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Review Article

Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential

Ran Guo[1,†], Li-Hua Chen[2,†], Chungen Xing[4,*] and Tong Liu[3,5,*]

[1]Department of Pain, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, China, [2]Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Department of Nutrition and Food Hygiene, School of Public Health, Suzhou, China, [3]Jiangsu Key Laboratory of Neuropsychiatric Diseases and Institute of Neuroscience, Soochow University, Suzhou, China, [4]Department of General Surgery, The Second Affiliated Hospital of Soochow University, Suzhou, China and [5]College of Life Sciences, Yanan University, Yanan, China

*Corresponding authors. E-mails: xingcg@suda.edu.cn, liutong80@suda.edu.cn

†R. Guo and L.-H. Chen contributed equally to this work.

Summary

The relationship between gut microbiota and neurological diseases, including chronic pain, has received increasing attention. The gut microbiome is a crucial modulator of visceral pain, whereas recent evidence suggests that gut microbiota may also play a critical role in many other types of chronic pain, including inflammatory pain, headache, neuropathic pain, and opioid tolerance. We present a narrative review of the current understanding on the role of gut microbiota in pain regulation and discuss the possibility of targeting gut microbiota for the management of chronic pain. Numerous signalling molecules derived from gut microbiota, such as by-products of microbiota, metabolites, neurotransmitters, and neuromodulators, act on their receptors and remarkably regulate the peripheral and central sensitisation, which in turn mediate the development of chronic pain. Gut microbiota-derived mediators serve as critical modulators for the induction of peripheral sensitisation, directly or indirectly regulating the excitability of primary nociceptive neurones. In the central nervous system, gut microbiota-derived mediators may regulate neuroinflammation, which involves the activation of cells in the blood–brain barrier, microglia, and infiltrating immune cells, to modulate induction and maintenance of central sensitisation. Thus, we propose that gut microbiota regulates pain in the peripheral and central nervous system, and targeting gut microbiota by diet and pharmabiotic intervention may represent a new therapeutic strategy for the management of chronic pain.

Keywords: inflammation; gut–brain axis; microbiome; microbiome–gut–brain axis; pain; pharmabiotic

Editor's key points

- The gut microbiota influence many types of chronic pain, including visceral, inflammatory, headache, neuropathic pain, and affects opioid tolerance.
- The gut microbiota can directly modulate dorsal root ganglia neuronal excitability, and regulate neuroinflammation in the peripheral and central nervous systems under chronic pain conditions.
- Targeting gut microbiota through dietary intervention, pharmabiotic approaches, or faecal microbiota transplantation, represents a novel and potentially fruitful strategy for chronic pain management.

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[1] Pain is a subjective experience, which involves not only nociception, but also

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emotional, cognitive, and social components.[1] Acute pain serves as an alarm system to protect us from tissue damage.[2] Patients with congenital insensitivity to pain often cannot avoid tissue damage and succumb to medical problems.[3] In contrast, chronic pain is debilitating and significantly reduces the quality of life of afflicted patients.[4] Based on the aetiology, pain can be classified as nociceptive, nociplastic, inflammatory, or neuropathic.[5,6] The molecular and cellular mechanisms underlining chronic pathological pain are not fully understood. Clinically, there is still a lack of safe, tolerable, and effective therapeutic approaches for the management of chronic pain.[7]

Pain is initiated by the activation of nociceptors that populate peripheral organs, including skin, muscle, bones, joints, and deep visceral tissues.[1,8] Nociceptors refer to peripheral free nerve endings of a subset of primary sensory neurones, whose soma are located in dorsal root ganglia (DRG) and trigeminal ganglia (TG); they are Aδ and C fibres.[8] They convert noxious stimuli (e.g. inflammatory stimuli, noxious heat or cold, and mechanical injury) into nerve impulses, and then transmit the nociceptive signalling to the spinal cord dorsal horn.[1,9] Neural circuits for nociceptive signalling processing in the spinal cord contain multiple types of neurones, including projection neurones, excitatory interneurones, and inhibitory interneurones. Through spinothalamic and spinoparabrachial tracts, spinal nociceptive neurones project to supraspinal brain regions (e.g. thalamus, somatosensory cortex, and anterior cingulate cortex [ACC]) for processing the sensory and affective components of pain.[1,10] In addition, the descending pathways from the brain exert either inhibitory or facilitatory influences on pain processing in the spinal cord dorsal horn.[1,11,12] Notably, increasing evidence has demonstrated that non-neuronal cells, such as glial cells, immune cells, keratinocytes, and tumour cells, are also considered critical regulators of pain in the peripheral and central nervous system.[13,14] A better understanding of the mechanisms underlying the regulation of pain by non-neuronal cells will undoubtedly lead to novel therapeutic approaches for the management of chronic pain.

As the most complex and populous micro-ecological system in our body, the gut microbiota consists of bacteria, archaea, yeast, single-celled eukaryotes, and helminth parasites or viruses, or both.[15,16] The number of organisms comprising the gut microbiota in humans is approximately 10^{14}, and the total number of genes is about 100 times that of the human genome.[17] The complexity and diversity of the gut microbiota are established early in the first few years of life and are influenced by a number of external factors, including delivery (vaginal or Caesarean section), whether breastfed or formula fed, weaning, diet, antibiotic medication, infections, and stress.[18,19] The homeostasis between gut microbiota and host is essential for maintenance of health, including energy regulation, gut-barrier integrity, protection from pathogens, brain development, and immune system function.[20,21] However, dyshomeostasis between gut microbiota and host will lead to a variety of diseases, such as metabolic diseases, cardiovascular diseases, and neurological diseases.[15,22] Although neurological conditions are traditionally considered brain disorders, it is becoming appreciated that they may have the aetiology in the periphery, especially in the gut microbiota. In recent years, it was revealed that the gut microbiota participates in the regulation of many neurological diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), traumatic brain injury, depression, and chronic pain.[23,24] Recently, the emerging roles of gut microbiota in the regulation of pain have been attracting more attention.[25–28] Although it is well appreciated that gut microbiota play a key role in visceral or abdominal pain,[25] their roles in other types of chronic pain have only been recently recognised, such as inflammatory pain, neuropathic pain, headache, and opioid tolerance. In this article, we will address the role of the gut microbiota in pain regulation and discuss the possibility for pain therapy by targeting the gut microbiota.

This narrative review was accomplished by the following literature search strategy. An electronic database search of PubMed was performed with several key terms on July 8, 2019. We used key terms which included microbiota, microbiome, bacteria, probiotics, prebiotics, pain, and hyperalgesia. For specific pain conditions, we applied the following search: (microbiota OR microbiome OR bacteria OR probiotics OR prebiotics) AND ('visceral pain' OR 'abdominal pain' OR 'visceral hypersensitivity'); (microbiota OR microbiome OR bacteria OR probiotics OR prebiotics) AND ('migraine' OR 'headache'); (microbiota OR microbiome OR bacteria OR probiotics OR prebiotics) AND ('inflammatory pain' OR 'gout' OR 'arthritic pain'); (microbiota OR microbiome OR bacteria OR probiotics OR prebiotics) AND ('neuropathic pain' OR 'chemotherapy-induced pain' OR 'Trigeminal Neuralgia'); (microbiota OR microbiome OR bacteria OR probiotics OR prebiotics) AND ('opioid tolerance' OR 'opioid-induced hyperalgesia' OR morphine). For literature search in PubMed, the filters (such as Full text, English, and Title/Abstract) were activated. Finally, after reading abstracts for relevance, 80 papers were included (Fig. 1). In addition, the reference lists of selected studies and relevant reviews were also checked to ensure a complete collection.

The following questions were addressed or discussed in constructing the review. Are the changes of gut microbiota associated with several types of chronic pain? How does the gut microbiota regulate peripheral and central sensitisation under chronic pain? Does modulation of the gut microbiota represent a new therapeutic strategy for chronic pain management?

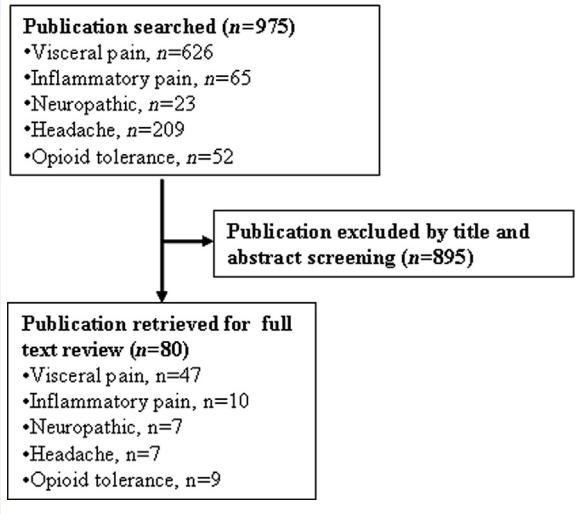

Fig 1. Flowchart of publication selection.

