با نام خدا

Microbiota in Health and Disease

Narges Hosseinmardi, Ph.D.
SBMU

- Introduction to the human gut microbiota
- Microbiota in Health and Disease
- Microbiota in Disease

Introduction to the human gut microbiota

- The human (GI) tract: one of the largest interfaces (250–400 m2) between the host, environmental factors and antigens in the human body.
- In life time, 60 tonnes of food pass through the human GI tract, along with an abundance of microorganisms from the environment which impose a huge threat on gut integrity .
- The collection of bacteria, archaea and eukarya colonising the GI tract is termed the '**gut microbiota**'
- **Gut microbiota** has co-evolved with the host over thousands of years to form an intricate and mutually beneficial relationship.

- The number of microorganisms inhabiting the GI tract: **exceed** 10^{14}

- Which encompasses ~10 times more bacterial cells than the number of human cells and over 100 times the amount of genomic content (microbiome) as the human genome.

- The ratio of human:bacterial cells is actually closer to 1:1.

- As a result of the vast number of bacterial cells in the body, the host and the microorganisms inhabiting it are often referred to as a '**superorganism**.

- Many benefits of microbiota to the host:

  - Strengthening gut integrity or shaping the intestinal epithelium,
  - Harvesting energy,
  - Protecting against pathogens
  - Regulating host immunity

- There is potential for these mechanisms to be disrupted as a result of an altered microbial composition, known as **dysbiosis**.

- Role for the microbiota in a large number of intestinal and extra-intestinal diseases has become steadily apparent.

Composition of the human GI microbiota

- 2172 species isolated from human beings, classified into 12 different phyla

- 93.5% belonged to
  - Proteobacteria,
  - Firmicutes,
  - Actinobacteria
  - Bacteroidetes

- In humans, 386 of the identified species are strictly anaerobic and be found in mucosal regions such as the oral cavity and the GI tract.

- Study identified the presence of country-specific microbial signatures
- So, gut microbiota composition is shaped by environmental factors, such as diet, and possibly also by host genetics.
- Microbiotas that differ in terms of composition may share some degree of functional redundancy, yielding similar protein or metabolite profiles

Development of the human GI microbiota

- Begin from birth,
- Microbes were detected in womb tissues, such as the placenta .
- After birth, chaotic shifts in the microbiota.
- The mode of delivery affect the microbiota composition
- Vaginally delivered infants: high abundance of lactobacilli during the first few days.
- Microbiota of infants delivered by C-section is depleted and delayed in the colonisation of the Bacteroides genus, but colonised by facultative anaerobes such as Clostridium species.
- Faecal microbiota of 72% of vaginally delivered infants resembles that of their mothers'
- Faecal microbiota, in babies delivered by C-section, this percentage is reduced to only 41% .

- In early stages of development, the microbiota is low in diversity
- Dominated by two main phyla, Actinobacteria and Proteobacteria.
- During the first year of life, the microbial diversity increases
- Microbiota composition converges towards a distinct adult-like microbial profile
- By around 2.5 years of age, the composition, diversity and functional capabilities of the infant microbiota resemble those of adult microbiota
- Although, in adulthood, the composition of the gut microbiota is relatively stable, it is still subject to perturbation by life events.

- Over the age of 65, the microbial community shifts, with an increased abundance of Bacteroidetes phyla and Clostridium cluster IV
- Diversity of the microbiota of centenarians was significantly reduced
- In the elderly population, a significant relationship has been identified between diversity and living arrangements
- Capacity of the microbiota to carry out metabolic processes such as short-chain fatty acid (SCFA) production and amylolysis is reduced in the elderly, whilst proteolytic activity is increased.
- Given the increasing evidence for the role of SCFAs as key metabolic and immune mediators, it was postulated that the decrease in SCFAs may nurture the inflamm-ageing process in the intestine of aged people

Biogeography of the human microbiota in the GI tract

- Microbiota composition in the GI tract is reflective of the physiological properties in a given region and is stratified on both a transverse and longitudinal axis.
- The density and composition of the microbiota are affected by chemical, nutritional and immunological gradients along the gut.
- In the small intestine, there are high levels of acids, oxygen and antimicrobials, and a short transit time.
- These properties limit bacterial growth, such that only rapidly growing, facultative anaerobes with the ability to adhere to epithelia/ mucus are thought to survive .
- In mice, the small-intestine microbial community is largely dominated by Lactobacillaceae.
- In contrast, colonic conditions support a dense and diverse community of bacteria, mainly anaerobes with the ability to utilise complex carbohydrates which are undigested in the small intestine.
- In the colon Prevotellaceae, Lachnospiraceae and Rikenellaceae have been shown to dominate.

- In contrast with the differing microbiota composition between varying GI organs, the microbiota of different colorectal mucosal regions within the same individual is spatially conserved in terms of both composition and diversity.

- This feature is apparent even during periods of localised inflammation.

- On the other hand, the faecal/luminal and mucosal compositions are significantly different .

- outer mucus of the large intestine forms a unique microbial niche and that bacterial species present in the mucus show differential proliferation and resource utilisation compared with the same species in the intestinal lumen.

- These observations highlight the need for careful consideration in choosing a sampling method when analysing the microbiota composition.

Factors shaping the GI microbiota

- The microbiota composition is subject to shaping by host and environmental selective pressures.
- To protect from injury and maintain homeostasis, the GI tract limits exposure of the host immune system to the microbiota by recruitment of a multifactorial and dynamic intestinal barrier.
- The barrier comprises several integrated components including physical (the epithelial and mucus layers), biochemical (enzymes and antimicrobial proteins) and immunological (IgA and epithelia-associated immune cells) factors .
- An individual microbe's longevity is determined by whether it is contributing to the range of essential functions on which host fitness relies.

- Gut microbes must be adapted to a certain type of lifestyle due to the relatively fewer number of biochemical niches available in the gut, compared with other microbial-rich environments.

- In the gut, energy can generally be derived through processes such as fermentation and sulphate reduction of dietary and host carbohydrates.

- The organisms that can survive in the gut are therefore limited by their phenotypic traits.

- Current research suggests that **diet** exerts a large effect on the gut microbiota.

- Ileal microbiota is driven by the capacity of the microbial members to metabolise simple sugars, reflecting adaptation of the microbiota to the nutrient availability in the small intestine.

- Shaping of the colonic microbiota is subject to the availability of microbiota-accessible carbohydrates (MACs) that are found in dietary fibre.

- Extreme 'animal-based' or 'plant-based' diets result in wide-ranging alterations of the gut microbiota in humans.

- Diets high in resistant starch or in non-starch polysaccharide fibre (wheat bran) resulted in the strong and reproducible enrichment of different bacterial species in the human gut.

- **Feeding methods** can also affect the abundance of some bacterial groups in the gut microbiota of infants.

- For example, fucosylated oligosaccharides present in human milk can be utilised by Bifidobacterium longum and several species of Bacteroides allowing them to outcompete other bacteria such as E. coli and Clostridium perfringens.

- Whilst the abundance of Bifidobacterium spp. in breast-fed infant microbiota is typically high, this is reduced in formula-fed infants.

- Furthermore, formula-fed infant microbiota has an increased diversity and altered levels of other groups such as E. coli, Clostridium difficile, Bacteroides fragilis and lactobacilli.

- The microbiota of undernourished infants is immature, dysbiotic and contains greater numbers of enteropathogens, such as Enterobacteriaceae.

- Infants from rural Africa, with a diet dominated by starch, fibre and plant polysaccharides, harbour a microbiota that is abundant in the Actinobacteria (10.1%) and Bacteroidetes (57.7%) phyla.

- In contrast, in European children, whose diet is rich in sugar, starch and animal protein, the abundance of these groups is reduced to just 6.7 and 22.4%.

- Some SCFA producers, such as Prevotella, were exclusive to the microbiota of African children.

- This trend was also apparent in healthy individuals consuming high amounts of carbohydrates and simple sugars.

- A decreased SCFA output is also evident in individuals consuming a low MAC diet, a notable effect since SCFAs play an important role in host health via, for example, anti-inflammatory mechanisms.
- The abundance of MACs is substantially reduced in the Western diet.
- Administration of a low MAC diet to mice results in a reduction in microbial diversity.
- Restoration of diversity requires administration of MACs
- Certain microbial species can be used to restore growth impairments
- Using these species as a therapeutic intervention to counteract the negative effects of undernutrition.

