Vasc Health Risk Manag. 10: 75-87.
10. Cramer S.C., Chopp M., 2000. Recovery recapitulates ontogeny. Trends Neurosci. 23(6):265-71.
11. NIDCD. Aphasia. 2015 2015-08-18; Available from: https://www.ncbi.nlm.nih.gov/pubmed/

12. Slujitoru A.S., Enache A.L., Pintea I.L., Rolea E., Stocheci C.M., Pop O.T., Predescu A., 2012. Clinical and morphological correlations in acute ischemic stroke. Rom J Morphol Embryol. 53(4): 917-26.
13. Musuka T.D., Wilton S.B., Traboulsi M., Hill M.D., 2015. Diagnosis and management of acute ischemic stroke: speed is critical. CMAJ. 187(12):887-93.
14. Broughton B.R., Reutens D.C., Sobey C.G., 2009. Apoptotic mechanisms after cerebral ischemia. Stroke. 40(5): e331-9.
15. Woodruff T.M., Thundyil J., Tang S.C., Sobey C.G., Taylor, S.M., Arumugam T.V., 2011. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener. 6(1):11.
16. Gelderblom M., Leypoldt F., Steinbach K., Behrens D., Choe C.U., Siler D.A., Arumugam T.V., Orthey E.,Gerloff C., Tolosa E., Magnus T., 2009. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. Stroke. 40(5):1849-57.
17. Suh S.W., Shin B.S., Ma H., Hoecke M.V., Brennan A.M., Yenari M.A., Swanson R.A., 2008. Glucose and NADPH oxidase drive neuronal superoxide formation in stroke. Ann. Neurol. 64(6):654-63.
18. Qureshi A.I., Ali Z., Suri M.F., Shuaib A., Baker G., Todd K., Guterman L.R., Hopkins L.N., 2003. Extracellular glutamate and other amino acids in experimental intracerebral hemorrhage: An in vivo microdialysis study. Crit. Care Med. 31(5):1482-9.
19. Wang J., Fields J. Zhao C., Langer J., Thimmulappa R.K., Kensler T.W., Yamamoto M., Biswal S., Doré S., 2007. Role of Nrf2 in protection against intracerebral hemorrhage injury in mice. Free Radic Biol Med. 43(3):408-14.
20. Flaherty M.L., Woo D., Haverbusch M., Sekar P., Khoury J., Sauerbeck L., Moomaw C.J., Schneider A., Kissela B., Kleindorfer, D., Broderick J.P., 2005. Racial variations in location and risk of intracerebral hemorrhage. Stroke. 36(5):934-7.
21. Testai F.D., Aiyagari V., 2008. Acute hemorrhagic stroke pathophysiology and medical interventions: Blood pressure control, management of anticoagulant-associated brain hemorrhage and general management principles. Neurol Clin. 26(4):963-85.
22. Aronowski J., Zhao X., 2011. Molecular pathophysiology of cerebral hemorrhage: Secondary brain injury. Stroke. 42(6):1781-6.
23. Qin C.; Yang S., Chu Y.H., Zhang H., Pang X.W., Lian Chen, Zhou L.Q., Chen M., Tian D.S., Wang W.,2022. Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 7(1):215.
24. Chen B., Jin W., 2023. A comprehensive review of stroke-related signaling pathways and treatment in western medicine and traditional Chinese medicine. Front Neurosci. 7:17:1200061.
25. Eskandari S., Sajadimajd S., Alaei L., Soheilikhah Z., Derakhshankhah H., Bahrami G., 2021. Targeting Common Signaling Pathways for the Treatment of Stroke and Alzheimer's: a Comprehensive Review. Neurotox Res. 39(5):1589-1612.
26. Roger V.L., Go A.S., Lloyd-Jones D.M., Adams R.J., Berry J.D., Brown T.M., Carnethon M.R., Dai S., Simone G.D., Ford E.S., 2011. Heart disease and stroke statistics–2011 update: A report from the American Heart Association. Circulation. 123(4): e18-e209.
27. George M.G., Tong X., Kuklina E.V., Labarthe D.R., 2011. Trends in stroke hospitalizations and associated risk factors among children and young adults, 1995–2008. Ann Neurol. 70(5):713-21.
28. Kapral M.K., Fang J., Hill M.D., Silver F., Richards J., Jaigobin C., Cheung A.M., Investigators of the Registry of the Canadian Stroke Network, 2005. Sex differences in stroke care and outcomes: Results from the Registry of the Canadian Stroke Network. Stroke. 36(4):809-14.