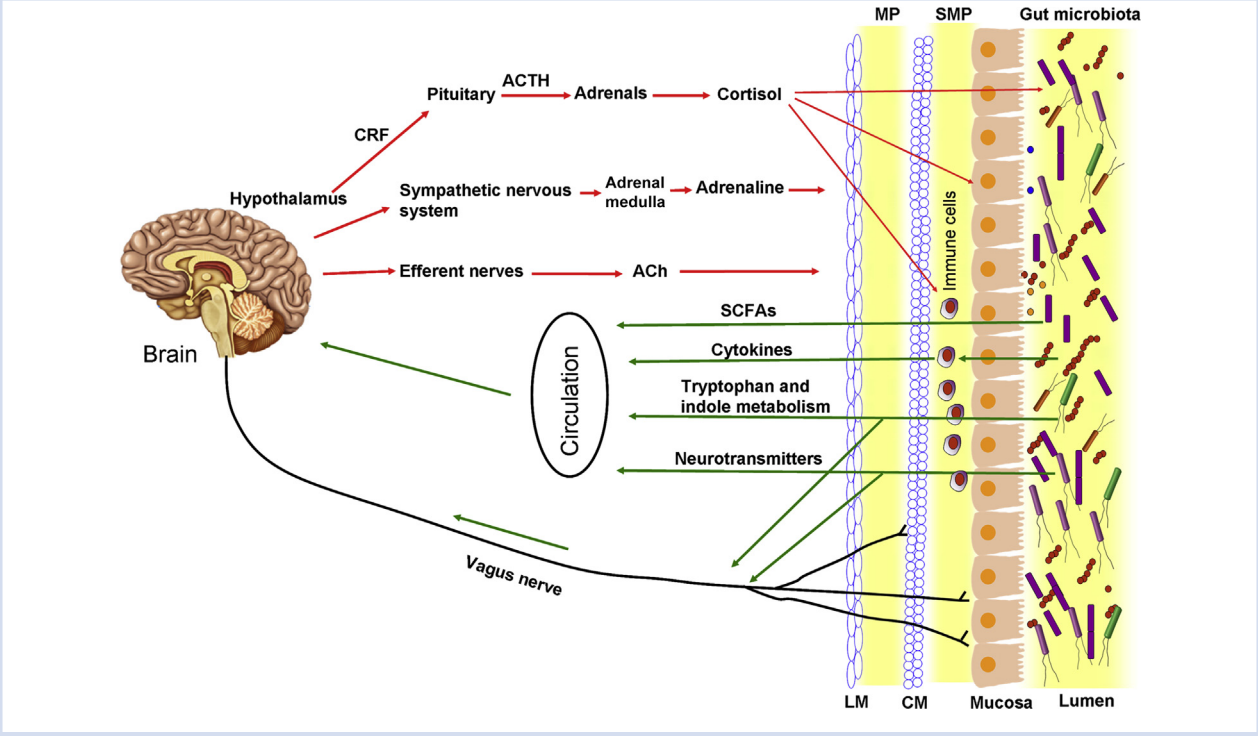

Fig 2. Bidirectional communication between the gut microbiota and the brain. Endocrine (cortisol), immune (cytokines) and neural (vagus nerve and enteric nervous system) are major pathways mediating the bidirectional communication between the gut microbiota and the brain. ACh, acetylcholine; ACTH, adrenocorticotropic hormone; CM, circular muscle; CRF, corticotropin-releasing factor; LM, longitudinal muscle; MP, myenteric plexus; SCFA, short-chain fatty acid; SMP, submucosal plexus.

Microbiome–gut–brain axis

Gut–brain axis refers to bidirectional communication between the gut and the brain, which is traditionally considered to integrate immunological, neural, and hormonal signals.[29] However, the gut microbiota is now considered a key gastrointestinal (GI) factor that modifies the gut–brain axis. Thus, a new concept of 'microbiota–gut–brain axis' is established (Fig. 2).[30] The microbiota–gut–brain axis comprises multiple organs, including the brain, glands, gut, immune cells, and intestinal microbiota, which bidirectionally communicate to maintain homeostasis.[30] Historically, studies on bidirectional communications of microbiota–gut–brain axis focused on its involvement in functional GI syndromes, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).[31] In recent years, dysregulation of bidirectional communication of microbiota–gut–brain axis was shown to be involved in the pathogenesis of many other pathological conditions, such as metabolic diseases (e.g. obesity and diabetes),[32,33] liver disease,[34,35] and neurological diseases (e.g. AD, PD, autism, depression, and pain).[29,36]

Gut microbiota and pain

Visceral pain

Visceral pain refers to pain of internal organs, such as abdominal pain caused by IBS, IBD, functional dyspepsia, functional abdominal pain syndrome, infantile colic, and interstitial cystitis.[26] It has been demonstrated that dyshomeostasis of gut microbiota and host is associated with the pathogenesis of many GI disorders, such as IBS, IBD, celiac disease, and food allergies.[25] In recent years, mounting evidence from preclinical animal studies and human clinical trials supported a crucial role of the gut microbiota in the regulation of pain associated with GI disorders.

Preclinical studies have determined the involvement of gut microbiota in the pathogenesis of visceral pain.[25,26] By using antibiotic and probiotic treatment, it was found that gut microbiota play a key role in visceral hypersensitivity in preclinical animal models. For example, Aguilera and colleagues[37] showed antibiotic treatment decreased visceral pain elicited by intraperitoneal injection of acetic acid or intracolonic infusion of capsaicin in mice, possibly by inducing alteration of GI microbiota. In contrast, antibiotics administered early in life were shown to produce long-lasting enhancement of visceral pain in adult mice via disturbance of the gut microbiota.[38] Treatment using vancomycin in early life also led to increased visceral pain by colorectal distension (CRD) in rats.[39] Preclinical studies also provided evidence that probiotic administration can regulate visceral pain.[40] For example, application of *Bifidobacteria* reversed CRD-induced visceral hypersensitivity in mice.[41] Administration of *Lactobacillus acidophilus* induced analgesic-related receptors, such as μ-opioid and cannabinoid receptors, in colonic epithelial cell lines and epithelium in rodents.[42] Neonatal maternal deprivation (NMD) led to visceral pain and a reduced diversity of faecal microbiota and administration of *Faecalibacterium prausnitzii* reduced visceral pain after NMD in rats.[43] Treatment with *Lactobacillus*

paracasei NCC2461 also ameliorated NMD-induced visceral hypersensitivity after CRD in mice.[44] Administration of the probiotic *Lactobacillus* GG or the prebiotic combination of polydextrose/galacto-oligosaccharide (PDX/GOS) reduced neonatal inflammation-induced visceral hypersensitivity in rats.[45] Studies using germ-free (GF) mice also provided strong evidence that gut microbiota critically contributes to visceral pain. Luczynski and colleagues[46] found that GF mice displayed visceral hypersensitivity accompanied by up-regulation of Toll-like receptors (TLRs) and cytokines in the spinal cord, which were abolished by postnatal colonisation with microbiota from conventionally colonised. In the brain of GF mice, the volume of the ACC was decreased, whereas the volume of the periaqueductal grey (PAG) was enlarged, suggesting gut microbiota may be involved in the development of brain regions related to pain processing.[46] Recolonisation of microbiota rescued the excitability changes of the neurones, suggesting commensal microbiota was necessary for normal excitability of sensory neurones.[47] Faecal microbiota transplantation (FMT) studies showed that visceral hypersensitivity in rats was induced by the transplantation of the faecal microbiota from constipation-predominant IBS patients.[48] These studies have brought some insights into the mechanisms of pain regulation by gut microbiota, although the translational significance of non-human animal studies on gut microbiota and pain is still uncertain.

Clinical studies further indicate that targeting gut microbiota may be a promising strategy for visceral pain management in patients with GI disorders. The antibiotic rifaximin reduces pain in IBS patients.[49] Treatment with *Lactobacillus rhamnosus* GG reduced abdominal pain in children with functional GI disorders,[50] and a mixture of *Bifidobacterium infantis* M-63, *breve* M-16V, and *longum* BB536 improved abdominal pain in children with IBS.[51] *L. acidophilus* NCFM reduced functional abdominal pain in adults.[52] Although it is difficult to integrate the data on probiotic interventions owing to differences in the study design, probiotic dose, and strain used, a systematic review showed specific probiotics were beneficial in certain GI disorders, including overall symptom burden, abdominal pain, bloating/distension and bowel movement frequency.[53] Dietary intervention strategies have shown to be effective in IBS possibly via modulation of gut microbiota, such as a diet of low-FODMAP (foods high in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols).[28,54] FMT strategies also showed efficiency for alleviation of chronic constipation, IBS and IBD with varied success, effective in 36% of patients, mildly improved discomfort in 16%, and non-effective in the rest (47%).[55,56] Thus, both preclinical and clinical evidence strongly supports the critical involvement of gut microbiota in visceral pain associated with GI disorders.