- **Intestinal mucus** also provides a source of carbohydrates to the gut microbiota.
- The intestinal mucus layers are built around the large highly glycosylated gel-forming mucin MUC2 (Muc2 in mouse) secreted by goblet cells.
- The glycan structures present in mucins are diverse and complex and account for up to 80% of the total molecular mass of Muc2/MUC2.
- Mucus is present throughout the GI tract and is thickest in the colon where it is crucial in mediating the host–microbiota relationship.
- Mucus and mucin glycosylation are therefore key in shaping the microbiota and allow for the selection of the most optimal microbial species to mediate host health.
- A depletion of MACs from the diet of mice can result in thinner mucus in the distal colon, increased proximity of microbes to the epithelium and heightened expression of the inflammatory marker REGIIIβ.
- The microbiota can also be shaped by the **host immune system**.

- **Several environmental factors** have been implicated in shaping the microbiota including
  - geographical location,
  - surgery,
  - smoking,
  - depression
  - living arrangements (urban or rural).

- Xenobiotics, such as antibiotics, shape the physiology and gene expression of the active human gut microbiome.

- Antibiotic treatment dramatically disrupts both short- and long-term microbial balance, including decreases in the richness and diversity of the community.

- Clindamycin, clarithromycin and metronidazole, and ciproflaxin have all been demonstrated to affect the microbiota structure for varying lengths of time.

- The exact effects and the time for recovery of the microbiota following antibiotic administration appear to be individual-dependent, a likely effect of the inter-individual variation in the microbiota prior to treatment.

Role of the GI microbiota in health

- Owing to its large genomic content and metabolic complement, the gut microbiota provides a range of beneficial properties to the host.

- Some of the most important roles of these microbes are to help to maintain the **integrity of the mucosal barrier**, to provide nutrients such as vitamins or to protect against pathogens.

- In addition, the interaction between commensal microbiota and the mucosal immune system is crucial for proper immune function.

- Colonic bacteria express carbohydrate-active enzymes, which endow them with the ability to ferment complex carbohydrates generating metabolites such as **SCFAs**.

- Three predominant SCFAs, propionate, butyrate and acetate, are typically found in a proportion of 1:1:3 in the GI tract.

- These SCFAs are rapidly absorbed by epithelial cells in the GI tract where they are involved in the regulation of cellular processes such as gene expression, chemotaxis, differentiation, proliferation and apoptosis.

- Acetate is produced by most gut anaerobes, whereas propionate and butyrate are produced by different subsets of gut bacteria following distinct molecular pathways.

- Propionate is primarily absorbed by the liver, whilst acetate is released into peripheral tissues.
- The role of SCFAs on human metabolism has recently been reviewed.
- Butyrate is known for its **anti-inflammatory and anticancer activities.**
- Butyrate is a particularly important **energy source for colonocytes** .
- A gradient of butyrate from lumen to crypt is suggested to control intestinal epithelial turnover and homeostasis by promoting colonocyte proliferation at the bottom of crypts, whilst increasing apoptosis and exfoliation of cells closer to the lumen.
- Butyrate can attenuate bacterial translocation and enhance gut barrier function by affecting tight-junction assembly and mucin synthesis.

- SCFAs also appear to regulate hepatic lipid and glucose homeostasis via complementary mechanisms.

- In the liver, propionate can activate gluconeogenesis, whilst acetate and butyrate are lipogenic.

- SCFAs also play a role in regulating the immune system and inflammatory response.
- They influence the production of cytokines, for example, stimulating the production of IL-18, an interleukin involved in maintaining and repairing epithelial integrity.
- Butyrate and propionate are histone deacetylase inhibitors that epigenetically regulate gene expression.
- SCFAs have also been shown to modulate appetite regulation and energy intake via receptor-mediated mechanisms,
- Propionate has beneficial effects in humans acting on β-cell function and attenuating reward-based eating behaviour via striatal pathways.
- Microbial metabolites other than SCFAs have been reported to have an impact on intestinal barrier functions, epithelium proliferation and the immune system.

- The GI microbiota is also crucial to the de novo **synthesis of essential vitamins** which the host is incapable of producing.
- Lactic acid bacteria are key organisms in the production of vitamin B12, which cannot be synthesised by either animals, plants or fungi.
- Bifidobacteria are main producers of folate, a vitamin involved in vital host metabolic processes including DNA synthesis and repair.
- Further vitamins, which gut microbiota have been shown to synthesise in humans, include vitamin K, riboflavin, biotin, nicotinic acid, panthotenic acid, pyridoxine and thiamine.
- Colonic bacteria can also metabolise bile acids that are not reabsorbed for biotransformation to secondary bile acids.
- All of these factors will influence host health.
- For example, an alteration of the co-metabolism of bile acids, branched fatty acids, choline, vitamins (i.e. niacin), purines and phenolic compounds has been associated with the development of metabolic diseases such as obesity and type 2 diabetes.

- There are many lines of evidence in support of a role for the gut microbiota in influencing epithelial homeostasis.
- A role has been demonstrated for bacteria in promoting cell renewal and wound healing
- Furthermore, several species have been implicated in promoting epithelial integrity
- In addition to modulating epithelial properties, bacteria are proposed to modulate mucus properties and turnover.

- The GI microbiota is also important for the development of both the intestinal mucosal and systemic immune system as demonstrated by the deficiency in several immune cell types and lymphoid structures exhibited by germ-free animals.

- A major immune deficiency exhibited by germ-free animals is the lack of expansion of CD4+ T-cell populations.

- A. muciniphila has been correlated with protection against several inflammatory diseases, suggesting that this strain possesses anti-inflammatory properties although the underlying mechanisms have not been completely elucidated.

- The physical presence of the microbiota in the GI tract also influences pathogen colonisation by, for example, competing for attachment sites or nutrient sources, and by producing antimicrobial substances.

Conclusion

- Given the close symbiotic relationship existing between the gut microbiota and the host, it is not surprising to observe a divergence from the normal microbiota composition (generally referred to as dysbiosis) in a plethora of disease states ranging from chronic GI diseases to neurodevelopmental disorders.

- At a functional level, a potential way to describe a 'dysbiotic microbiota' might be one which fails to provide the host with the full complement of beneficial properties.

- Whether dysbiosis of the microbiota is a cause or a consequence of the disease is therefore likely to exacerbate the progression of the disease and affect the type of strategies needed to restore symbiosis.

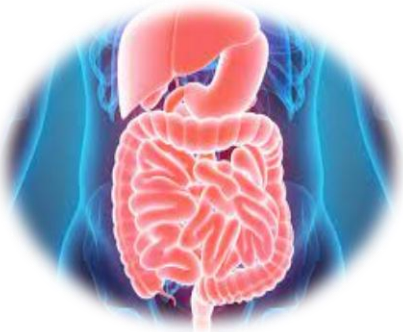
Role and Mechanism of Gut Microbiota in Human Disease

- The human gut microbiota (GM) is considered as the “essential organ” of human body.
- As the largest micro-ecosystem in the human body, GM is symbiotic with the host and maintains normal physiological processes in a dynamic equilibrium state.
- Firmicutes/Bacteroidetes ratio is an important parameter to reflect GM disorder.
- In addition, the abundance, diversity and evenness of GM are also important indicators to reflect the composition of intestinal flora.

Once the GM disorder occurs, the structure and function of the intestinal flora will change and even cause the occurrence or development of some diseases.

A great deal of research has produced evidence that **GM disorder** and its **metabolites** play a key role in maintaining host intestinal homeostasis and influencing the development of many diseases, including:

- neurodegenerative diseases
- cardiovascular diseases
- metabolic diseases
- gastrointestinal diseases



- The imbalance of GM will affect the health of the host through many ways, such as
  - energy absorption,
  - choline,
  - short chain fatty acids (SCFAs),
  - gut–brain axis,
  - bile acids (BAs), and so on.

- However, the mechanism of GM on human diseases has not yet been completely elucidated.

GM AND NEURODEGENERATIVE DISEASES

- An increasing number of studies suggested that GM can modulate nervous, endocrine, and immune communication through the gut-brain axis which takes part in the occurrence and development of central nervous system diseases, especially in Parkinson disease (PD) and Alzheimer disease (AD).

The gut microbiota–brain axis in behaviour and brain disorders

- Gut bacteria cooperate with their animal hosts to regulate the development and function of the immune, metabolic and nervous systems through dynamic bidirectional communication along the 'gut–brain axis'.

- These processes may affect human health, as certain animal behaviours appear to correlate with the composition of gut bacteria, and disruptions in microbial communities have been implicated in several neurological disorders.

