OPEN ACCESS

Gastrointestinal microbiota and inflammasomes interplay in health and disease: a gut feeling

Roberto De Luca,[1] Valentina Arrè,[2] Stefano Nardone ,[3] Sandra Incerpi,[4] Gianluigi Giannelli,[5] Pankaj Trivedi,[6] Eleni Anastasiadou,[7] Roberto Negro [2]

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/gutjnl-2025-334938).

For numbered affiliations see end of article.

Correspondence to
Dr Roberto Negro; roberto.negro@irccsdebellis.it and Roberto De Luca; rdeluca@bidmc.harvard.edu

RDL and VA are joint first authors.
GG, PT, EA and RN are joint senior authors.

Received 12 February 2025
Accepted 12 May 2025

ABSTRACT

The intricate interplay between the gut microbiota and the GI tract has garnered significant attention, as growing evidence has identified the inflammasome as a crucial yet underexplored master regulator in microbiota-driven diseases. Triggered by a variety of dangers, inflammasomes are supramolecular complexes that regulate immune response. A large number of bacterial-derived inducers have been characterised so far. Although structurally divergent, threats are neutralised by the inflammasome, which is then classified into three families: (1) nucleotide-binding oligomerisation domain, leucine-rich repeat-containing proteins, (2) absent in melanoma 2-like receptors and (3) pyrin. An unbalanced microbiota composition, expressed by a dysbiotic phenotype, might therefore induce undesired inflammasome activation, altering the local host homeostasis. Recent studies on the 'microbiota-inflammasome axis' have uncovered unexpected roles for inflammasome signalling in various types of GI cancer and IBD. Additionally, beyond local gut functions, microbiota influences stress responses and neurological health through aberrant secretion of inflammasome-processed cytokines, linking gut-derived signals to systemic diseases via the vagus nerve and the hypothalamic-pituitary-adrenal axis. Besides the standard experimental approaches, this complex network of interactions is now being addressed by Artificial intelligence, which emphasises the profound impact of the gut microbiota on GI health, cancer progression and brain function, opening new avenues for therapeutic intervention in GI diseases, cancer and neurological disorders. Ultimately, microbiota-inflammasome interactions manage a regulatory framework that influences inflammation, cancer progression and systemic diseases, positioning it as both a mediator and a promising therapeutic target in GI malignancies and systemic diseases of the central nervous system.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The GI tract regulates nutrient digestion, absorption, excretion and exerts key immune and endocrine functions.
⇒ Unbalanced microbiota (dysbiosis) can lead to inflammasome activation, disrupting the host homeostasis.
⇒ Dysbiosis is linked to multiple GI diseases, including IBD, obesity, cardiovascular disease, cancer and neurological disorders such as Parkinson's and Alzheimer's disease.

WHAT THIS STUDY ADDS

⇒ The microbiota-inflammasome axis influences GI cancer and neurological health, linking gut signals to systemic diseases through the vagus nerve and hypothalamic-pituitary-adrenal axis.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Artificial Intelligence is being used to explore dysbiosis-inflammasome interactions, offering new insights into therapeutic possibilities.

INTRODUCTION

The GI tract is a remarkable body part that performs various essential tasks, working in harmony across different systems and timeframes. In recent years, scientists have made significant progress in understanding how the GI tract cells, tissues and organs work together to perform its essential functions. The primary role of the GI tract is to regulate nutrient digestion, absorption, excretion and immune protection.[1] In particular, it manages the body's balance of essential electrolytes and acid-base through the digestion and assimilation of food, along with key immune, endocrine and barrier responsibilities. The proper function of the GI tract depends on a highly integrated system that includes the microbiota—a diverse community of bacteria, protozoa, viruses, archaea and fungi and myogenic, neural, humoral and immune components that regulate anatomical, endocrine, metabolic and immunological processes.[1] The human microbiota consists of trillions of microorganisms primarily residing in the gut; it is established after birth and shapes the immune system.[2] With over 100 bacterial phyla and about 150 times more genes than the human genome, the microbiota performs essential protective functions that influence nearly every aspect of human biology in a symbiotic relationship with its host, serving as a competitor against pathogenic microbes.[2] In healthy subjects, Firmicutes and *Bacteroides* constitute over 90% of the entire gut microbiota, followed by *Proteobacteria* and *Actinobacteria*.[2] Microbial colonisation in the gut begins at birth and progresses through a succession of taxonomic changes until it achieves balanced, adult-like diversity.[3] Increased evidence over the past decade has shown that early dysbiosis modulates physiological, metabolic and immunological functions across several regions, including the GI tract and neurological tissues. These effects span a range of conditions from obesity and diabetes to intestinal, cardiovascular or neurological diseases,