Inflammatory pain

Inflammatory pain, such as arthritic pain, afflicts millions of people and represents a major health problem.[57] Pain can be elicited and enhanced by inflammatory reactions, which refers to a decrease in pain threshold and an increase in pain response.[2] Under inflammatory conditions, noxious stimuli can cause enhanced pain (hyperalgesia), whereas non-noxious stimuli (e.g. light touch) can cause pain (allodynia).[58–60] The so-called 'inflammatory soup' comprises many pro-inflammatory mediators, such as adenosine 5′-triphosphate (ATP), H^+, prostaglandin E2 (PGE2), tumour necrosis factor-alpha (TNF-α), interleukin 1β (IL-1β), C–C motif chemokine ligand 2 (CCL2), and chemokine (C-X-C motif) ligand 1 (CXCL1), which are released by infiltrating immune cells or resident cells. Once released, they activate or sensitise peripheral nociceptors to cause peripheral pain hypersensitivity.[61–64] Subsequently, activation of the intracellular downstream signalling pathways, such as cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), PKC, and mitogen-activated protein kinase (MAPK), lead to phosphorylation of certain receptors and ion channels in primary sensory neurones, resulting in neuronal hyperexcitability (peripheral sensitisation).[65–67]

So far, there are mainly preclinical animal studies indicating that gut microbiota may play an important role in inflammatory pain. Amaral and colleagues[68] found that the inflammatory pain induced by carrageenan, lipopolysaccharide (LPS), TNF-α, IL-1β, and chemokine CXCL1 was reduced in GF mice compared with conventional mice. However, no difference was identified in prostaglandins and dopamine-induced inflammatory pain between the GF and conventional mice.[68] Carrageenan-induced inflammation was also reduced in the GF mice and was reversed by reposition of the microbiota or systemic administration of LPS.[68] The reduced pain hypersensitivity of the GF mice was significantly aggravated after being transplanted with the stool of conventional mice. Notably, decreased pain hypersensitivity in GF mice was associated with enhanced expression of interleukin 10 (IL-10) upon stimulation and can be reversed by anti-IL-10 neutralising antibody.[68] Recently, Yan and colleagues[69] demonstrated that neonatally LPS-challenged rats showed increased mechanical hypersensitivity during adolescence, and the administration of a broad spectrum antibiotic cocktail attenuated this mechanical hypersensitivity. Injection of monosodium urate monohydrate (MSU) crystals caused joint inflammation, hypernociception, and production of IL-1β and CXCL1, which were substantially decreased in GF mice, mice treated with antibiotics, and GPR43-deficient mice, suggesting commensal microbiota play a critical role in gout-induced acute inflammation and pain.[70] Vitamin D deficiency in mice, which represents a 'proinflammatory' state, induced mechanical allodynia associated with neuronal hyperexcitability and a lower microbial diversity characterised by an increase in Firmicutes and a decrease in Verrucomicrobia and Bacteroidetes.[71] However, it was recently found that oral probiotics *Lactobacillus reuteri* LR06 or *Bifidobacterium* BL5b had no significant antinociceptive effects on complete Freund's adjuvant (CFA)-induced inflammatory pain in rats.[72]

Nevertheless, these results indicated that targeting gut microbiota or using specific probiotics may be promising for attenuating pain hypersensitivity in many inflammatory settings. Based on the critical regulation of host immune function by gut microbiota, the role of gut microbiota in inflammatory pain warrants further investigation.

Neuropathic pain

Neuropathic pain is pain caused by damage (e.g. nerve trauma or chemotherapy drugs) or disease (e.g. diabetes or spinal stenosis) affecting the somatosensory nervous system, including peripheral and central nervous systems.[5] Neuropathic pain is associated with abnormal sensations (dysaesthesia) or normally non-painful stimuli-evoked pain (allodynia).[5,73] Chemotherapy-induced peripheral neuropathy (CIPN) is caused by certain chemotherapy drugs (vincristine, paclitaxel, platinum etc.) during anti-cancer treatment.[74] More

than 30% of patients with CIPN experience peripheral neuropathic pain.[75] Neuropathic pain caused by CIPN continues to worsen for months and even years. Thus, many cancer patients are unable to receive adequate chemotherapy dosages because of CIPN-induced pain. Recent studies have found that gut microbiota not only played an important role in the therapeutic effect of chemotherapeutic drugs for inhibiting tumour growth,[76] but also contributed to the pathogenesis of the CIPN pain.[77,78]

Shen and colleagues[79] found that pre-treatment with antibiotics reduced the bacterial load in mouse faeces by two times when it was given for 3 weeks. Meanwhile, oxaliplatin-induced mechanical hyperalgesia was reduced in GF mice and in mice pre-treated with antibiotics. This study further found that after oxaliplatin treatment, the infiltration of macrophages, and cytokines (IL-6 and TNF-α) in the DRGs of antibiotics-treated mice were lower than those of mice fed with water.[79] The administration of exogenous LPS in antibiotics-treated mice can abolish the protective effect induced by the removal of gut microbiota.[79] They further confirmed that TLR4 expressed by bone marrow-derived macrophages mediated the protective effect induced by the removal of gut microbiota in oxaliplatin-induced mechanical hyperalgesia.[79] Therefore, it is speculated that LPS derived from the gut microbiota promoted and aggravated the release of pro-inflammatory factors by macrophages, leading to the sensitisation of peripheral nociceptors. Another study found that the intestinal mucosal structure of the jejunum, ileum, and colon of mice were damaged after irinotecan chemotherapy, causing increased permeability of intestinal mucosa.[80] Bacterial translocation led to the activation of astrocytes and microglia though release of LPS and triggered persistent pain, which was ameliorated in TLR4 knockout mice.[80] Another study also demonstrated that paclitaxel-induced neuropathic pain can be counteracted by the probiotic DSF, a high concentration probiotic formulation (450 billion bacteria per sachet).[81] Thus, these data point towards the use of probiotic as a possible adjuvant agent for counteracting pain associated CINP.

A recent study also demonstrated that gut microbiota may play an important role in neuropathic pain induced by peripheral nerve trauma.[82] It was found that abnormal composition of gut microbiota may contribute to neuropathic pain and anhedonia susceptibility induced by spared nerve injury (SNI) in rats.[82] Interestingly, FMT from SNI rats with or without anhedonia can alter pain and depression-like phenotypes in the pseudo-GF mice.[82] However, it was also found that oral probiotics *L. reuteri* LR06 or *Bifidobacterium* BL5b had no significant antinociceptive effects on chronic constriction injury (CCI)-induced neuropathic pain in rats.[72] This indicated that gut microbiota may play a key role in neuropathic pain induced by nerve injury or CIPN, and pain comorbidities (such as depression).[82]

Headache

Headache can be a sign of stress or emotional distress, or it can result from a medical disorder, such as migraine, high blood pressure, anxiety, or depression. Migraine refers to a group of repeated and unilateral headaches.[83] So far, the relationship between gut microbiota and headache is still not clear. Interestingly, recent studies have found that migraine was associated with GI disorders.[84] A Norwegian survey found that patients with frequent migraine had more complaints about GI symptoms than normal controls.[85,86] Supplementation of probiotics protected the intestinal barrier and intestinal permeability, and regulated the immune responses, possibly via the intervention of gut microbiota.[15,87] Interestingly, supplementation of probiotics (including *L. acidophilus*, *Lactobacillus bulgaricus*, *Enterococcus faecium*, and *Bifidobacteria*) was shown to improve the quality-of-life scores in migraine patients.[17] Another study found that the therapeutic effect of supplementation of probiotics daily for 12 weeks in patients with migraine was comparable with that of antiepileptic drugs and antihypertensive drugs, and no obvious side-effects were found.[22] However, a randomised placebo-controlled study did not confirm significant benefit from a multispecies probiotic compared with a placebo on the outcome parameters of migraine.[88] Overall, although the direct evidence linking gut microbiota and migraine is still lacking, it was suggested that gut microbiota may have a possible role in the pathogenesis of headache, especially migraine, based on the fact that gut microbiota have a significant impact on brain function.