The gut microbiota–brain axis

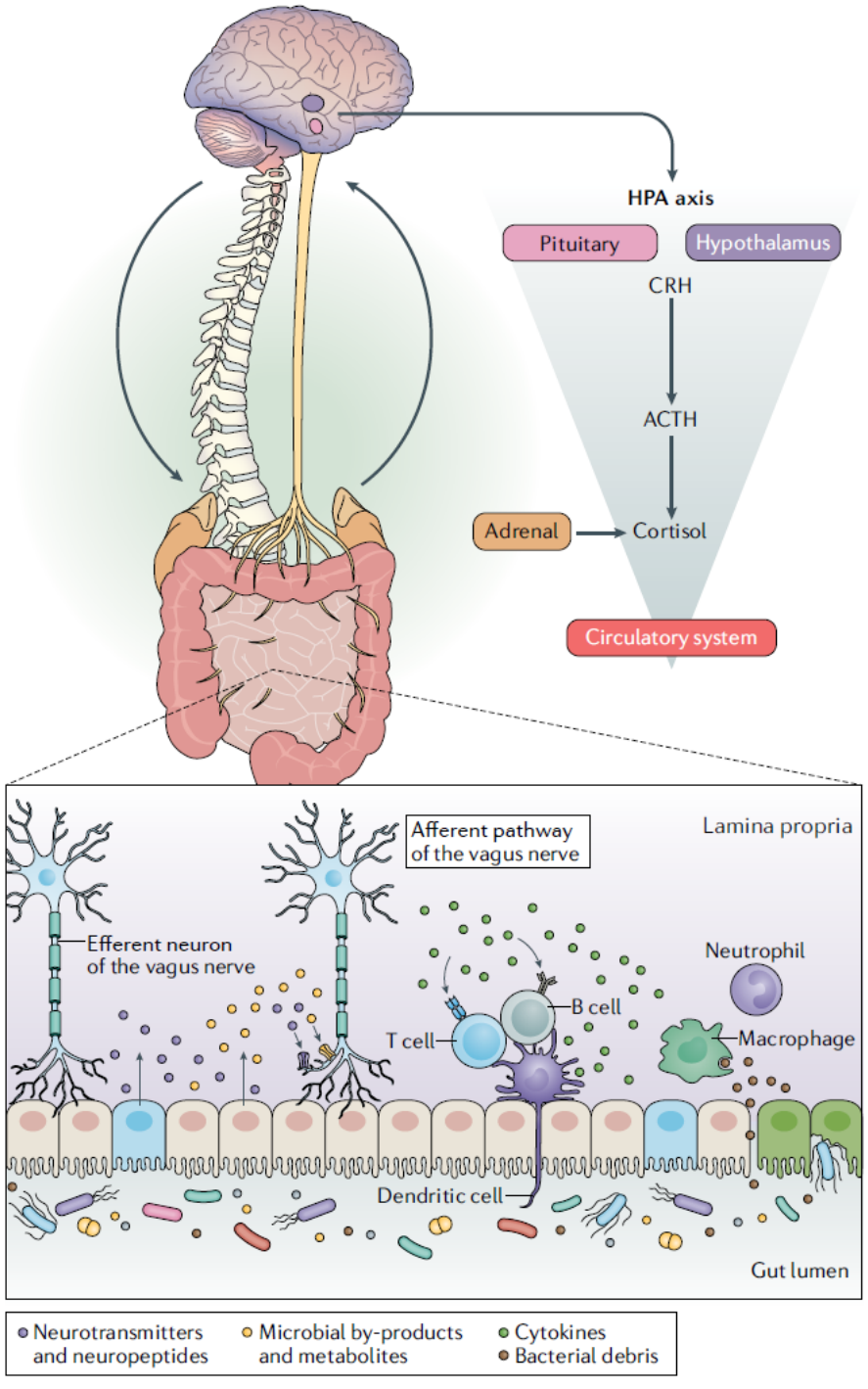
- how microorganisms influence the brain through their ability to produce and modify many metabolic, immunological and neurochemical factors in the gut that ultimately impact the nervous system

The ‘gut–microbiota–brain axis

- **Refers to the network of connections involving multiple biological systems that allows bidirectional communication between gut bacteria and the brain**

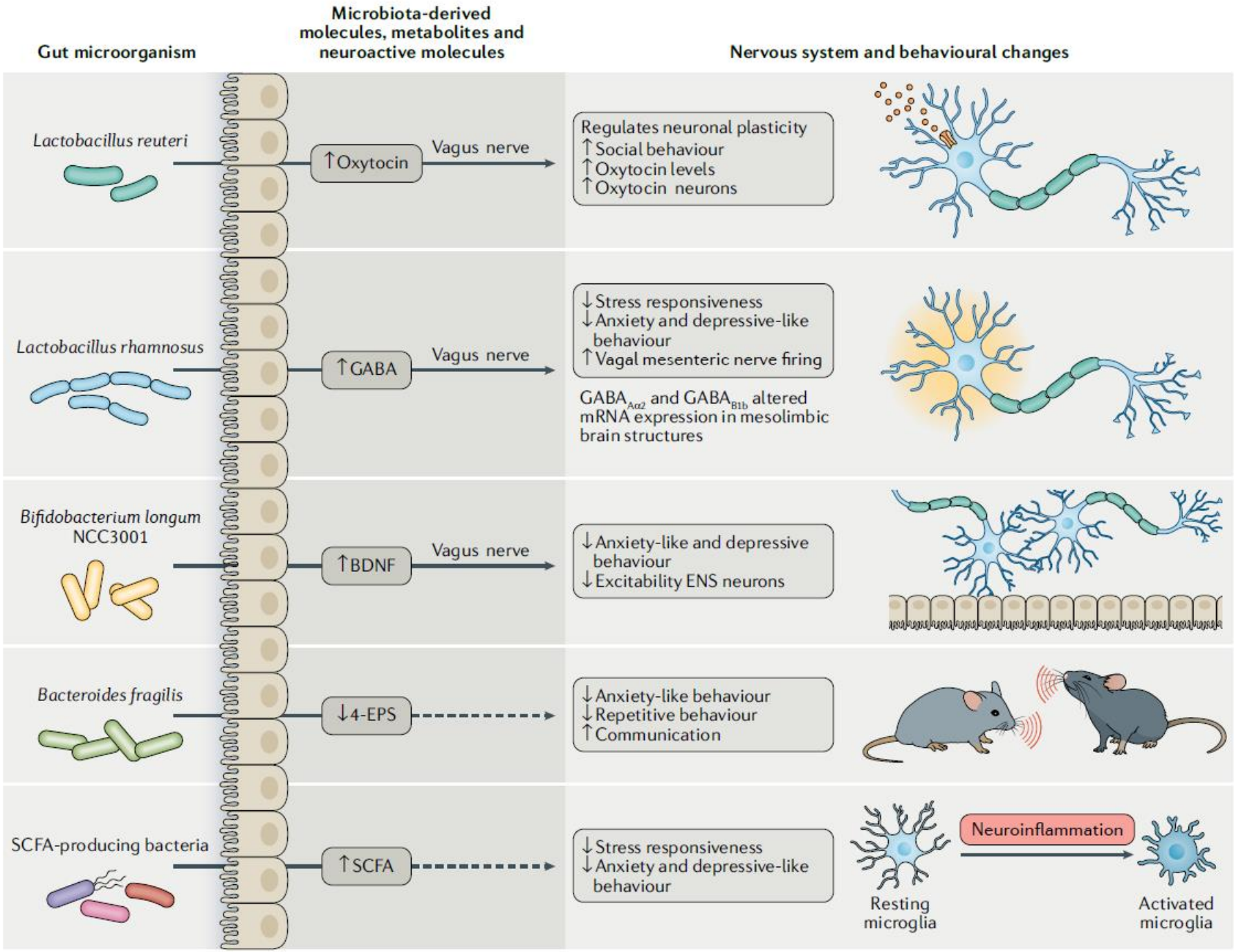
- is crucial in maintaining homeostasis of the gastrointestinal, central nervous and microbial systems of animals.

- The communication pathways in these biological networks include both direct and indirect signalling via chemical transmitters, neuronal pathways and the immune system.


HPA axis
Pituitary
Hypothalamus
CRH
ACTH
Adrenal
Cortisol
Circulatory system
Afferent pathway of the vagus nerve
Lamina propria
Efferent neuron of the vagus nerve
Neutrophil
B cell
T cell
Macrophage
Dendritic cell
Gut lumen
Neurotransmitters and neuropeptides
Microbial by-products and metabolites
Cytokines
Bacterial debris

Chemical signalling between the gut and the brain.