29. Ferro J.M., Falcão I., Rodrigues G., Canhão P., Melo T.P., Oliveira V., Pinto A.N., Crespo M., Salgado A.V., 1996. Diagnosis of transient ischemic attack by the nonneurologist. A validation study. Stroke. 27(12):2225-9.
30. Easton J.D., Saver J.L., Albers G.W., Alberts M.J., Chaturvedi S., Feldmann E., Hatsukami T.S., Higashida R.T., Johnston S.C., Kidwell, C.S., Lutsep H.L., Miller E., Sacco R.L., 2009. Definition and evaluation of transient ischemic attack: A scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. Stroke. 2009, 40(6):2276-93.
31. Seshadri S., Beiser A., Pikula A., Himali J.J., Kelly-Hayes M., Debette S., DeStefano A.L., Romero J.R., Kase C.S., Wolf P.A., 2010. Parental occurrence of stroke and risk of stroke in their children: The Framingham study. Circulation. 121(11):1304-12.
32. Touzé E., Rothwell P.M., 2008. Sex differences in heritability of ischemic stroke: A systematic review and meta-analysis. Stroke. 39(1):16-23.
33. Matarin M., Brown W.M., Singleton A., Hardy J.A., Meschia J.F., 2008. For the ISGS Investigators. Whole genome analyses suggest ischemic stroke and heart disease share an association with polymorphisms on chromosome 9p21. Stroke. 39(5):1586-9.
34. O'Donnell M.J., Xavier D., Liu L., Zhang H., Chin S.L., Rao-Melacini P., Rangarajan S., Islam S., Pais P., McQueen M.J., Mondo C., Damasceno A., Lopez-Jaramillo P., Hankey G.J., Dans A.L., Yusoff K., Truelsen T., Diener H.C., Sacco R.L., Ryglewicz D., Czlonkowska A., Weimar C., Wang X., Yusuf S., 2010. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): A case-control study. Lancet. 376(9735):112-23.
35. Lewington S., Clarke R., Qizilbash N., Peto R., Collins R., Collaboration P.S., 2002. Age-specific relevance of usual blood pressure to vascular mortality: A meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 360(9349):1903-13.
36. Vermeer S.E., Sandee W., Algra A., Koudstaal P.J., Kappelle L.J., Dippel D.W., Dutch TIA Trial Study Group., 2006. Impaired glucose tolerance increases stroke risk in nondiabetic patients with transient ischemic attack or minor ischemic stroke. Stroke. 37(6):1413-7.
37. Banerjee C., Moon Y.P., Paik M.C., Rundek T., Mora-McLaughlin C., Vieira J.R., Sacco R.L., Elkind M.S.,2012. Duration of diabetes and risk of ischemic stroke: The Northern Manhattan Study. Stroke. 43(5):1212-7.
38. Romero J.R., Morris J., Pikula A., 2008. Stroke prevention: Modifying risk factors. Ther Adv Cardiovasc Dis. 2(4):287-303.
39. Iribarren C., Jacobs D.R., Sadler M., Claxton A.J., Sidney S., 1996. Low total serum cholesterol and intracerebral hemorrhagic stroke: Is the association confined to elderly men? The Kaiser Permanente Medical Care Program. Stroke. 27(11):1993-8.
40. Denti L., Cecchetti A., Annoni V., Merli M.F., Ablondi F., Valenti G., 2003. The role of lipid profile in determining the risk of ischemic stroke in the elderly: A case-control study. Arch Gerontol Geriatr. 37(1):51-62.
41. Iso H., Jacobs D.R., Wentworth D., Neaton J.D., Cohen J.D., 1989. Serum cholesterol levels and six-year mortality from stroke in 350,977 men screened for the multiple risk factor intervention trial. N Engl J Med. 320(14):904-10.
42. Gill J.S., Zezulka A.V., Shipley M.J., Gill S.K., Beevers D.G., 1986. Stroke and alcohol consumption. N Engl J Med. 315(17):1041-6.