Opioid tolerance

The clinical application of morphine and other opioids is limited by their side-effects, including tolerance, hyperalgesia, addiction, nausea, vomiting, and constipation.[89] Since opium was first used, the effects of opioids on GI function have been well recognised. These GI-related symptoms are collectively referred to as 'opioid-induced intestinal dysfunction'.[90,91] Recent studies have shown that long-term use of opioids is associated with microbial dysbiosis in humans[92,93] and mice.[94–97] In mice, tolerance was associated with microbial dysbiosis with selective depletion in *Bifidobacteria* and Lactobacillaceae. Long-term use of morphine caused disruption of the intestinal epithelial barrier (leaky gut) and enhanced bacterial translocation in the ileum[95] and colon of mice.[96] An increased risk of infection may result from bacterial translocation in the colon, leading to sepsis[98] and immune disorders.[99] Clinical studies have shown that the use of opioid analgesics could worsen the condition of patients with Crohn's disease (CD) and may be associated with the recurrence after remission.[100] The Crohn TREAT registry, containing more than 6000 CD patients, showed a 1.5-fold increase in mortality and a 3-fold increased risk of infection with opioid analgesic administration, compared with those who did not take opioids.[101,102] Meng and colleagues[95] reported that the intestinal epithelial barrier was impaired because of the activation of morphine-mediated TLRs in epithelial cells, leading to the transfer of bacterial products. Both Gram-positive and Gram-negative bacteria could activate TLRs of immune cells and enteric glial cells.[103]

Enteric glia, a key factor regulating GI function, mainly participate in immune regulation by interacting with intestinal neurones. Bhave and colleagues[104] found that the purinergic pathway in enteric glial cells activated by the bacterial product LPS is an important source of cytokines during long-term morphine therapy. Long-term treatment with morphine leads to the up-regulation of P2X purinergic receptors and enhances ATP-induced currents in mice enteric glial cells *in vivo*. The P2X receptor is a cation channel that allows calcium to enter, and the calcium channel is an important component of cytokine release. In addition, in enteric glial cells, the bacterial product LPS up-regulates the expression of connexin43 (Cx43), which is the hemichannel for ATP release. Carbenolone inhibits Cx43 and reverses the

Table 1 Chronic pain regulated by gut microbiota in preclinical and clinical studies. ABX, antibiotic cocktail; CFA, complete Freund's adjuvant; GF, germ-free; IBS, irritable bowel syndrome; MSU, monosodium urate monohydrate; NMD, neonatal maternal deprivation; PAG, periaqueductal grey; PFC, prefrontal cortex; TLR, Toll-like receptor; PDX/GOS, polydextrose/galacto-oligosaccharide.

Pain condition	Reference	Type of study	Involvement of microbiota
Visceral pain	Pimentel and colleagues[49]	Human clinical trial	Antibiotic rifaximin reduced IBS-related pain.
	Verdu and colleagues[38]	Preclinical animal study	Visceral pain to colorectal distension was increased by antibiotic cocktail and reverses by *Lactobacillus paracasei* in mice.
	Saulnier and colleagues[51]	Human clinical trial	A mixture of *Bifidobacterium infantis* M-63, *breve* M-16V, and *longum* BB536 improved abdominal pain in paediatric IBS patients.
	Ringel-Kulka and colleagues[52]	Human clinical trial	*Lactobacillus acidophilus* NCFM reduced functional abdominal pain in adults.
	Kannampalli and colleagues[45]	Preclinical animal study	Administration of the probiotic *Lactobacillus* GG or the prebiotic combination of PDX/GOS reduced neonatal inflammation-induced visceral hypersensitivity in rats.
	O'Mahony and colleagues[39]	Preclinical animal study	Treatment with antibiotic vancomycin and cocktail in early life lead to an increased visceral pain by colorectal distension in rats.
	Aguilera and colleagues[37]	Preclinical animal study	Antibiotic cocktail reduced visceral pain by acetic acid and intracolonic capsaicin in mice.
	Perez-Burgos and colleagues[108]	Preclinical animal study	Administration of *Lactobacillus reuteri* DSM 17938 reduced jejunal spinal nerve firing evoked by gastric distension or injection of capsaicin in rodents.
	Miquel and colleagues[43]	Preclinical animal study	NMD leads to visceral pain and a reduced diversity of faecal microbiota and administration of *Faecalibacterium prausnitzii* reduces visceral pain after NMD in rats.
	Weizman and colleagues[109]	Human clinical trial	A randomised, double-blind, placebo-controlled trial found administration of *Lactobacillus reuteri* DSM 17938 is beneficial in functional abdominal pain of childhood.
	Spiller and colleagues[110]	Human clinical trial	Randomised, double-blind, placebo-controlled trial found *Saccharomyces cerevisiae* CNCM I-3856 at the dose of 1000 mg day^{-1} does not improve intestinal pain and discomfort in general IBS patients.
	Luczynski and colleagues[46]	Preclinical animal study	GF mice displayed visceral hypersensitivity accompanied by up-regulation of TLRs and cytokines in the spinal cord, which were abolished by postnatal colonisation with microbiota from conventionally colonised.
	Pokusaeva and colleagues[111]	Preclinical animal study	Oral administration of a GABA-producing *Bifidobacterium* strain (*B. dentium* ATCC 27678) reduced visceral hypersensitivity in a rat faecal retention model.
	Zhao and colleagues[112]	Preclinical animal study	Treatment with *Clostridium butyricum* exerted a beneficial effect on visceral hypersensitivity of IBS by inhibiting low grade inflammation of colonic mucous in mice.
	Zhang and colleagues[113]	Preclinical animal study	Butyrate-producing Lachnospiraceae exerted a beneficial effect of on stress-induced visceral hypersensitivity in rats.
	Newlove-Delgado and colleagues[114]	Human clinical trial	Treatment with probiotic preparations improved recurrent abdominal pain in children.
	Li and colleagues[115]	Preclinical animal study	The probiotic VSL#3 decreases visceral hypersensitivity in rat, possible related with the mast cell—PAR2—TRPV1 signalling pathway.
Inflammatory pain	Amaral and colleagues[68]	Preclinical animal study	Inflammatory pain induced by carrageenan, lipopolysaccharide, TNF-alpha, IL-1beta, and CXCL1 was reduced in GF mice.
	Vieira and colleagues[70]	Preclinical animal study	Injection of MSU crystals caused joint inflammation, hypernociception, and production of IL-1beta and CXCL1, which were greatly decreased in GF mice, mice treated with antibiotics, and GPR43-deficient mice.
	Yan and colleagues[69]	Preclinical animal study	Neonatal treatment with LPS increased pain sensitivity associated with decreased Oprm1 expression in the PFC and PAG of rats.
	Guida and colleagues[71]	Preclinical animal study	Vitamin D deficiency in mice induced tactile allodynia associated with neuronal hyperexcitability and a lower microbial diversity characterised by an increase in Firmicutes and a decrease in Verrucomicrobia and Bacteroidetes.
	Huang and colleagues[72]	Preclinical animal study	Oral probiotics *L. reuteri* LR06 or *Bifidobacterium* BL5b had no significant antinociceptive effects on CFA-induced inflammatory pain in rats.

Continued

Table 1 Continued

Pain condition	Reference	Type of study	Involvement of microbiota
Neuropathic pain	Shen and colleagues[79]	Preclinical animal study	Oxaliplatin-induced neuropathic pain was reduced in GF mice and antibiotic-treated mice. Restoring the microbiota of GF mice abolished the effects. TLR4 expressed on macrophages appear to be partially involved.
	Castelli and colleagues[81]	Preclinical animal study	Probiotic formulation attenuated paclitaxel-induced neuropathic pain.
	Yang and colleagues[82]	Preclinical animal study	Transplantation of faecal microbiota from anhedonia susceptible rats into antibiotics-treated pseudo-GF mice exaggerated pain and depression-like behaviours. Transplantation of faecal microbiota from resilient rats into antibiotics-treated pseudo-GF mice improved pain and depression-like behaviours.
	Wardill and colleagues[80]	Preclinical animal study	TLR4 deficient mice attenuated chemotherapy drug irinotecan-induced gut toxicity and pain.
	Huang and colleagues[72]	Preclinical animal study	Oral probiotics *L. reuteri* LR06 or *Bifidobacterium* BL5b had no significant antinociceptive effects on chronic constriction injury-induced neuropathic pain in rats.
Headache	de Roos and colleagues[22]	Human clinical trial	An open-label pilot study the multispecies probiotic mixture may decrease migraine.
	de Roos and colleagues[88]	Human clinical trial	This study did not confirm significant benefit from a multispecies probiotic compared with a placebo on the outcome parameters of migraine.
Opioid tolerance	Meng and colleagues[95]	Preclinical animal study	Morphine induced gut epithelial barrier dysfunction and subsequent bacteria translocation, which was mediated by TLR signalling in mice.
	Kang and colleagues[96]	Preclinical animal study	ABX treatment prevented the development of antinociceptive tolerance to chronic morphine in mice. ABX reduced gut microbiota and prevented chronic morphine induced increases in gut permeability, colonic mucosal destruction, and colonic IL-1 beta expression in mice.
	Lee and colleagues[116]	Preclinical animal study	Intermittent morphine treatment decreased the relative abundances of *Lactobacillus* spp. and increased *Ruminococcus* spp., whereas sustained morphine treatment showed significant increases in the genus *Clostridium* and Rikenellaceae. Depletion of the microbiome via oral antibiotics resulted in altered microglia morphology and produced opioid tolerance.
	Zhang and colleagues[97]	Preclinical animal study	Morphine tolerance was significantly attenuated in GF mice. Reconstitution of GF mice with naïve faecal microbiota reinstated morphine tolerance. Morphine tolerance was associated with microbial dysbiosis with depletion in *Bifidobacteria* and Lactobacillaeae. The probiotic VSL#3 attenuated morphine tolerance in mice.