- The gut microbiota can help modulate homeostasis and behaviour in its animal host through chemical communication with the nervous system, including both ‘direct’ and ‘indirect’ signalling.


Gut microorganism
Microbiota-derived molecules, metabolites and neuroactive molecules
Nervous system and behavioural changes
Lactobacillus reuteri
↑Oxytocin
Vagus nerve
Regulates neuronal plasticity
↑Social behaviour
↑Oxytocin levels
↑Oxytocin neurons
Lactobacillus rhamnosus
↑GABA
Vagus nerve
↓Stress responsiveness
↓Anxiety and depressive-like behaviour
↑Vagal mesenteric nerve firing
GABA$_{A\alpha2}$ and GABA$_{B1b}$ altered mRNA expression in mesolimbic brain structures
Bifidobacterium longum NCC3001
↑BDNF
Vagus nerve
↓Anxiety-like and depressive behaviour
↓Excitability ENS neurons
Bacteroides fragilis
↓4-EPS
↓Anxiety-like behaviour
↓Repetitive behaviour
↑Communication
SCFA-producing bacteria
↑SCFA
↓Stress responsiveness
↓Anxiety and depressive-like behaviour
Neuroinflammation
Resting microglia
Activated microglia

Neuronal pathways for gut-brain interactions.

- Neuronal pathways physically link the gut and the brain.
- Chief amongst these neuronal pathways is the vagus nerve, which extends from the brainstem to innervate the gut and the enteric nervous system (ENS).
- Development and function of the ENS is partially mediated by the gut microbiota
- Neuronal innervation of the colonic epithelium is reduced in GF mice and restored by microbial colonization.
- In addition, gut microbiota regulate the development of enteric glial cells in mice, which are important for regulating gut homeostasis and maintenance of neuronal networks.
- The gut microbiota can affect the function of enteric neurons through chemical signalling,

- Microbial products including bacterial cell wall components, SCFAs and others have been shown to influence ENS activity and regulate gut motility in rodents

- The effects of microbial products on neurons can even extend to the brain via neuronal pathways.

- Furthermore, activation of this neuronal pathway, which helps mediate gut motility, can be suppressed by administration of SCFA producing gut microorganisms, supporting a role for the gut microbiota in regulation of gut motility.

- These findings demonstrate that intestinal bacteria can, through microbial metabolites, modulate neuronal pathways of the gut–brain axis.

- The gut microbiota also communicates with the brain via the vagus nerve, which is the most direct and well- studied pathway between the gut and the CNS.

- Vagus nerve fibres innervate the muscle and mucosa layers of the gastrointestinal tract, detect sensory signals and then relay these signals to the CNS.

- The transmission of signals from the peripheral ends of the vagus nerve to the CNS occurs though activation of mechanoreceptors that can sense luminal volume or chemoreceptors triggered by chemical stimuli such as hormones, neurotransmitters and metabolites produced by EECs, which may themselves be influenced by the gut microbiota.

- Vagus nerve efferent fibres propagate information from the brain to the viscera and have an important role in immune function and metabolism.

- These factors, in turn, may alter the gut microbiota by affecting the environment of the gut, which implicates the vagus nerve as an important mediator of bidirectional communication both to and from the brain.

- Microbiota–brain communication via the vagus nerve is also important in modulating host behaviour, as demonstrated in several studies using animal models.

Gut microbiota–brain signalling through the immune system

- Both the CNS and the gut microbiota directly affect, and are affected by, the immune system.
- The gut microbiota is a crucial factor in modulating the development and function of the peripheral immune system.
- The microbiota is also necessary for healthy development, maturation and activation of microglia, innate immune cells of the brain.
- gut microbiota may influence human neurological diseases through effect mediated by microglia

- The gut microbiota and the brain also interact through the systemic immune system via circulating cytokines.

- Changes to systemic immunity drive altered immune signalling within the brain and increased peripheral inflammation is found in many neuropsychiatric diseases, including depression, anxiety and …

- Cytokines and chemokines can either be produced by brain- resident immune cells or access the CNS through direct transport across the BBB.

- Importantly, there is evidence that the permeability of the BBB is influenced by the gut microbiota,

- Infections, autoimmune disease and injury can alter BBB integrity, thus increasing accessibility of the brain to microbial products in the circulatory system and sensitizing the brain to subsequent pathology.

- In fact, elevated BBB permeability is a defining feature of many neuropathological conditions, further highlighting the potential impact of connections between systemic immunity and outcomes in the brain.

The gut microbiota–brain axis in disease

- The composition of the gut microbiota in individuals with various neurological diseases is different relative to healthy individuals

- Studies have led to the characterization of putative probiotics that can ameliorate disease symptoms as well as the identification of bacteria and bacterial factors that influence disease progression in mice, providing a template for further investigations in humans.

- Importantly, communication along the gut microbiota–brain axis occurs throughout life, as can be seen in diseases of neurodevelopment (for example, ASD), neurodegeneration (for example, PD and AD) and behaviour (for example, depression and anxiety).

- what is currently known about the role of bacteria in neurological diseases and their cognate preclinical models.

Gut microbiota–brain axis in autism spectrum disorder.

- ASD is a group of neurodevelopmental disorders that manifest early in life,
- Symptoms of ASD are heterogeneous but are currently characterized by changes in behavioural domains such as social communication, social interaction and repetitive behaviours.
- Additionally, gastrointestinal dysfunction is more prevalent in individuals with ASD, including increased susceptibility to intestinal inflammation and altered gut permeability.
- Intriguingly, there is a positive correlation between the severity of behavioural and gastrointestinal symptoms, which suggests a link between the gut and the brain in neurodevelopment.

- Importantly, numerous studies report that the composition of the gut microbiota differs between individuals with ASD and neurotypical individuals.

- In addition, research in mice has shown that gut microorganisms are capable of influencing behaviours that are core features of ASD.

- the importance of the gut microbiota in the
- development of social behaviour, a key domain of ASD pathology.

- Recent research has also reported beneficial effects of faecal microbiota transplantation therapy for individuals with ASD.

- Animal models have been used to highlight the multiple pathways of communication between the gut microbiota and the brain, and show how chemical, neuronal and immune- based signalling can influence ASD- like phenotypes.

- Numerous observational human studies have shown altered metabolism in ASD, with different levels of urinary, blood and faecal metabolites
- compared with neurotypical controls.

Neurodegenerative disorders and the gut.

- PD is the second most common neurodegenerative disorder after AD, affecting 0.3% of individuals in the general population and more than 1% of the elderly population worldwide.
- PD is a progressive neurodegenerative disorder characterized by an inability to control voluntary movements due to profound changes in the functional organization of the substantia nigra and striatum regions of the brain.
- These changes include degeneration of dopaminergic neurons, aggregation of phosphorylated forms of the neuronal protein α- synuclein (αSyn), mitochondrial dysfunction, excessive reactive oxygen species and an increase in microglia activation.

- Intriguingly, gastrointestinal issues, primarily in the form of constipation, can occur in up to 80% of individuals with PD.

- The presence of gut inflammation, increased intestinal permeability and early accumulation of phosphorylated αSyn in the ENS and in the dorsal motor nucleus of the vagus nerve, a gateway between the gut and the brain, all suggest that PD pathology may start in the gut and reach the brain by navigating through neuronal pathways of the vagus nerve.

- Recent studies suggest a link between the gut microbiota and PD,
- as both the composition of the microbial community and the metabolic profile in the serum of individuals with PD are distinct from those of healthy individuals,
- with increasing levels of Enterobacteriaceae and a loss of gut microorganisms associated with antiinflammatory properties.
- Increased abundance in Enterobacteriaceae positively correlates with the severity of certain PD symptoms.
- Enterobacteriaceae are also associated with gut inflammation in Crohn's disease (a form of inflammatory bowel disease), and individuals with Crohn's disease are at increased risk for developing PD, whereas individuals with Crohn's disease who are treated with anti- inflammatory drugs are partially protected from PD
- Suggesting that inflammation in the gut could be a driver of PD pathology.
- Interestingly, PD- like symptoms can be exacerbated by gastrointestinal infection in mice.