Check for updates

To cite: De Luca R, Arrè V, Nardone S, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2025-334938

with long-term implications.[4–10] After childhood, the unique proportion of the various species remains quasi-stable in the individual life span, although in the elderly a shift has been observed from Firmicutes towards *Proteobacteria* and *Alistipes*.[11] The microbiota's recognised roles include preservation of: (1) the homeostatic intestinal mucosal barrier, which provides deterrence against pathogenic microbes, and is therefore vital for overall health and disease prevention[12]; (2) the fine balance between the pro-inflammatory and anti-inflammatory local and systemic responses, ensuring the maintenance of the immune system in a perpetually vigilant state for successful protection against insults[12]; (3) the neutralisation of harmful pathogens through the release of antimicrobial metabolites[12]; (4) the management of nutrient fermentation, synthesis of vitamins and drug metabolism[12] and (5) the delivery of essential nutrients, such as short-chain fatty acids to colonic epithelial cells,[12] along with important functions in brain development.[4] Over the past decade, research has greatly expanded our understanding of the biochemical connections between the central nervous system (CNS), the autonomic nervous system (ANS) and the enteric nervous system, often referred to as the 'second brain', forming what is now known as the gut-brain axis (GBA).[12] This crosstalk, mediated by nerves within the GI tract, connects the gut to the brain, allowing the latter to influence intestinal activities and the gut to modulate neural tasks.[13] The vagus nerve, or cranial nerve X, is the longest nerve of the ANS and is primarily responsible for the gut-brain dialogue between the CNS and the GI tract. It regulates parasympathetic functions and transmits motor signals to organs such as the pancreas, bile ducts, spleen, stomach, GI tract, lungs, heart and bronchial structures. It also receives sensory feedback from these organs and plays a key role in the inflammatory reflex, controlling innate immunity and the inflammatory response during infection and tissue damage.[14] Thus, the balance between the different microbial strains, a condition called eubiosis, which mirrors the host's health status, along with the crosstalk between the GI and anatomically distant districts, is crucial in maintaining the proper GI functions and homeostasis, aspects aimed at reducing the risk of developing critical illness.[15] The eubiosis phenotype is age-dependent and constantly influenced by the host genome, prolonged broad-spectrum antibiotic usage and environmental and lifestyle factors, which define the biological diversity among individuals.[16] Hence, a shift towards a more pronounced dysbiotic scenario, a condition characterised by a compromised composition and abundance of specific phyla, might alter the natural individual's susceptibility to various diseases, framing the so-called 'leaky gut' syndrome and, consequently, microbial dissemination in the host bloodstream.[17] The best-characterised microbiota-related GI diseases can be subdivided into six macro areas, summarised in figure 1: (1) the GI and hepatic diseases, which encompass IBS, IBD and liver diseases; (2) metabolic diseases, such as obesity and diabetes[18 19]; (3) cardiovascular diseases, including heart failure, atherosclerosis and hypertension; (4) immune-related disorders, such as allergy,[18] eczema,[20] ocular disorders,[21] asthma[18]; (5) oncological diseases, such as colorectal cancer (CRC),[18] hepatocellular carcinoma,[22] cholangiocarcinoma,[23] pancreatic ductal adenocarcinoma (PDA),[18] gastric cancer (GC)[18]; (6) neurological, neuroinflammatory and psychiatric disorders, like autism spectrum disorder (ASD), anxiety, depression, Parkinson's disease (PD) and Alzheimer's disease (AD),[18] multiple sclerosis (MS),[24] stress[25] and addiction.[26] While beneficial strains strive to support the host's life, dysfunctional microbiota manipulates the innate immune system, sabotaging the individual's health. A key mechanism involves the persistent activation of inflammasomes, a complex of receptors and sensors within innate immune cells.[27] Although inflammasomes are essential for maintaining cellular integrity and tissue homeostasis, chronic activation or dysbiotic microbiota can harm the GI tract and GBA. Artificial intelligence (AI) techniques have now emerged as a powerful tool for deciphering the complexities of the microbiome in the context of several GI diseases. The AI-based approach facilitates predictive modelling, biomarker discovery and a deeper understanding of underlying biological mechanisms that traditional methods may overlook.[28] This review explores the fascinating interplay between the gut microbiota and inflammasomes, highlighting how this relationship affects GI health and other body systems, such as the GBA. We also explore potential AI implementation strategies to harness this knowledge as a tool helping to develop innovative therapies.