constipation caused by the long-term use of opioids in mice.[104] Therefore, the long-term use of morphine up-regulates Cx43 and P2X receptors in enteric glial cells and can significantly amplify the inflammatory responses.[105]

A previous study found that oral administration of antibiotic cocktails to mice prevented tolerance to chronic morphine analgesia in mice.[96] It was also shown that oral bioavailability of vancomycin was sufficient to prevent tolerance, indicating that microbial translocation, particularly Gram-positive bacteria, was intricately involved in this process.[106] Recently, it was found that morphine analgesic tolerance was significantly attenuated in GF mice, and reconstitution of GF mice with naïve faecal microbiota reinstated morphine analgesic tolerance.[97] Patch clamping studies on cultured neurones of the DRG revealed that morphine incubation did not reduce neuronal excitability in chronic morphine-treated DRG neurones, suggesting that morphine tolerance occurred.[106] However, in primary cultured neurones from antibiotic-pretreated mice, incubation of morphine reduced neuronal excitability, suggesting morphine tolerance was reversed. These findings demonstrated that peripheral sensory neurones are key cellular targets of gut microbiota under opioid tolerance conditions.[106] Corder and colleagues[107] provided strong evidence supporting the view that the peripheral nervous system may play a key role in mediating opioid tolerance. They observed that chronic morphine analgesic tolerance was lost in mice in which μ-opioid receptors in DRG neurones were conditionally knocked out, although the acute analgesic effect of morphine remained intact in these mice, indicating that chronic opioid tolerance involves peripheral nociceptors, whereas systemic morphine analgesia is mediated by the central nervous system. These factors

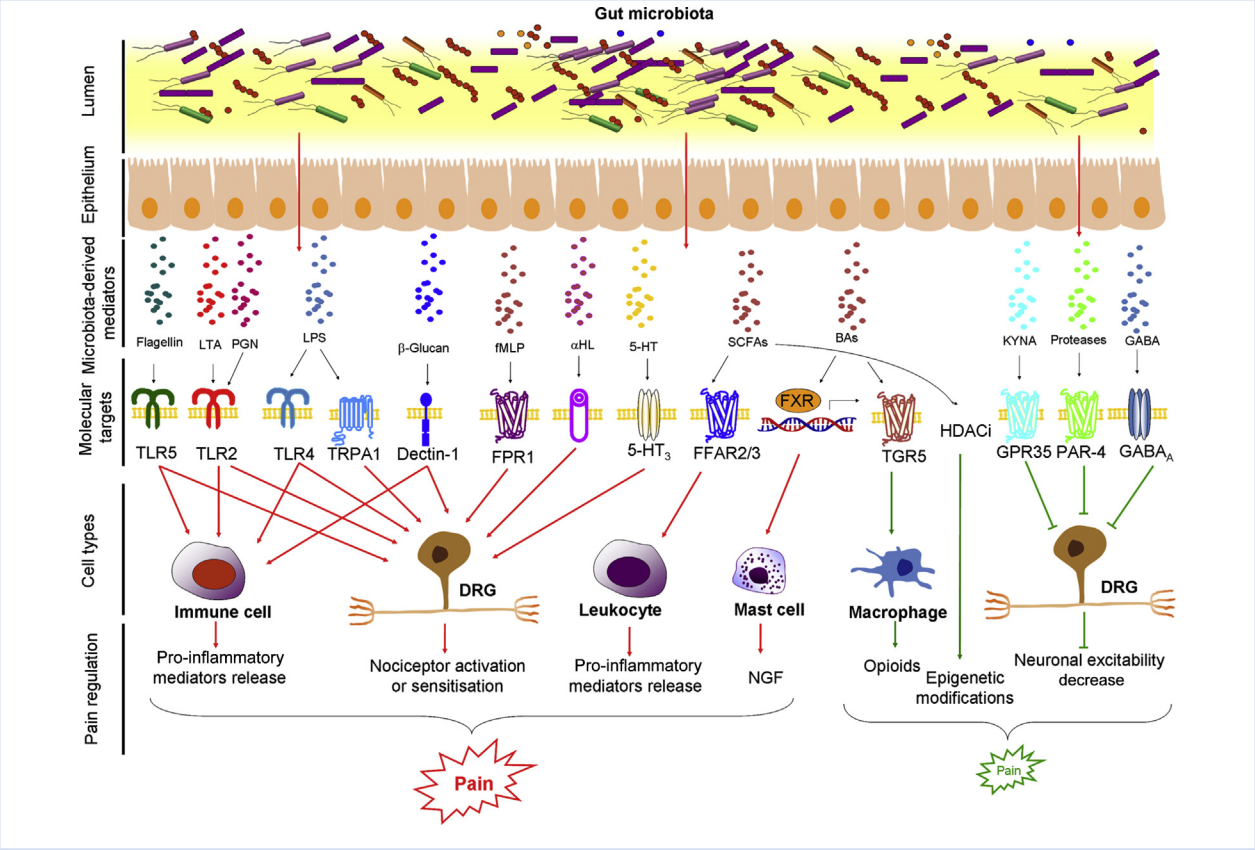

Fig 3. Gut microbiota-derived mediators directly or indirectly regulate peripheral sensitisation of pain. Gastrointestinal microbiota can directly or indirectly modulate peripheral sensitisation underlying chronic pain through multiple gut microbiota-derived mediators, including microbial by-products (e.g. PAMPs), metabolites (e.g. SCFAs, BAs), and neurotransmitters or neuromodulators release (e.g. GABA). Some microbiota-derived mediators (e.g. TLRs agonists and FPR1 agonists) can directly activate or sensitise primary nociceptive neurones in dorsal root ganglia (DRG) to enhance pain, whereas other microbiota-derived mediators (e.g. KYNA and proteases) can directly decrease the excitability of DRG neurones to inhibit pain. Gut microbiota-derived mediators (e.g. TLRs agonists and SCFAs) can indirectly increase the excitability of DRG neurones by inducing pro-inflammatory factors release from immune cells to enhance pain, whereas other mediators (e.g. BAs) can indirectly decrease the excitability of DRG neurones by release opioids from immune cells to inhibit pain. α-HL, α-haemolysin; BA, bile acid; DRG, dorsal root ganglion; FXR, farnesoid X receptor; GABA, γ-aminobutyric acid; HDACi, histone deacetylase inhibitor; KYNA, kynurenic acid; LPS, lipopolysaccharide; LTA, Lipoteichoic acid; NGF, nerve growth factor; PAMP, pathogen-associated molecular pattern; PAR-4, proteinase-activated receptor 4; PGN, peptidoglycan; SCFA; short-chain fatty acid; TRPA1, transient receptor potential cation channel, subfamily A, member 1.

together demonstrate that the gut microbiota is an important regulator of the efficacy of chronic application of opioids, possibly through inducing gut dysbiosis, disruption of the gut barrier, and bacterial translocation, initiating TLRs-mediating gut inflammation and releasing pro-inflammatory cytokine, and regulation of neuronal excitability in the peripheral nervous system (Table 1).