AD

- The gut microbiota appears to have a role in other neurodegenerative diseases, namely AD, the leading cause of dementia worldwide.
- Shifts in the gut microbiota composition have been identified in individuals with AD, including a decreased abundance of Firmicutes and *Bifidobacterium* spp. and increased Bacteroidetes, *Escherichia* and *Shigella* spp.
- *Shigella* spp. have been associated with inflammation and increased expression of amyloid proteins in AD, in a manner that is very similar to PD.
- The role of the microbiota in AD pathogenesis has been studied in transgenic mice, which are used to model neuronal loss, cognitive deficits and immune alterations similar to human AD.
- Transgenic mice exhibit marked changes in the gut microbiota and metabolism of amino acids.

- Similarly, links between the gut microbiota, immune system and neurodegeneration have been reported in multiple sclerosis, an autoimmune demyelinating disease characterized by degeneration of neuronal signalling throughout an individual's lifetime.

- Thus, the gut microbiota may be a driver of disease pathogenesis across many neurodegenerative diseases.

Gut microbiota–brain axis in stress, depression and anxiety.

- Stress, depression and anxiety are highly co-morbid conditions and have overlapping biological mechanisms and manifestations.
- The connection between the brain and the gut microbiota in stress is bidirectional,
- as chronic stress is associated with lasting alterations in the composition and function of the gut microbiota,
- including a correlation between reduced *Lactobacillus* spp.

- Depression affects millions of people worldwide and is associated with neurological symptoms of cognitive dysfunction, anhedonia and despair.

- Major depressive disorder is associated with physiological changes throughout the body, including changes to gut epithelial permeability and increased systemic inflammation with elevated levels of C-reactive protein, interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF).

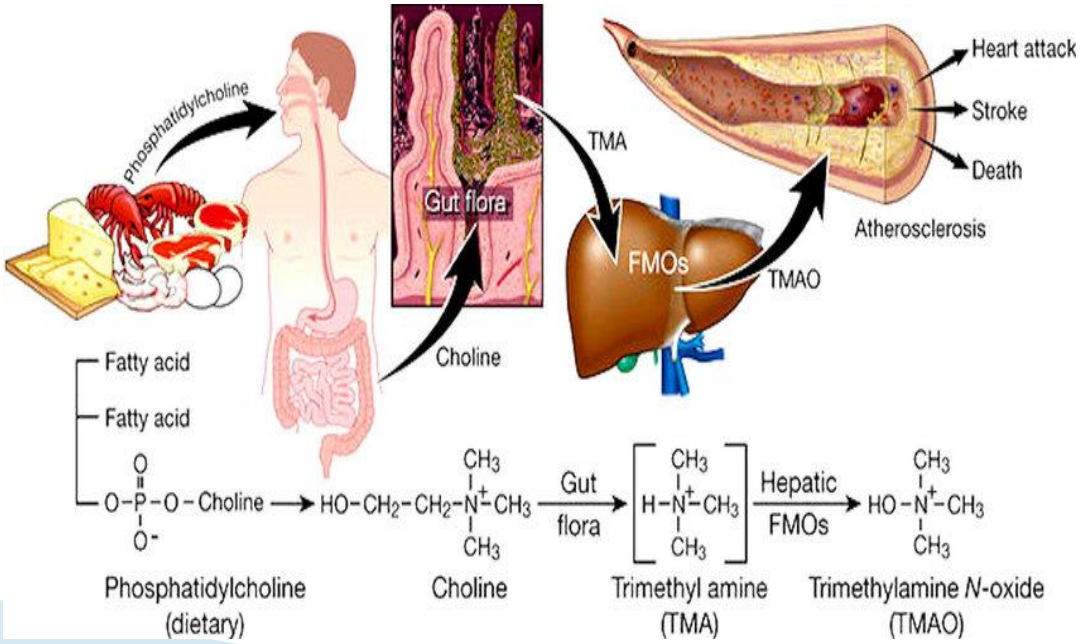
- Recent evidence has shown that individuals diagnosed with major depression disorder have altered gut bacterial species relative to healthy adults

- Increased likelihood of developing an anxiety disorder in individuals previously exposed to intestinal infection, implicating the gut microbiota as a potential ‘trigger’ for a subsequent anxiety disorder.

GM AND CARDIOVASCULAR DISEASES

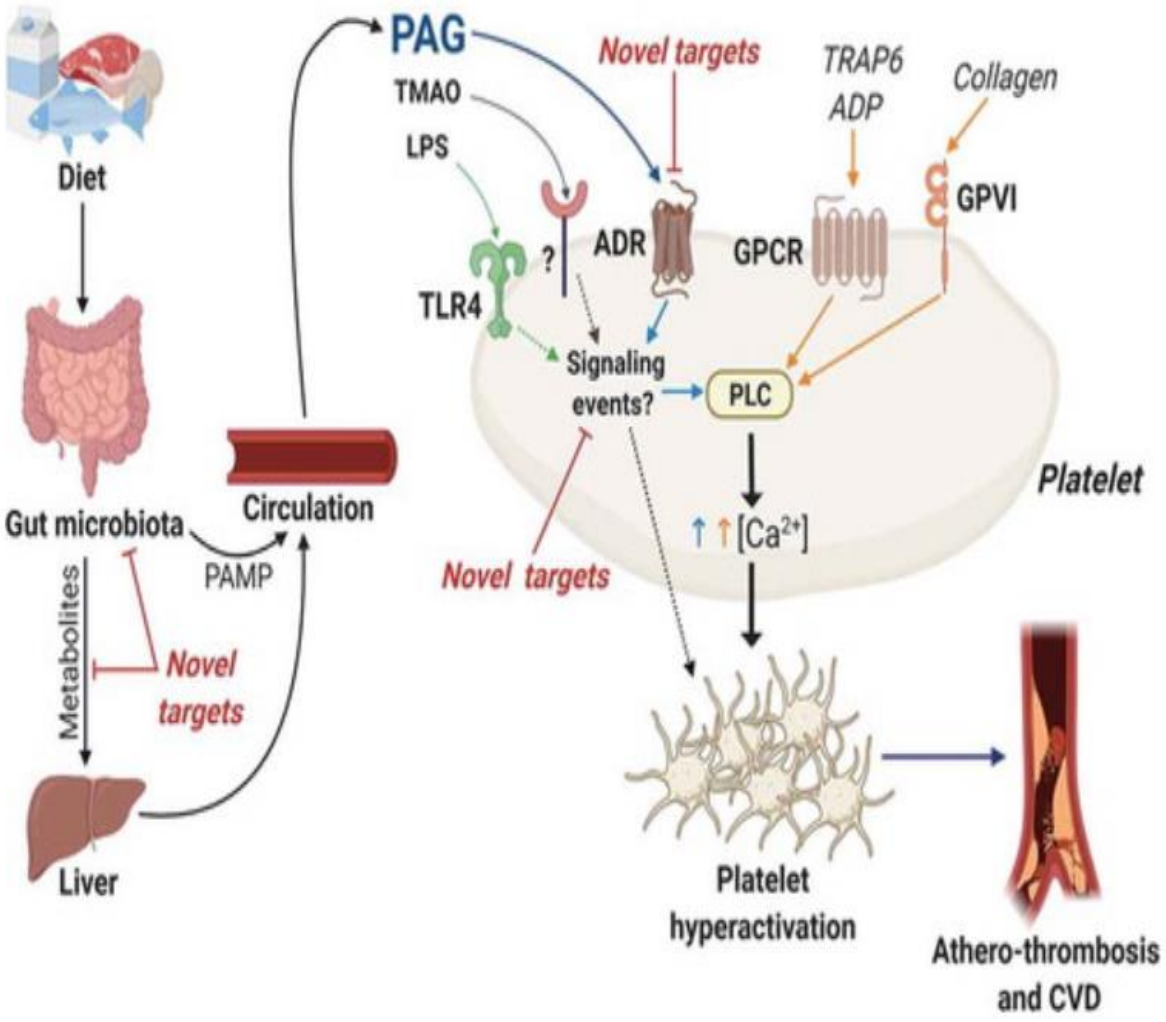
Cardiovascular diseases (CVD), such as **hypertension**, **atherosclerosis** and **heart failure**, are a major cause of death worldwide.

A growing number of studies have exposed that GM and its metabolic products interact with the host in many different ways to influence the development and occurrence of CVD. The role of Trimetlylamine oxide (TMAO), Bile acids, and SCFAs in CVD has been supported by a large number of studies, which are metabolic products of the gut microbiome.