43. Hillbom M., Numminen H., Juvela S., 1999. Recent heavy drinking of alcohol and embolic stroke. Stroke. 30(11):2307-12.
44. Klatsky A.L., Armstrong M.A., Friedman G.D., Sidney S., 2001. Alcohol drinking and risk of hospitalization for ischemic stroke. Am. J Cardiol. 88(6):703-6.
45. Bhat V.M., Cole J.W., Sorkin J.D., Wozniak M.A., Malarcher A.M., Giles W.H., Stern B.J., Kittner S.J., 2008. Dose-response relationship between cigarette smoking and risk of ischemic stroke in young women. Stroke. 39(9):2439-43.
46. Song Y.M., Cho H.J., 2008. Risk of stroke and myocardial infarction after reduction or cessation of cigarette smoking: A cohort study in korean men. Stroke. 39(9):2432-8.
47. Shinton R., Beevers G., 1989. Meta-analysis of relation between cigarette smoking and stroke. BMJ. 298(6676):789-94.
48. Zhou M.L., Zhu L., Wang J., Hang C.H., Shi J.X., 2007. The inflammation in the gut after experimental subarachnoid hemorrhage. J Surg Res. 137(1):103-8.
49. Manson J.E., Colditz G.A., Stampfer M.J., Willett W.C., Krolewski A.S., Rosner B., Arky R.A., Speizer F.E., Hennekens C.H., 1991. A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. Arch Intern Med. 151(6):1141-7.
50. Larsson S.C., Orsini N., Wolk A., 2011. Dietary potassium intake and risk of stroke: A dose-response meta-analysis of prospective studies. Stroke. 42(10):2746-50.
51. Estruch R., Ros E., Martínez-González M.A., 2013. Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med. 369(7):676-7.
52. Appel L.J., Brands M.W., Daniels S.R., Karanja N., Elmer P.J., Sacks F.M., Association A.H., 2006. Dietary approaches to prevent and treat hypertension: A scientific statement from the American Heart Association. Hypertension. 47(2):296-308.
53. Li X.Y., Cai X.L., Bian P.D., Hu L.R., 2012. High salt intake and stroke: Meta-analysis of the epidemiologic evidence. CNS Neurosci Ther. 18(8):691-701.
54. He J., Ogden L.G., Vupputuri S., Bazzano L.A., Loria C., Whelton P.K., 1999. Dietary sodium intake and subsequent risk of cardiovascular disease in overweight adults. JAMA. 282(21):2027-34.
55. Liu S., Zhen G., Meloni B.P., Campbell K., Winn H.R., 2009. Rodent stroke model guidelines for preclinical stroke trials (1st edition). J Exp Stroke Transl Med. 2(2):2-27.
56. VE, O.C.; GA, D.; MR, M.; DW, H. Animal models of stroke versus clinical stroke: Comparison of infarct size, cause, location, study design, and efficacy of experimental therapies. In Animal Models for the Study of Human Disease; Michael Conn, P., Ed.; Academic Press: Waltham, MA, USA, 2013; pp. 531–568.
57. Connolly E.S., Winfree C.J., Stern D.M., Solomon R.A., Pinsky D.J., 1996. Procedural and strain-related variables significantly affect outcome in a murine model of focal cerebral ischemia. Neurosurgery. 38(3):523-31
58. Popa-Wagner A., Schröder E., Schmoll H., Walker L.C., Kessler C., 1999. Upregulation of MAP1B and MAP2 in the rat brain after middle cerebral artery occlusion: Effect of age. J Cereb Blood Flow Metab. 19(4):425-34.
59. Sugimori H., Yao H., Ooboshi H., Ibayashi S., Iida M., 2004. Krypton laser-induced photothrombotic distal middle cerebral artery occlusion without craniectomy in mice. Brain Res Brain Res Protoc. 13(3):189-96.
60. McAuley M.A., 1995. Rodent models of focal ischemia. Cerebrovasc Brain Metab Rev. 7(2):153-80.
61. Watson B.D., Dietrich W.D., Busto R., Wachtel M.S., Ginsberg M.D., 1985. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol. 17(5):497-504.