INFLAMMASOMES, NOVEL INSIGHTS ON THE MOLECULAR MECHANISM OF ACTIVATION

The inflammasome is an intracellular multiprotein complex thought to play a crucial role in GI disorders mediated by microbiota dysfunctions. Structurally defined as an inducible supramolecular complex, the inflammasome recognises various dangers, which ultimately licence its activation. This complex comprises three key components: a sensor unit, an adapter molecule and an effector component that work together to detect and respond to cellular stress or microbial signals. To date, the inflammasome is broadly classified into three families of sensor proteins: (1) nucleotide-binding oligomerisation domain, leucine-rich repeat-containing proteins (NLRs), (2) absent in melanoma 2 (AIM2)-like receptors and (3) pyrin. Differences in the N-terminus effector domain further classify the NLR family into two subgroups: NLRP, containing a pyrin domain and NLRC, containing a CARD domain, with a caspase activation and recruitment domain (figure 2). Distinct cell types, such as immune cells (eg, monocytes, macrophages, dendritic cells) and non-immune cells (eg, intestinal epithelial cells, fibroblasts), express various combinations of these inflammasomes. This enables comprehensive detection of mechanistically disparate microbial inputs. On engagement by exogenous insults, such as pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs), the sensor proteins begin to self-assemble and signal through the inflammasome complex. Surprisingly, a crucial hallmark of inflammasome activation is the formation of a single supramolecular punctum (also known as a speck or pyroptosome) per cell. Although chemically diverse, all sensor proteins converge on the same adaptor protein, named apoptosis-associated speck-like protein containing a CARD (ASC), which forms an intracellular filament measuring approximately 1–3 µm in length that, in turn, signals to the cysteine protease, caspase-1. Active caspase-1 (p20 fragment) operates on three substrates: pro-inflammatory interleukin (pro-IL)-1β, pro-IL-18 and the effector protein gasdermin D (GSDMD). Caspase-1 cleaves the N-terminus fragment of GSDMD, which oligomerises into a ring-shaped structure able to permeate the plasma membrane, thus allowing the release of the bioactive cytokines IL-1β and IL-18 into the stream, concomitantly with a form of inflammatory cell death, known as pyroptosis. Moreover, a recent study by Kayagaki *et al* showed that efficient plasma membrane rupture (PMR) relies on a parallel process mediated by ninjurin 1 (NINJ1). However, the detailed molecular mechanism of activation on induction of lytic cell death is still under intense investigation. Likewise, GSDMD-executed plasma membrane ring-shaped pores, structural and imaging