➢ Hypertension

- ✓ Hypertension is one of the most common risk factors of CVD.
- ✓ Hypertension is associated with altered gut function, altered gut bacterial populations and changes in gut–nervous system connectivity.
- ✓ In hypertensive patients, microbial richness, diversity, and evenness were significantly decreased while the ratio of Firmicutes/ Bacteroidetes ratio is remarkably increased.
- ✓ The change of intestinal flora plays a key role in the regulation of blood pressure, and the change in the production of gut microbial metabolites may be a key mechanism.
- ✓ circulating (serum or plasma) TMAO concentration was significantly dose-dependent associated with hypertension risk and was associated with cardiovascular metabolic risk indicators such as HDL cholester.


Diet
Gut microbiota
Metabolites
Novel targets
Liver
PAMP
Circulation
PAG
TMAO
LPS
TLR4
?
ADR
Novel targets
Signaling events?
Novel targets
TRAP6
ADP
GPCR
Collagen
GPVI
PLC
↑ ↑ [Ca2+]
Platelet
Platelet hyperactivation
Athero-thrombosis and CVD

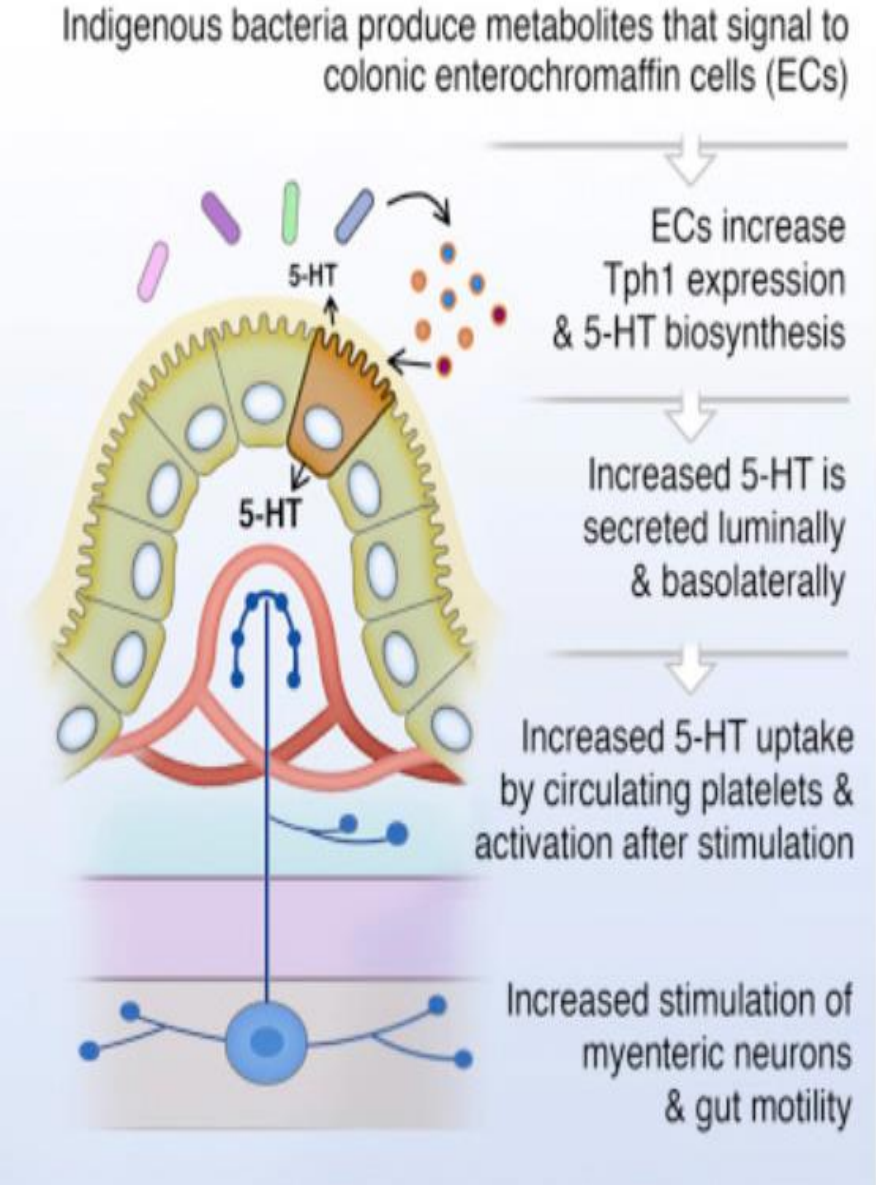
- ✓ GM metabolites can be absorbed into the blood and transported through the blood-brain barrier to regulate brain function.

- ✓ The imbalance of **SCFA** caused by GM dysbiosis can stimulate intestinal chromaffin cells to produce 5-hydroxytryptamine (**5- HT**), which can act on the 5-HT3 receptor of intestinal vagus nerve and inhibit the afferent activity of vagus nerve from intestine to brain.

- ✓ 5-HT released into blood can lead to vasoconstriction. Then 5-HT and SCFA can affect blood pressure through blood circulation and blood brain barrier damage caused by hypertension.

- ✓ In addition, **Hydrogen sulfide** (H2S) produced by GM has an important impact on human health. H2S can regulate a variety of physiological processes, including vasorelaxation, angiogenesis, and hypotension.

  - ✓ H2S deficiency occurs before the occurrence of hypertension. Exogenous H2S donor can protect spontaneously hypertensive rat from hypertension, which indicates that H2S deficiency plays an important role in the regulation of blood pressure.


Indigenous bacteria produce metabolites that signal to colonic enterochromaffin cells (ECs)
5-HT
5-HT
ECs increase Tph1 expression & 5-HT biosynthesis
Increased 5-HT is secreted luminally & basolaterally
Increased 5-HT uptake by circulating platelets & activation after stimulation
Increased stimulation of myenteric neurons & gut motility

- ✓ study reported that antibiotic treatment in rats can rebalance the dysbiotic hypertension GM by reducing the Firmicutes/Bacteroidetes ratio, which then attenuates high blood pressure.

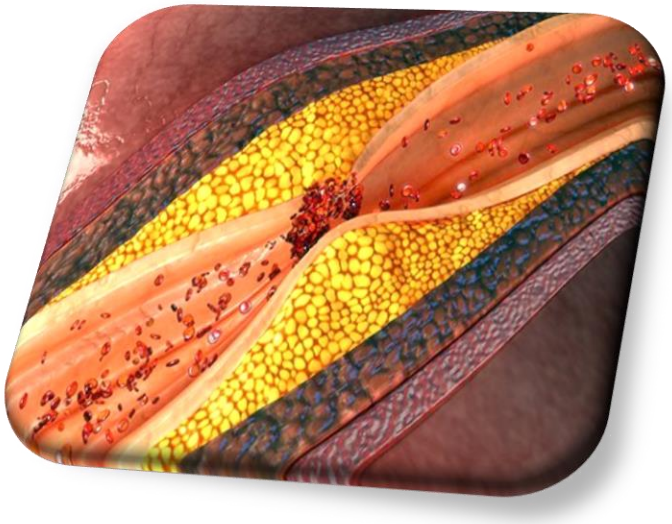
- ✓ the ablation of the entire GM remarkably reduced the incidence of hypertension-related aneurysms suggesting that GM contributes to the pathophysiology of aneurysms by regulating inflammatory and immune response.

- ✓ dietary intervention rich in polyphenols can significantly improve the intestinal permeability of the elderly, increase the number of cellulolytic and butyrate producing bacteria in the intestinal flora, and reduce blood pressure

- ✓ The richness, diversity, and evenness of intestinal microbes were significantly reduced in patients with hypertension.

- ✓ The effect of GM on hypertension is not only through the regulation of intestinal tract itself but also through a change in the production of its metabolites.
- ✓ These findings highlight a strong linkage between hypertension and GM, suggesting that correcting the balance of GM could be a promising therapeutic strategy for hypertension.

➢ Atherosclerosis



- ✓ Atherosclerosis, which accounts for approximately 50% of CVD, is the principal cause of coronary heart disease, cerebral infarction and peripheral vascular disease.
- ✓ GM is involved in the development and progression of atherosclerosis.
- ✓ GM composition varies significantly among atherosclerosis patients and healthy controls.
- ✓ The abundance of **Enterobacteriaceae** and **Enterobacter aerogenes** is much higher in atherosclerosis patients.