Molecular mechanisms underlying regulation of pain by gut microbiota

Peripheral mechanisms

In the past decades, the mechanisms underlying pain regulation by the gut microbiota have been gradually uncovered. So far, current studies have focused on peripheral sensitisation mechanisms underlying the regulation of pain by the gut microbiota (Fig. 3). On one hand, microbiota-derived mediators can directly regulate the neuronal excitability of primary sensory neurones in DRGs, through activation or sensitisation of the pain-related receptors or ion channels, including TLRs, transient receptor potential (TRP) channels, γ-aminobutyric acid (GABA) receptors, and acid-sensing ion channels (Table 2).[13,14,135] For example, live luminal *L. reuteri* (DSM 17938), and its conditioned medium, dose-dependently reduced jejunal spinal nerve firing evoked by distension or capsaicin, which was blocked by a specific TRPV1 channel antagonist or in TRPV1 knockout mice.[108] On the other hand, microbiota-derived mediators can indirectly regulate the neuronal excitability of primary sensory neurones in DRGs, through activation of non-neuronal cells (such as immune cells) to release pro-inflammatory cytokines (e.g. TNF-α, IL-1β, and IL-6), chemokines (e.g. CCL2 and CXCL1), anti-inflammatory cytokines (e.g. IL-4), or neuropeptides (e.g.

Table 2 Gut microbiota-derived mediators regulate peripheral sensitisation underlying chronic pain. α-HL, α-haemolysin; BAs, bile acids; CLR, C-type lectin receptors; DRG, dorsal root ganglia; GABA, γ-aminobutyric acid; HDAC, histone deacetylase; KYNA, kynurenic acid; LTA, lipoteichoic acid; LPS, lipopolysaccharide; PAMPs, pathogen-associated molecular patterns; PAR-4, protease-activated receptor-4; PGN, peptidoglycan; SCFAs, short-chain fatty acids; TLR, Toll-like receptor.

Type of mediators	Reference	Mediators	Actions of mediators on pain
PAMPs	Diogenes and colleagues[117]; Qi and colleagues[118]; Meseguer and colleagues[119]	TLR4 ligands, e.g. LPS	LPS directly activates or sensitises primary sensory neurones, possible through TLR4—TRPV1 or TRPA1-mediated mechanisms. LPS activates immune cells via TLR4 to release pro-inflammatory mediators, indirectly leading to sensitisation of primary sensory neurones.
	Miller and colleagues[120]	TLR2 ligands, e.g. LTA or PGN	TLR2 agonists directly activate primary sensory neurones through TLR2. TLR2 agonists activate immune cells via TLR2 to release pro-inflammatory mediators, indirectly leading to sensitisation of primary sensory neurones.
	Das and colleagues[121]; Xu and colleagues[122]	TLR5 ligands, e.g. flagellin	Flagellin activates primary sensory neurones through TLR5 in A-fibres. Activation of TLR5 in immune cells leads to release pro-inflammatory mediators, indirectly causing sensitisation of primary sensory neurones and allodynia.
	Maruyama and colleagues[123]; Maruyama and colleagues[124]	CLR ligands, e.g. β-glucan	β-Glucan directly activates primary sensory neurones, possible through Dectin-1 and TRPV1/A1 axis.
	Chiu and colleagues[125]	N-formylated peptides	N-formylated peptides activate primary sensory neurones through FPR-1.
Toxins	Chiu and colleagues[125]	α-HL	α-HL is sufficient to depolarise nociceptive neurones in DRGs via inserting into cell membranes after non-selective entry of cations, leading to pain hypersensitivity.
Metabolites	Vinolo and colleagues[126]; Kukkar and colleagues[127]	SCFAs	SCFAs act on their receptors FFAR2/3 to regulate leucocyte functions, such as production of cytokines, eicosanoids and chemokines. Butyrate, as a HDAC inhibitor, attenuated nerve injury-induced pain.
	Cosi and colleagues[128]; Resta[129]	KYNA	KYNA reduces excitability of primary sensory neurones via GPR35, and its activation led to analgesia.
	Alemi and colleagues[130]; Lieu and colleagues[131]	BAs	Activation of TGR5 by bile acids in the DRG neurones caused extracellular calcium influx, action potentials, and itch responses in mice. Activation of TGR5 by bile acids in macrophages leads to analgesia via endogenous opioids release.
Neurotransmitters/ neuromodulators	Du and colleagues[132]	GABA	Activation $GABA_A$ receptor in the DRG neurones by GABA depolarised the majority of sensory neuronal soma, but produced a net inhibitory effect on the nociceptive transmission because of the filtering effect at nociceptive fibre T-junctions.
	Cortes-Altamirano and colleagues[133]	5-HT	5-HT acts on 5-HT_1 receptor produces hyperpolarising effect, whereas 5-HT acts 5-HT_2 and 5-HT_3 produces depolarising effect on primary nociceptive neurones.
	Sessenwein and colleagues[134]	Serine proteases	Serine proteases reduce action potential discharge from colonic afferent nerves, partial through activation PAR-4.

opioids)[135–137] (Fig. 3). Thus, this suggests that the gut microbiota may play a key role in the direct or indirect regulation of neuronal excitability of peripheral nervous system under chronic pain conditions.

Pathogen-associated molecular patterns (PAMPs) derived from gut microbiota are considered to be important contributors to peripheral sensitisation under chronic pain conditions.[138] PAMPs obtained from gut microbiota including bacterial cell wall components, such as LPS, lipoteichoic acid (LTA), peptidoglycan (PGN), and β-glucan, are released locally and transferred into the circulation and bind to pattern recognition receptors (PRR), including TLRs, expressed on immune cells and sensory neurones located in DRGs,[120,121] participating in peripheral sensitisation under chronic pain conditions. On one hand, these PAMPs act on immune cells to release pro-inflammatory cytokines and chemokines, which are indirectly activated or sensitised primary sensory neurones in DRGs. On the other hand, primary sensory neurones in DRGs can be directly activated or sensitised by PAMPs.[138] For example, LPS can bind to TLR4 to induce activation and sensitisation of nociceptive neurones in DRGs, partially via a TRPV1-mediated mechanism.[117,118] Moreover, LPS directly activates TRPA1 channel and induces release of calcitonin gene-related peptide (CGRP), calcium flux, and action potentials in nociceptive sensory neurones through a TLR4-independent manner.[119] Activation TLR2 of primary sensory neurones by its ligands, such as PGN and aggrecan fragment, also causes neuronal hyperexcitability and pain behaviours in animals.[120] Fungal *Candida albicans*-derived β-glucan can bind Dectin-1, which is expressed in primary sensory neurones in DRGs to produce pain behaviours in mice.[123,124] Further analysis revealed that Dectin-1-mediated PLC-TRPV1/TRPA1 axis contributed to the pronociceptive role of β-glucan.[123,124] Recently, some by-products of bacteria, such