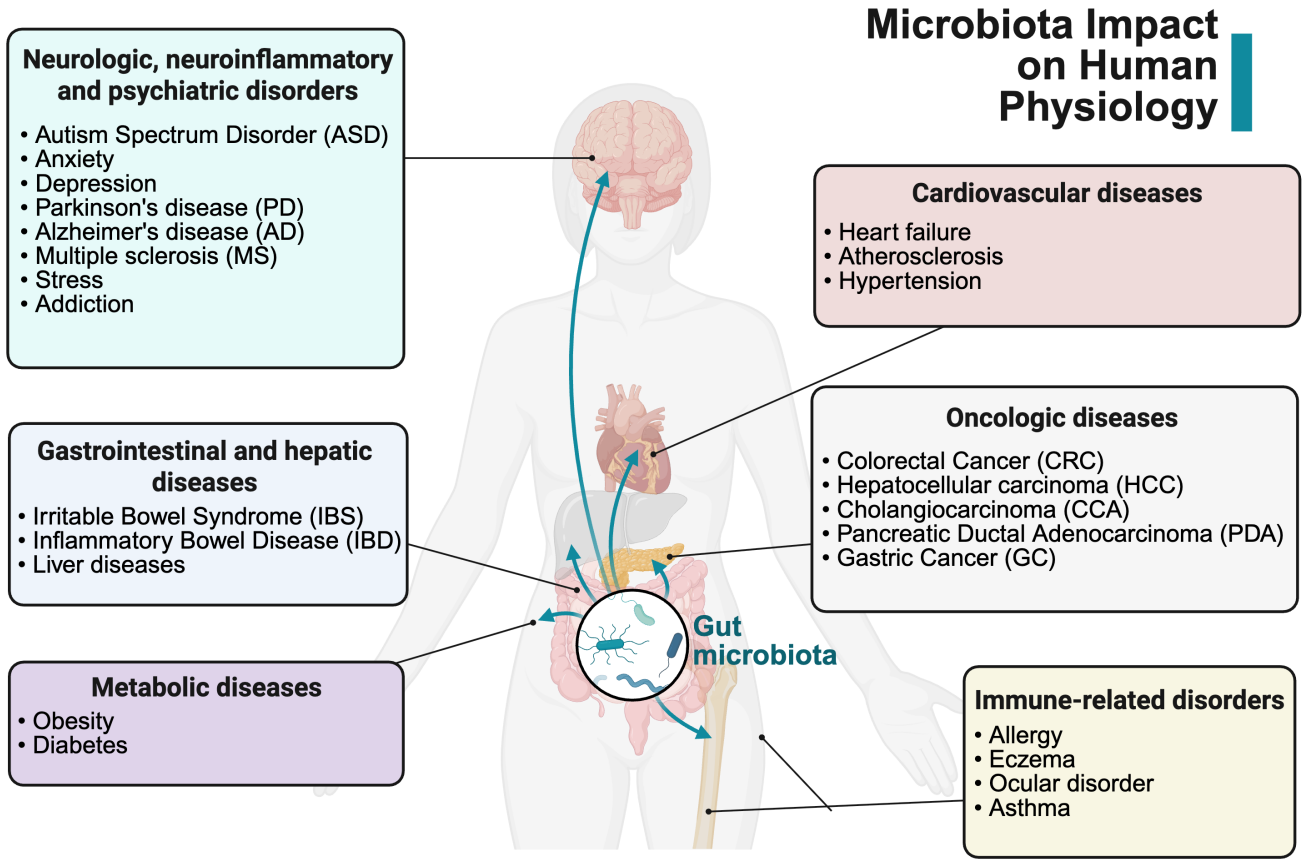

Figure 1 Overview of the microbiota-derived pathological outcomes. Several pathological conditions have been associated with changes in microbiota composition, encompassing metabolic diseases, such as obesity and diabetes; cardiovascular diseases, including heart failure, atherosclerosis and hypertension; immune-related disorders, such as allergy, eczema, ocular disorders and asthma; oncological diseases, such as colorectal cancer (CRC), hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), pancreatic ductal adenocarcinoma (PDA) and gastric cancer (GC); neurological, neuroinflammatory and psychiatric disorders, which involve autism spectrum disorder (ASD), anxiety, depression, Parkinson's disease and Alzheimer's disease (PD and AD), multiple sclerosis (MS), stress and addiction.

data demonstrate the presence of 'cookie''-like disks of oligomerised NINJ1 encircling and releasing membrane patches, ultimately resulting in membrane fragmentation and complete PMR.[29 30] Several inflammasome cell context-specific types have so far been characterised in mammals: NLRP1, NLRP3, NLRP6, NLRP7, NLRP9, NLRP10, NLRP11, NLRP12, NLRC4, NLRC5, AIM2, IFI16 and pyrin, among which the NLRP3 activation mechanism is the most well-studied (figure 3).