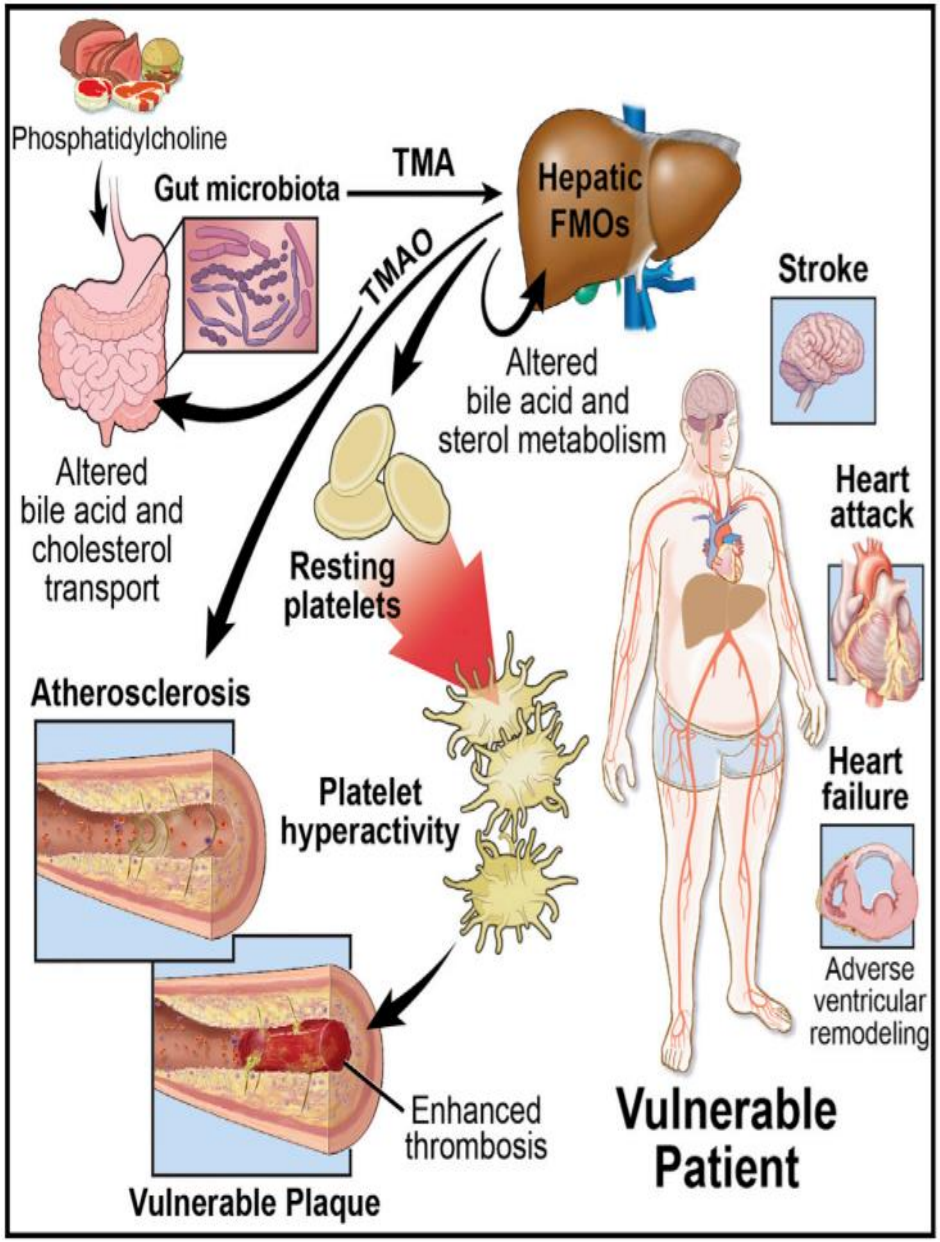
- ✓ The human GM can produce a wide variety of enzymes that ferment dietary fibers into SCFAs such as acetate, propionate, and butyrate.

- ✓ **SCFAs** as signal molecules activate G-protein-coupled receptors mainly include GPR41 and Olfr78, which can promote the release of peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), thus reduce blood pressure and inhibit the occurrence of atherosclerosis.

- ✓ **Bile acids**, as the end product of cholesterol catabolism, have an anti atherosclerotic effect.

- ✓ BAs are one of the major classes of metabolites modified by GM, and they can activate the Farnesoid X receptor (FXR) then reduce the expression of inflammatory factors on monocytes, macrophages and dendritic cells, and reduce the level of inflammation, thus inhibiting the occurrence of atherosclerosis

- ✓ **TMAO** is another metabolite of GM with a major role in the formation of atherosclerosis.
- ✓ TMAO has become a new indicator to identify the risk of cardiovascular events.
- ✓ A positive correlation between high-level TMAO and the incidence of atherosclerosis has been indicated.
- ✓ High levels of TMAO promote the migration of macrophages and accelerate the transformation of macrophages into foam cells, increasing the expression of pro-inflammatory cytokines, thereby promoting the formation of atherosclerosis.
- ✓ TMAO also impairs vascular reactivity and causes oxidative stress, leading to endothelial dysfunction and result in atherosclerosis.
- ✓ TMAO can also activate platelets to promote thrombosis and atherosclerotic plaque rupture.


Phosphatidylcholine
Gut microbiota
TMA
Hepatic FMOs
TMAO
Stroke
Altered bile acid and sterol metabolism
Altered bile acid and cholesterol transport
Heart attack
Resting platelets
Atherosclerosis
Platelet hyperactivity
Heart failure
Adverse ventricular remodeling
Enhanced thrombosis
Vulnerable Plaque
Vulnerable Patient

GM AND METABOLIC DISEASES

Although the incidence of metabolic diseases is related to genetic and environmental factors, the incidence of metabolic diseases in people with the same genetic background and energy intake is correlated with the presence of intestinal flora.

In recent years, increasing studies have suggested that GM dysbiosis is closely associated with many metabolic disorders including:

- obesity
- Diabetes
- non-alcoholic fatty liver disease (NAFLD)

➢ Obesity

- ✓ More and more studies have demonstrated that GM plays an important role in the development of obesity.
- ✓ The GM dysbiosis is prevalent in obesity, which is manifested as a reduction in gut microbiome diversity and richness in obese.
- ✓ The abundance of Akkermansia muciniphila, Faecalibacterium prausnitzii, and Bacteroides decreased, while the abundance of Phylum Firmicutes increased significantly.
- ✓ germ-free mice transplanted with GM from obesity mice gained higher fat and more weight than lean mice, suggesting that GM dysbiosis may contribute to obesity.

- ✓ GM has the ability to ferment indigestible carbohydrates to the important metabolites such as SCFAs and succinate.

- ✓ these metabolites play a significant role in obesity and its comorbidities. SCFAs regulate energy balance and prevent obesity by suppressing appetite and increasing energy expenditure.

- ✓ GLP-1 and PYY by enteroendocrine L-cells have a link to increase satiety and reduced food intake.

- ✓ SCFAs can regulate GLP-1 and PYY by regulating immune cell function through receptors GPR41 and GPR43.

- ✓ Additionally, SCFAs can also induce the up-regulation of heat generating proteins (PPARg, PGC1a, UCP1) and lipid oxidation-related proteins (CPT-I, UCP2), thereby increasing energy consumption and lipid oxidation to prevent obesity.