62. Robinson M.J., Macrae I.M., Todd M., Reid J.L., McCulloch J., 1990. Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. Neurosci. Lett. 118(2):269-72.
63. Biernaskie J., Corbett D., Peeling J., Wells J., Lei H., 2001. A serial MR study of cerebral blood flow changes and lesion development following endothelin-1-induced ischemia in rats. Magn Reson Med. 46(4):827-30.
64. Hossmann K.A., 2008. Cerebral ischemia: Models, methods and outcomes. Neuropharmacology. 55(3):257-70.
65. Gerriets T., Li F., Silva M.D., Meng X., Brevard M., Sotak C.H., Fisher M., 2003. The macrosphere model: Evaluation of a new stroke model for permanent middle cerebral artery occlusion in rats. J Neurosci Methods. 122(2):201-11.
66. Overgaard K., Sereghy T., Boysen G., Pedersen H., Høyer S., Diemer N.H., 1992. A rat model of reproducible cerebral infarction using thrombotic blood clot emboli. J Cereb Blood Flow Metab. 12(3):484-90.
67. Mosca A., Leclerc M., Hugot J.P., 2016. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? Front Microbiol. 31:7:455.
68. Quigley E.M.M., 2013. Gut Bacteria in Health and Disease. Gastroenterol Hepatol. 9(9):560-9.
69. Borre Y.E., O'Keeffe G.W., Clarke G., Stanton C., Dinan T.G., Cryan J.F., 2014. Microbiota and Neurodevelopmental Windows: Implications for Brain Disorders. Trends Mol Med. 20(9):509-18.
70. Donaldson G.P., Lee S.M., Mazmanian S.K., 2016. Gut Biogeography of the Bacterial Microbiota. Nat Rev Microbiol. 14(1):20-32.
71. Sender R., Fuchs S., Milo R., 2016. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 14(8):e1002533.
72. Carabotti M., Scirocco A., Maselli M.A., Severi C., 2015. The Gut-Brain Axis: Interactions between Enteric Microbiota, Central and Enteric Nervous Systems. Ann Gastroenterol. 28(2):203-209.
73. Grenham S., Clarke G., Cryan J.F., Dinan T.G., 2011. Brain–Gut–Microbe Communication in Health and Disease. Front Physiol. 7:2:94.
74. Vogt N.M., Kerby R.L., Dill-McFarland K.A., Harding S.J., Merluzzi A.P., Johnson S.C., Carlsson C.M., Asthana S., Zetterberg H., Blennow K., Bendlin B.B., Rey F.E., 2017. Gut Microbiome Alterations in Alzheimer's Disease. Sci Rep. 7(1):13537.
75. Bauer K.C., Huus K.E.; Finlay B.B., 2016. Microbes and the Mind: Emerging Hallmarks of the Gut Microbiota-Brain Axis. Cell Microbiol. 18(5):632-44.
76. Sampson T.R., Mazmanian S.K., 2015. Control of Brain Development, Function, and Behavior by the Microbiome. Cell Host Microbe. 17(5):565-76.
77. Dinan T.G., Cryan J.F., 2015. The Impact of Gut Microbiota on Brain and Behaviour: Implications for Psychiatry. Curr Opin Clin Nutr Metab Care. 18(6):552-8.
78. Sartor R.B., 2008. Microbial Influences in Inflammatory Bowel Diseases. Gastroenterology. 134(2):577-94.
79. Benakis C., Brea D., Caballero S., Faraco G., Moore J., Murphy M., Sita G., Racchumi G., Ling L., Pamer E.G., Iadecola C., Anrather J., 2016. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat Med. 22(5):516-23.
80. Ballway J.W., Song B.J., 2021. Translational Approaches with Antioxidant Phytochemicals against Alcohol-Mediated Oxidative Stress, Gut Dysbiosis, Intestinal Barrier Dysfunction, and Fatty Liver Disease. Antioxidants. 10(3):384.
81. Chidambaram S.B., Essa M.M., Rathipriya A.G., Bishir M., Ray B., Mahalakshmi A.M., Tousif A.H., Sakharkar M.K., Kashyap R.S., Friedland R.P., Monaghan T.M., 2022. Gut