THE INFLAMMASOME: A MISSING MASTER REGULATOR OF MICROBIOTA-DRIVEN GI PATHOPHYSIOLOGY

It is well accepted that most of the GI functions may rely on microbial components that colonise the digestive tract of every individual. When tissue homeostasis at the GI level is disrupted, the microorganisms can shift from commensals to pathogenic species. The host genome, diet, lifestyle and antibiotic consumption are among the key factors responsible for altered phenotypes that could radically unbalance the commensal proportions and lead to dysbiosis. Changes in this composition are crucial for the initiation and maintenance of the inflammatory milieu that guides a broad spectrum of GI-related diseases such as IBD, including Crohn's disease (CD) and UC, coeliac disease, various types of GI cancer[30] and diabetes.[31 32] Inflammasomes represent a key bridge linking microbiota-driven molecular events to the disease onset. While inflammasome-derived signals are considered beneficial against threats, chronic activation may lead to disease onset. Consequently, its regulation must be tightly tuned since an uncontrolled activation could result in widespread systemic inflammation. Given the high density of the microbial community within the GI tract, as well as the role of the host inflammasome as a microbial sensor, it is reasonable that these two components operate reciprocal regulation. Recent examples describing the direct and indirect crosstalk between dysbiotic microbiota and inflammasome regulation underlie some of the major classes of inflammasomes so far described (online supplemental table 1). Proteolytic cleavage by lethal toxin, derived from the Gram-positive bacteria *Bacillus anthracis*, triggers the oligomerisation of the NLRP1 C-terminus domain and caspase-1 activation.[33] In addition, IpaH7.8 E3 ubiquitin ligase secreted by *Shigella flexneri* triggers NLRP1 following ubiquitination.[34] As for indirect stimuli, two agents have been identified so far, including *Toxoplasma gondii*,[35] *Listeria monocytogenes*.[36] Contrasting literature has been reported on the role of NLRP1 in IBD. While Williams *et al* showed that NLRP1-deficient mice, which exhibit low levels of IL-1β and IL-18, are more vulnerable to developing colitis than their wild-type counterparts,[37] another