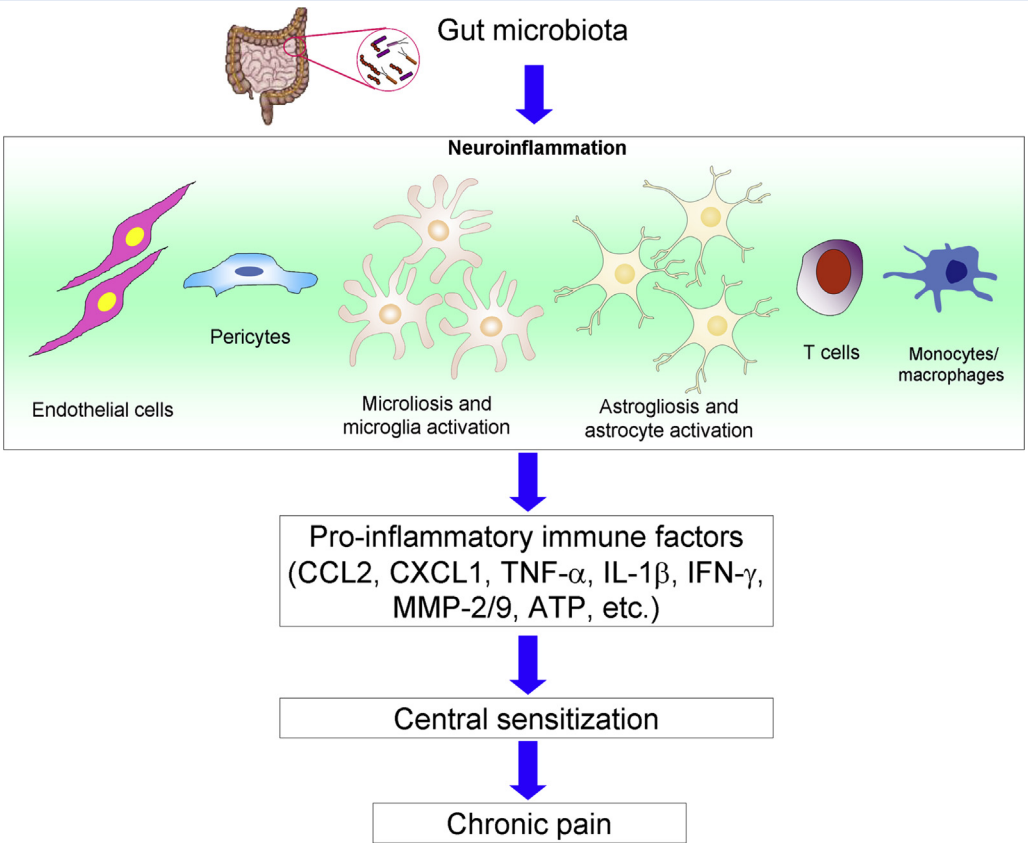

Fig 4. Gut microbiota may regulate neuroinflammation involved in central sensitisation underlying chronic pain. The cell types involved in neuroinflammation regulated by the gut microbiota are including endothelial cells, pericytes, microglia, astrocytes, T cells, and monocytes/macrophages. These cells secrete pro-inflammatory mediators to produce neuroinflammation, such as CCL2 (or MCP-1), CXCL-1, TNF-α, IL-1β, IFN-γ, MMP-2/9, and ATP. ATP, adenosine triphosphate; CCL2, C–C motif chemokine ligand 2; CXCL-1, C-X-C motif chemokine 1; IL-1β, interleukin-1β; IFN-γ, interferon-γ; MMP, matrix metalloprotein; TNF-α, tumour necrosis factor-α.

as *N*-formylated peptides and α-haemolysin, have been observed to regulate pain through distinct mechanisms.[125] *N*-formylated peptides are heat-stable components within bacteria and are recognised by formyl peptide receptors (FPRs), which are G-protein coupled receptors expressed on DRG and TG. *In vivo* and *in vitro* studies suggested that *N*-formylated peptides derived from *Staphylococcus aureus* bacteria activate nociceptor via FPR1. Another component pore-forming toxin, α-haemolysin (α-HL), directly depolarised nociceptive neurones in DRGs via inserting into cell membranes after the non-selective entry of cations.[125] α-HL elicited significant acute pain behaviours in mice and induced calcium flux in DRG neurones in a dose-dependent manner.[125] Thus, bacteria are able to produce pain via directly activating primary sensory neurones, which reveals an unsuspected role of primary sensory neurones in microbiome–host interaction. Therefore, it is suggested that PAMPs derived from gut microbiota may contribute to peripheral sensitisation via either directly acting on primary nociceptive neurones or indirectly acting on immune cells to induce neuronal hyperexcitability, leading to peripheral sensitisation (Fig. 3).

Besides PAMPs, there are several metabolites from gut microbiota; for example, short-chain fatty acids (SCFAs) are demonstrated to regulate pain sensation via multiple mechanisms. SCFAs (mainly including formic, pyruvic, butyric, lactic, and acetic acids) are derived primarily from bacterial fermentation of carbohydrates and proteins. Although the roles of SCFAs in controlling immune responses have been well recognised, their links to neurological disorders, especially chronic pain, have only recently been appreciated. SCFAs act on their receptors, FFAR2/3, and regulate leucocyte functions, such as the production of cytokines (TNF-α, IL-2, IL-6, and IL-10), eicosanoids and chemokines (e.g. CCL2).[126] Butyrate, also as a histone deacetylase (HDAC) inhibitor, attenuated pain behaviours and TNF-α level in a peripheral nerve injury model.[127] A recent study showed that butyrate, which decreased dramatically in faecal samples,[140] was highly effective in relieving peripheral nerve sensitisation and abdominal pain in patients with IBS and IBD.[141] Thus, this indicates that SCFAs may be important mediators derived from gut microbiota for regulation of pain through receptor-mediated mechanisms, epigenetic regulation mechanisms, or both (Fig. 3).

There is evidence supporting the hypothesis that gut microbiota also play a role in alternative routes of kynurenic acid (KYNA) production.[142] GPR35, an important receptor

activated by KYNA, was expressed in small-sized DRG neurones, and its activation led to a reduced excitability of DRG neurones *in vitro* and caused analgesia in a dose-dependent manner *in vivo*.[128,129] It was found that activation of GPR35 in DRG neurones reduced adenylate cyclase activity and inhibited hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels in DRG neurones.[129] Thus, kynurenic acid metabolism may represent a link between gut microbiota and chronic pain (Fig. 3).

The gut microbiota has emerged as a crucial player that influences bile acid (BA) metabolism.[143] As an endogenous membrane receptor for BAs, G protein-coupled bile acid receptor 1 (GPBAR1; TGR5) is expressed by both primary sensory neurones and macrophages. Activation of TGR5 by BAs, such as deoxycholic acid (DCA), in DRG neurones caused extracellular calcium influx, action potentials, and itch responses, via a TRPA1-dependent mechanism.[130,131] However, activation of TGR5 by BAs in peripheral macrophages leads to analgesia via endogenous opioids release. Thus, although the alteration in gut microbiota composition, and hence its derived secondary BA metabolites under chronic pain are still unknown, secondary BA metabolites may regulate pain, possibly via a TGR5-mediated mechanism (Fig. 3).

Many neurotransmitters and neuromodulators, which may remarkably affect pain signalling, can be generated by gut microbiota. For example, a chief inhibitory neurotransmitter, GABA, can be commensally produced via enzymatic decarboxylation of glutamate by GadB, possibly by *Lactobacillus* spp., *Bifidobacterium dentium*, and *Bifidobacterium* spp.[23] Interestingly, daily oral administration of GABA-producing *Bifidobacterium* strain modulated sensory neurone activity in a rat faecal retention model of visceral hypersensitivity.[111] Recent studies demonstrated that $GABA_A$ receptor was expressed by DRG neurones, and its activation by GABA depolarised the majority of sensory neuronal soma, but produced a net inhibitory effect on the nociceptive transmission because of the filtering effect at nociceptive fibre T-junctions.[132] Serotonin (5-HT), another important neurotransmitter, can be produced by *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. In peripheral tissues, 5-HT acts as a pain-inducing mediator and 5-HT may play a specific role in several types of headache, in particular migraine.[83] 5-HT receptor family is divided into seven subfamilies (5-HT_1 to 5-HT_7), comprising 15 receptor subtypes.[133] 5-HT acts on 5-HT receptors, such as 5-HT_{1a}, 5-HT_2, and 5-HT_3, to regulate peripheral pain sensitisation. In general, activation of the 5-HT_1 receptor produces a hyperpolarising effect, whereas activation of 5-HT_2 and 5-HT_3 produces a depolarising effect on primary nociceptive neurones.[133] Recent work demonstrated that a community of commensal GI bacteria derived from a healthy human donor (microbial ecosystem therapeutics [MET]-1) reduced the excitability of DRG neurones by significantly increasing rheobase, decreasing responses to capsaicin, and reducing action potential discharge from colonic afferent nerves, partly through the activation of protease-activated receptor-4 (PAR-4).[134] Furthermore, *F. prausnitzii* reproduced the effects of MET-1 on excitability of DRG neurones, suggesting that serine proteases derived from commensal bacteria can directly impact the excitability of DRG neurones, through PAR-4 activation.[134] Together, the complex interactions of microbiota and neurones may directly affect peripheral pain sensation (Fig. 3).

Central mechanisms

Both preclinical and clinical studies have implicated neuroimmune activation (also called neuroinflammation) as crucial mechanisms underlying central sensitisation of chronic pain induced by inflammation or nerve injury.[14,144] Activation of glia (e.g. microglia and astrocytes) can produce pro-inflammatory cytokines or chemokines, such as TNF-α, IL-1β, and CXCL1, and can result in elevated glutamatergic synaptic neurotransmission, decreased GABAergic synaptic neurotransmission, or both.[145–148] Both effects contribute to the development of central sensitisation, leading to pain hypersensitivity (Fig. 4).[149]

Intriguingly, recent work suggested that several cell types in the brain, including endothelial cells, pericytes, microglia, astrocytes, and infiltrated immune cells, are able to receive input from the periphery, including from the GI tract.[150–152] The activation of these cells contributes to the development of neuroinflammation (Fig. 4).[14] Notably, gut microbiota plays a pivotal role in the regulation of maturation, morphology, and immunological function of microglia.[153,154] Given that gut microbiota can participate in the pathogenesis of many neurological disorders, such as PD, via regulation of microglia by its metabolites, SCFAs,[155] it therefore warrants further investigation to determine whether neuroinflammation-mediated central sensitisation underlying chronic pain can be directly or indirectly regulated by gut microbiota.