Dysbiosis, Defective Autophagy and Altered Immune Responses in Neurodegenerative Diseases: Tales of a Vicious Cycle. Pharmacol Ther. 231:107988.
82. Samuelson D.R., Gu M., Shellito J.E., Molina P.E., Taylor C.M., Luo M., Welsh D.A., 2019. Intestinal Microbial Products from Alcohol-Fed Mice Contribute to Intestinal Permeability and Peripheral Immune Activation. Alcohol Clin Exp Res. 43(10):2122-2133.
83. Mayer E.A., Tillisch K., 2011. The Brain-Gut Axis in Abdominal Pain Syndromes. Annu Rev Med. 62:381-96.
84. Rhee S.H., Pothoulakis C., Mayer E.A., 2009. Principles and Clinical Implications of the Brain-Gut-Enteric Microbiota Axis. Nat Rev Gastroenterol Hepatol. 6(5):306-14.
85. Cryan J.F., Dinan T.G., 2012. Mind-Altering Microorganisms: The Impact of the Gut Microbiota on Brain and Behaviour. Nat Rev Neurosci. 13(10):701-12.
86. Cryan J.F., O'Mahony S.M., 2011. The Microbiome-Gut-Brain Axis: From Bowel to Behavior. Neurogastroenterol Motil. 23(3):187-92.
87. Principi N., Esposito S., 2016. Gut Microbiota and Central Nervous System Development. J Infect. 73(6):536-546.
88. Scott K.P., Jean-Michel A., Midtvedt T., van Hemert S., 2015. Manipulating the Gut Microbiota to Maintain Health and Treat Disease. Microb Ecol Health Dis. 2:26:25877.
89. Chidambaram S.B, Rathipriya A.G., Mahalakshmi A.M., Sharma S., Hediyal T.A., Ray B., Sunanda T., Rungratanawanich W., Kashyap R.S., Qoronfleh M.W., Essa M.M., Song B.J., Monaghan T.M., 2022. The influence of gut dysbiosis in the pathogenesis and management of ischemic stroke. Cells. 11(7):1239.
90. González-Fernández M., Ottenstein L., Atanelov L., Christian A.B., 2013. Dysphagia after stroke: an overview. Curr Phys Med Rehabil Rep. 1(3):187-196.
91. Cani P.D., Bibiloni R., Knauf C., Waget A., Neyrinck A.M., Delzenne N.M., Burcelin R., 2008. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet-Induced Obesity and Diabetes in Mice. Diabetes. 57(6):1470-81.
92. Kawai T., Akira S., 2010. The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-like Receptors. Nat Immunol. 11(5):373-84.
93. Neves A.L., Coelho J., Couto L., Leite-Moreira A., Roncon-Albuquerque R., 2013. Metabolic Endotoxemia: A Molecular Link between Obesity and Cardiovascular Risk. J Mol Endocrinol. 51(2): R51-64.
94. Singh V., Roth S., Llovera G., Sadler R., Garzetti D., Stecher B., Dichgans M., Liesz A., 2016. Microbiota Dysbiosis Controls the Neuroinflammatory Response after Stroke. J Neurosci. 36(28):7428-40.
95. Tuz A.A., Hasenberg A., Hermann D.M., Gunzer M., Singh V., 2022. Ischemic stroke and concomitant gastrointestinal complications- a fatal combination for patient recovery. Front Immunol. 10:13:1037330.
96. Benakis C., Liesz A., 2022. The gut-brain axis in ischemic stroke: its relevance in pathology and as a therapeutic target. Neurol Res Pract. 14;4(1):57.
97. Yadav M.K., Kumari I., Singh B., Sharma K.K., Tiwari S.K., 2022. Probiotics, prebiotics and synbiotics: safe options for next-generation therapeutics. Appl Microbiol Biotechnol. 106(2):505-521.
98. Markowiak P., Śliżewska K., 2017. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 15;9(9):1021.
99. Choi H.H., Cho Y.S., 2016. Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. Clin Endosc. 49(3):257-65.
100. Baunwall S.M.D., Lee M.M., Eriksen M.K., Mullish B.H., Marchesi J.R., Dahlerup J.F., Hvas C.L., 2020. Faecal microbiota transplantation for recurrent Clostridioides

difficile infection: An updated systematic review and meta-analysis. EClinicalMedicine. 23:29-30:100642.

101. Khodaparast N., Kilgard M.P., Casavant R., Ruiz A., Qureshi I., Ganzer P.D., Rennaker 2nd R.L., Hays S.A., 2016. Vagus nerve stimulation during rehabilitative training improves forelimb recovery after chronic ischemic stroke in rats. Neurorehabil Neural Repair. 30(7):676-84.

102. Zhang C., Xue P., Zhang H., Tan C., Zhao S., Li X., Sun L., Zheng H., Wang J., Zhang B., Weiya Lang, 2022. Gut brain interaction theory reveals gut microbiota mediated neurogenesis and traditional Chinese medicine research strategies. Front Cell Infect Microbiol. 8:12:1072341.

103. Brown J.M., Hazen S.L., 2017. Targeting of microbe-derived metabolites to improve human health: the next frontier for drug discovery. J Biol Chem. 292(21):8560-8568.