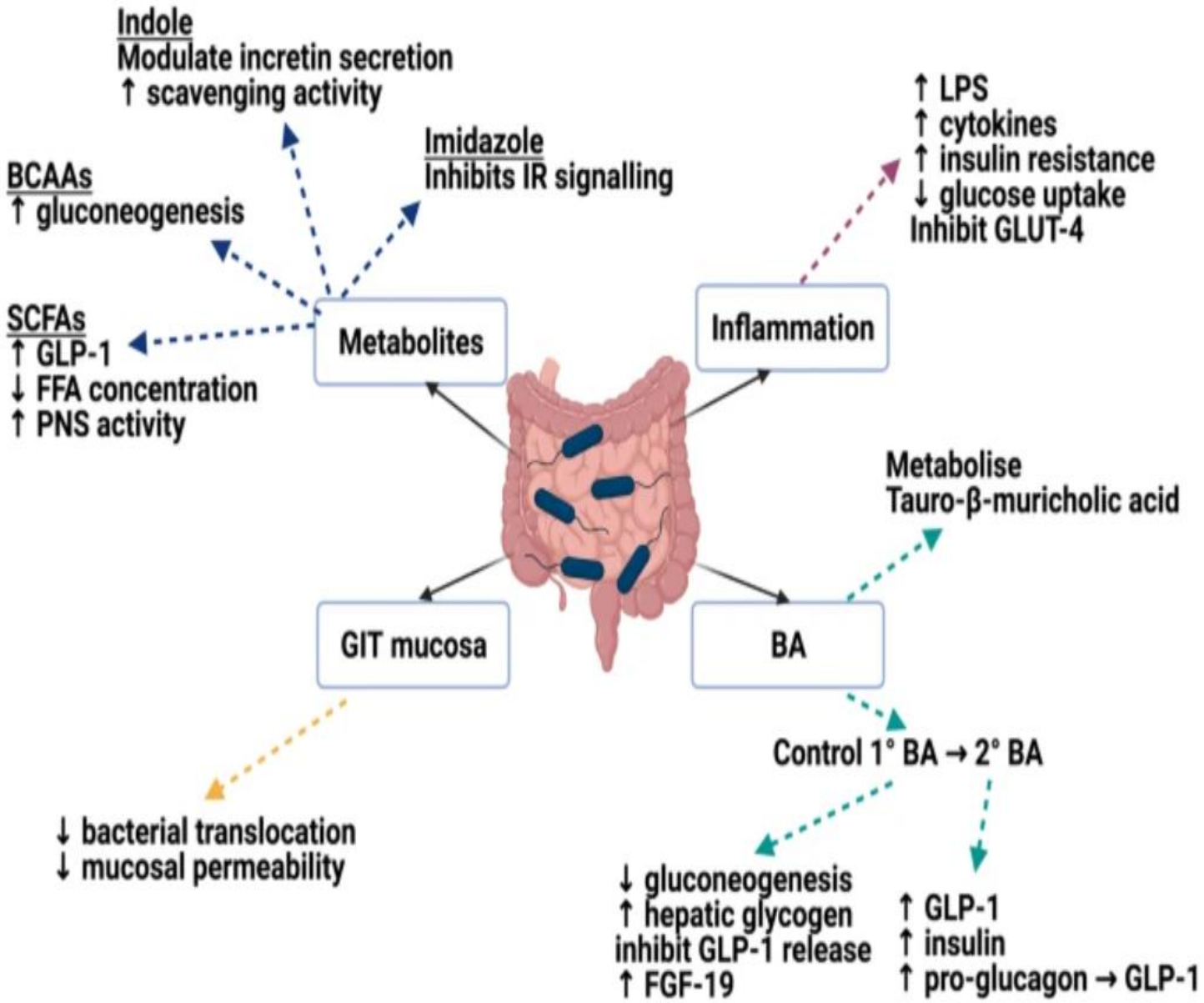
- ✓ The imbalance of intestinal flora, such as decreased diversity and richness of intestinal flora, is common in obese people.

- ✓ Some of the products from GM fermented indigestible carbohydrates prevent obesity by inhibiting appetite and increasing energy consumption, while others prevent obesity by increasing energy consumption and lipid oxidation.

- ✓ The above studies have clarified the role and mechanism of GM and its metabolites in the occurrence and development of obesity, which are of great significance for obesity prevention and treatment.

Diabetes

- ✓ Diabetes is a systemic metabolic disease characterized by high blood glucose, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM).
- ✓ There is accumulating evidence that GM is a risk factor in the occurrence and development of diabetes.
- ✓ GM has become one of the key environmental factors.
- ✓ Several studies have found that there are significant differences in the gut microbial composition between T1DM patients and healthy people.
- ✓ The diversity of GM in T1DM patients is reduced, which is manifested by the decreased amount of Clostridium and Prevotella.
- ✓ GM affects the pathological process of T1DM to a certain extent.


Indole
Modulate incretin secretion
↑ scavenging activity
Imidazole
Inhibits IR signalling
BCAAs
↑ gluconeogenesis
↑ LPS
↑ cytokines
↑ insulin resistance
↓ glucose uptake
Inhibit GLUT-4
SCFAs
↑ GLP-1
↓ FFA concentration
↑ PNS activity
Metabolites
Inflammation
Metabolise
Tauro-β-muricholic acid
GIT mucosa
BA
Control 1° BA → 2° BA
↓ bacterial translocation
↓ mucosal permeability
↓ gluconeogenesis
↑ hepatic glycogen
inhibit GLP-1 release
↑ FGF-19
↑ GLP-1
↑ insulin
↑ pro-glucagon → GLP-1

- ✓ T2DM is non-insulin-dependent diabetes, which is characterized by a decline in insulin secretion and insulin resistance.
- ✓ gut microbial diversity of T2DM patients was significantly reduced.
- ✓ the abundance of Bifidobacteria and Akkermansia in the gut of T2DM patients is decreased, while the abundance of Dallella is increased.
- ✓

Interestingly, 8 months after pregnancy, differences in the GM signatures are still detectable.

GM dysbiosis in GDM patients is associated with inflammation, obesity, and impaired glucose tolerance.

- ✓ Metabolites of GM are also involved in the occurrence and development of diabetes.

- ✓ Metabolites such as lipopolysaccharide, flagellin, lipoteichoic acid and peptidoglycan can destroy the tight junctions between intestinal epithelial cells, and induce inflammation through TRL2 and TRL4 signals.

- ✓ High levels of branched chain amino acids and TMAO can lead to increased insulin resistance and inflammation.

- ✓ Elevated levels of SCFAs can promote the secretion of GLP-1 and PYY to prevent intestinal transit and insulin resistance, and stimulate the secretion of glucagon-like peptide-2, which lead to decreased intestinal barrier function, endotoxemia, and inflammation.

Non-Alcoholic Fatty Liver Disease

GM is closely related to NAFLD.

The integrity of the gut barrier is critical to protect the liver from the invasion of intestinal microflora.

Intestinal permeability in patients with NAFLD was higher than that in healthy controls

The composition of gut microbes is different between NAFLD patients and healthy people.

The abundance of Lactobacillus, Dorea, Streptococcus in the intestine of NAFLD patients increased, while the presence of Rumenococcus, Prevotella, and Flavobacterium decreased

- ✓ SCFAs have beneficial effects on liver metabolism and function.
- ✓ Butyrate especially improves the intestinal barrier function and prevents toxic compounds to the liver by up-regulating the expression of tight junction proteins and mucus.
- ✓ The potential underlying mechanism is related to increased hepatic lipid oxidation through an AMPK–acetyl-CoA carboxylase pathway, reducing tumor necrosis factor expression, increasing glycogen storage and reducing liver fatty acid synthase activity.

GM AND GASTROINTESTINAL DISEASES

Accumulating evidence suggests that GM dysbiosis can cause digestive system diseases, including:

- Inflammatory bowel diseases (IBD)
- Colorectal cancer (CRC).

➢ Inflammatory Bowel Disease

- ✓ IBD consisting of ulcerative colitis and Crohn's are resultant of dysregulation of the immune system leading to intestinal inflammation and microbial dysbiosis.
- ✓ Various evidence indicated that GM contributes to driving intestinal inflammation.
- ✓ A loss of gut microbial diversity as one of the symbols of dysbiosis is commonly found in IBD patients.
- ✓ Compared with healthy individuals, the IBD GM displays a marked reduction in gut microbial diversity, which is manifested as a significant decrease in Firmicutes while a significant increase in Enterobacter and Proteobacteria.

- ✓ metabolites of gut microbes also play a role in the pathogenesis of IBD. SCFAs mediate multiple effects on mucosal immunity by promoting B cell development, maintenance of mucosal integrity via inflammasome activation and IL-18 production.

- ✓ BAs play an immunomodulatory role by direct stimulation of FXR, which exerts anti-inflammatory effects and protects in chemically induced colitis.

- ✓ Tryptophan is utilised by GM to produce indoles, which can regulate mucosal immunity by activating polycyclic aromatic hydrocarbon receptors.

- ✓ Furthermore, gut microbes can also regulate host cell functions through epigenetics.

➢ Colorectal Cancer

- ✓ Colorectal cancer (CRC) is one of the most common malignancies in the digestive system. Studies have shown that GM dysbiosis is related to CRC.

- ✓ Intestinal microbial Dysbiosis in CRC patients is characterized by a decrease in the species of intestinal probiotics (such as Bifidobacteria, Lactobacillus, and Bacteroides) and an increase in the number of pathogenic bacteria (such as Escherichia coli, Bacteroides fragilis, and Fusobacterium nucleatum).

- ✓ The pathogenic bacteria secrete toxic chemicals that damage intestinal epithelial cells and cause a chronic inflammatory response, which contributes to the development of CRC.

- ✓ studies have highlighted that the toxic metabolites produced by GM can directly participate in the occurrence of cancer, or indirectly participate in cancer through inflammation or immunosuppression.

Thanks for your attention