Therapeutic implications of targeting gut microbiota in chronic pain

Probiotics, prebiotics, and synbiotics

Probiotics are living bacteria which can provide health benefits when consumed.[156] Probiotics have enormous potential for modifying the gut microbiota. The beneficial effects of probiotics have been well demonstrated, including improved digestion, boosted immunity, and lowered risk of certain diseases.[157,158] Preclinical animal studies have demonstrated the beneficial effects of probiotics on visceral pain. A recent study showed that *Clostridium butyricum*, a common human and animal gut commensal bacterium, may exert a beneficial action on the visceral hypersensitivity of IBS by inhibiting colonic inflammation in mice.[112] Another study showed that *Roseburia hominis* may be a potential probiotic for treating stress-induced visceral hypersensitivity, because reduction in the abundance of butyrate-producing Lachnospiraceae was involved in the development of visceral hypersensitivity in rats.[113] The probiotic *B. infantis* 35624, but not *Lactobacillus salivarius* UCC118 and *Bifidobacterium breve* UCC2003, significantly reduced CRD-induced visceral pain in rats.[159] The probiotic VSL#3 decreases visceral hypersensitivity in rats, possibly involving the mast cell–PAR2–TRPV1 signalling pathway.[115] Probiotic *L. rhamnosus* GG attenuated chronic visceral pain induced by neonatally intracolonic administration of zymosan in rats.[45] A probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) prevents chronic stress-mediated brain function abnormalities by attenuating the hypothalamic–pituitary–adrenal (HPA) axis response.[160] Pre-treatment with the probiotic VSL#3 also attenuated morphine analgesic tolerance in mice.[97]

Clinical studies further demonstrated the beneficial effects of probiotics on chronic pain. A randomised, double-blind, placebo-controlled trial demonstrated that administration of

L. reuteri DSM 17938 significantly reduced the frequency and intensity of functional abdominal pain in children.[109] Probiotic preparations may improve abdominal pain in children in the short term.[114] However, another randomised, double-blind, placebo-controlled trial found that *Saccharomyces cerevisiae* CNCM I-3856 at a dose of 1 g day^{-1} does not improve intestinal pain and discomfort in patients with general IBS.[110] A multicentre, randomised, double-blind, placebo-controlled, crossover trial looking at a probiotic mixture of *B. infantis* M-63, *B. breve* M-16V, and *B. longum* BB536 demonstrated improved abdominal pain and quality of life in children with IBS.[161] Thus, there is both positive and negative evidence for the role of probiotics in pain associated with GI disorders.[162]

Probiotics can affect the inflammatory response affecting cytokines, including pro-inflammatory and anti-inflammatory cytokines.[163,164] Given the pivotal role of probiotics in regulating functions of immune system,[156] the possible analgesic effects of probiotics on inflammatory pain are expected. Indeed, treatment with probiotics was shown to alleviate pain and improve quality of life in patients with rheumatoid arthritis.[165] Moreover, probiotics might regulate pain through gene expression of pain-related receptors on epithelial cells.[42,166] For example, *L. acidophilus* NCFM up-regulated the expression of cannabinoid receptor 2 and colonic μ-opioid receptor, leading to reduced pain sensation.[52] Together, probiotics may be potential reagents for the treatment of chronic pain.

Prebiotics are considered as additional support for probiotics or as an alternative to them. Prebiotics may also exert beneficial effects on chronic pain. In humans, a prebiotic galacto-oligosaccharide mixture (B-GOS(R)) was shown to reduce abdominal pain associated with GI disorders in adults.[167] Prebiotics PDX/GOS attenuated chronic visceral pain induced by neonatally intracolonic administration of zymosan in rats.[45] A clinical study showed that the synbiotic containing *Bacillus coagulans* and fructo-oligosaccharides seems to be effective for the treatment of functional abdominal pain in children.[168] Notably, identification of personalised microbiota alterations, i.e. the identification of patient subsets with a distinct microbiota profile, may be necessary for developing a targeted approach to restore specific populations of beneficial bacteria for the management of chronic pain.

Low-FODMAP intervention

Dietary interventions refer to supplementing a specific ingredient, or a food from the diet, or excluding/restricting it, thereby modifying its intake.[169] For example, there are many dietary interventions that may have beneficial effects on health, such as fibre supplements,[170] dairy products or fructose,[171] and proteins.[172] It has been demonstrated that low-FODMAP (LFM) intervention exerted symptomatic benefits in IBS patients, such as decreasing abdominal pain by reducing fermentation and gas production.[54] Further investigation showed that a high-FODMAP (HFM) diet induced gut microbial dysbiosis and increased faecal LPS levels.[28] In contrast, an LFM diet reduced faecal LPS by modulating gut microbial composition.[28] Therefore, an LFM diet may be beneficial for reducing mucosal inflammation, restoring gut barrier function, and alleviating visceral pain.[28] Although studies consistently demonstrate the clinical effectiveness of LFM diet in patients with IBS, the impact of this dietary intervention on gut microbiota may be complicated. Luminal *Bifidobacteria* concentration, which is increased by probiotic supplementation, is associated with a reduction in IBS symptoms. Paradoxically, it was markedly reduced by LFM diet.[173] A randomised, double-blind trial showed that *psyllium* fibre reduced the number of abdominal pain episodes in children with IBS, whereas *psyllium* did not alter gut permeability or microbiome composition.[174] Given the heterogeneity of chronic pain and the complex and diverse nature of the microbiome, individual dietary interventions may be developed for management of chronic pain, in particular, visceral pain.[175,176]

Faecal microbiota transplantation

FMT, also called faecal bacteriotherapy or stool/faecal transplantation, is the infusion or engraftment of liquid filtrate faeces from a healthy donor into the gut of a recipient to treat many diseases, including *Clostridium difficile* infection, IBD, obesity, and insulin resistance.[55,177,178] An open-label study on FMT in IBS patients showed improvement in abdominal pain, which was associated with the relative abundance of *Akkermansia muciniphila*.[179] A patient with fibromyalgia, with a predominant symptom of pain, was reported to be in full recovery after FMT.[180] In this study, the most prominent changes at the genus level included an increase in faecal *Bifidobacterium* proportion from 0% to 5.23% and a reduction in *Streptococcus* from 26.39% to 0.15%.[180] Moreover, because colonic supernatants from chronic morphine exposure induced acute tolerance and neuronal hyperexcitability in naïve DRG neurones, engraftment of microbiota competing with indigenous gut microbiota may prevent or delay the development of morphine tolerance.[181] Several mechanisms underlying the therapeutic effects of FMT on chronic pain were proposed, including direct competition of pathogenic bacteria with commensal microbiota, protection of the intestinal barrier, restoration of secondary BA metabolism, and stimulation of the intestinal immune system. Therefore, it is suggested that FMT may become a promising approach to treatment of chronic pain, especially visceral pain related to GI disorders.

Concluding remarks and future perspectives

Although the emerging role of microbiota in the bidirectional communication in the gut–brain axis is attracting increasing attention, many questions remain. What is the characteristic of gut microbiota under chronic pain conditions? What is the distinct role of gut microbiota in different types of pain, including visceral pain, inflammatory pain, and neuropathic pain? What are the details of microbiota–gut–brain signalling in the pathogenesis of chronic pain? Do gut microbiota play any role in vulnerability or resilience towards developing chronic pain? Is it possible that the specific signature of gut microbiota is a biomarker of chronic pain?

Future investigations elucidating the molecular mechanisms underlying gut microbiota modulating pain may be necessary for the discovery of novel drug targets for pain relief.

It is noteworthy that gut microbiota may also play a critical role in depression and anxiety, which co-exist with pain comorbidities. Thus, an additional exciting frontier may be modulation of gut microbiota for management of pain comorbidities via the management of emotional illness. It remains to be seen whether FMT is a valuable therapeutic option for pain, especially visceral pain, in the future. Finally, given that dietary intervention is the most readily amenable strategy for modulation of gut microbiota, it will be of interest to

determine the effects of specific dietary interventions on management of chronic pain.

Study of the role of the human microbiome in health and disease is a fast-growing field in both academia and industry.[182] It is accepted that gut microbiota play a critical role in the development of neurological diseases, such as AD, PD, autism, depression, and chronic pain. Although our understanding of the role of gut microbiota in pain is still in its early stages, emerging evidence suggests that dysregulation of gut microbiota participates in visceral pain, inflammatory pain, neuropathic pain, migraine, and opioid tolerance. Therefore, we propose that modulation of gut microbiota by diet and pharmabiotic intervention offers a promising approach to the management of chronic pain.

Authors' contributions

Literature search: RG, LHC
Draft preparation: RG, LHC
Review of the manuscript and sharing of critical viewpoints: LHC, CX, TL
Project supervision: TL
Writing of the manuscript: TL
All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no conflicts of interest.

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