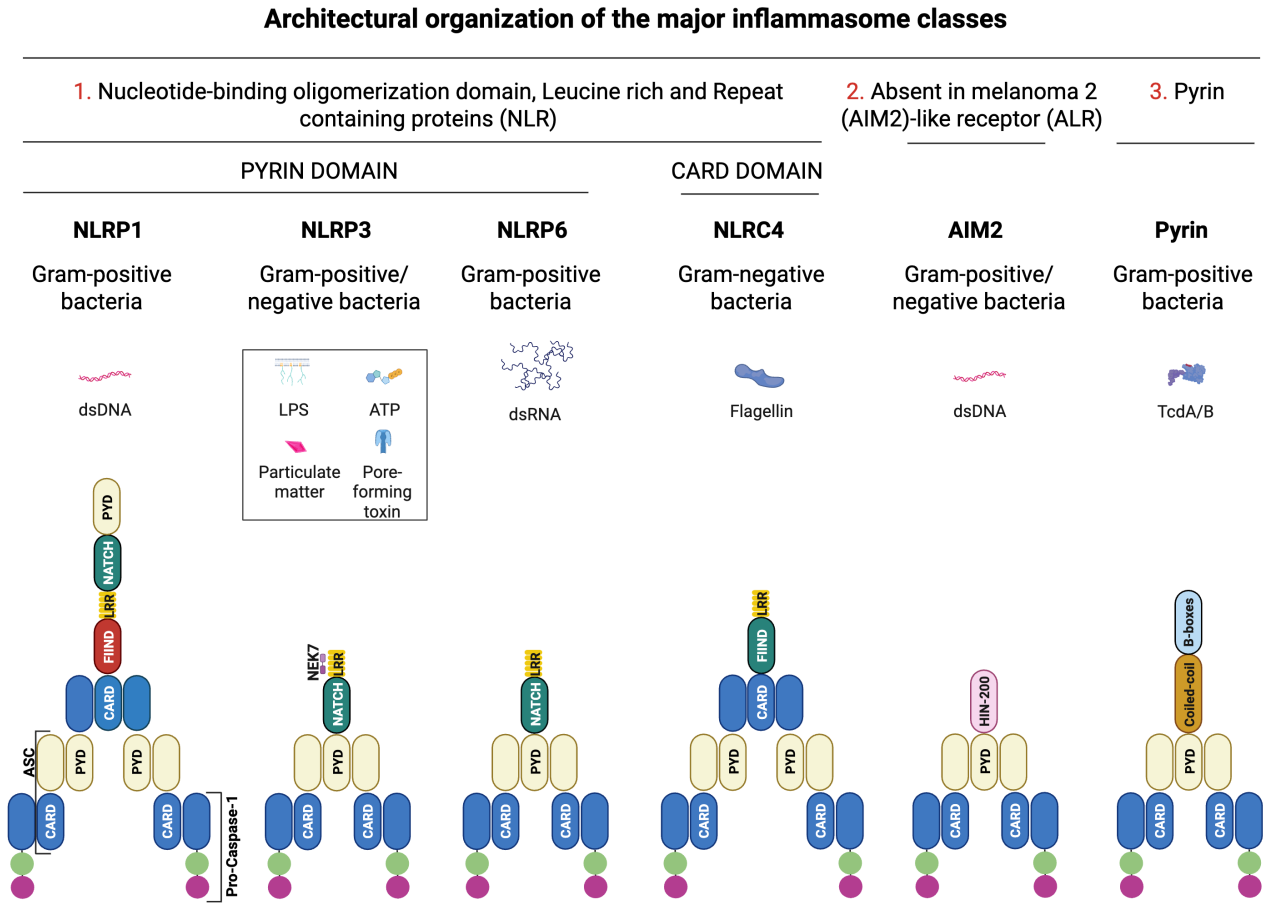

Figure 2 Domain architecture of different inflammasome sensors and their interacting ligands represented by distinct bacterial species. The NLR sensor proteins are composed of the effector domain for caspase-1 recruitment (CARD), pyrin domain (PYD) for apoptosis-associated speck-like protein containing a CARD (ASC) recruitment, a nucleotide-binding and oligomerisation domain NACHT and a leucine-rich repeats domain (LRR), indispensable for the binding to NEK7 and fully NLRP3 activation. In addition, the NACHT and the LRR domains are required for auto-inhibition of the sensor proteins in the absence of stimulation. Absent in melanoma 2-like receptors (ALRs) comprise a PYD domain and the double-stranded DNA-binding domain HIN200. Finally, the pyrin sensor necessitates a coiled-coil and B-box domain for activation. On recruitment and assembly via PYD domain, ASC then interacts with pro-caspase-1 via CARD/CARD interactions. Therefore, the bipartite nature of ASC represents the core structure of these inflammasomes. The inflammasome sensor NLRP1 recognises double-stranded RNA (dsRNA); NLRP3 is triggered by a variety of stimuli that converge to a potassium efflux and calcium intracellular mobilisation; NLRP6 binds to dsRNA; NLRC4 interacts with flagellin; AIM2 is triggered by dsDNA, whereas pyrin inflammasome senses *Clostridioides difficile* toxin A/B.

research group, led by Tye *et al* in 2018, disclosed competencies of NLRP1 in modulating the abundance of butyrate-producing commensals, which exacerbate IBD.[38] In particular, IL-18, but not IL-1β, derived from the activation of the NLRP1 inflammasome, alters the percentage of butyrate-producing bacteria (of the *Clostridiales* order), known to enhance mucus synthesis and the expression of tight junction proteins, both indispensable to preserve the epithelial intestinal barrier (EIB) functions, thus causing susceptibility to IBD.[39] Therefore, more confirmations are needed to attribute protective or harmful tasks to NLRP1 in the IBD background. Moreover, *Proteus mirabilis* (a colonising pathobiont residing in the GI tract) is capable—by synthesising haemolysin—of promoting robust NLRP3 inflammasome activation that induces the maturation and release of IL-1β, responsible for intestinal inflammation.[40] Similarly, *Aeromonas hydrophila*,[41] *Aeromonas dhakensis*[42] and *L. monocytogenes*[43] have been linked to the activation of the NLRP3 inflammasome. A form of oral dysbiosis caused by *Klebsiella aerogenes* or *Enterobacter* species causes robust production and release of pro-inflammatory cytokines via activation of the macrophage NLRP3, thus inducing periodontitis and colitis.[44] Likewise, NLRP1 and NLRP3, the NLRP6 inflammasome, is also subjected to the metabolic activity of the gut microbiome. Sulfur-containing amino acid taurine, histamine and spermine, deriving from commensal symbionts,[45] as well as lipoteichoic acid from Gram-positive pathogen *L. monocytogenes*, induce expression and secretion of IL-18 via the formation of intracellular liquid-like condensed phase (also known as liquid-liquid phase separation).[46] In this context, IL-18 performs a pivotal activity in enhancing immunity. In 2015, Levy *et al* demonstrated that IL-18 promotes antimicrobial peptides (AMPs) production from the intestinal epithelial cells to shape the intestinal microenvironment.[45] AMPs suppress the overgrowth of colitogenic pathobionts that are responsible for chronic intestinal inflammation, such as Prevotellaceae and those belonging to the phylum Saccharibacteria,[47] as well as *Akkermansia muciniphila*, whose colonisation degrades goblet cells-deriving mucin, the major component involved in the mucus layer homeostasis.[48] It has

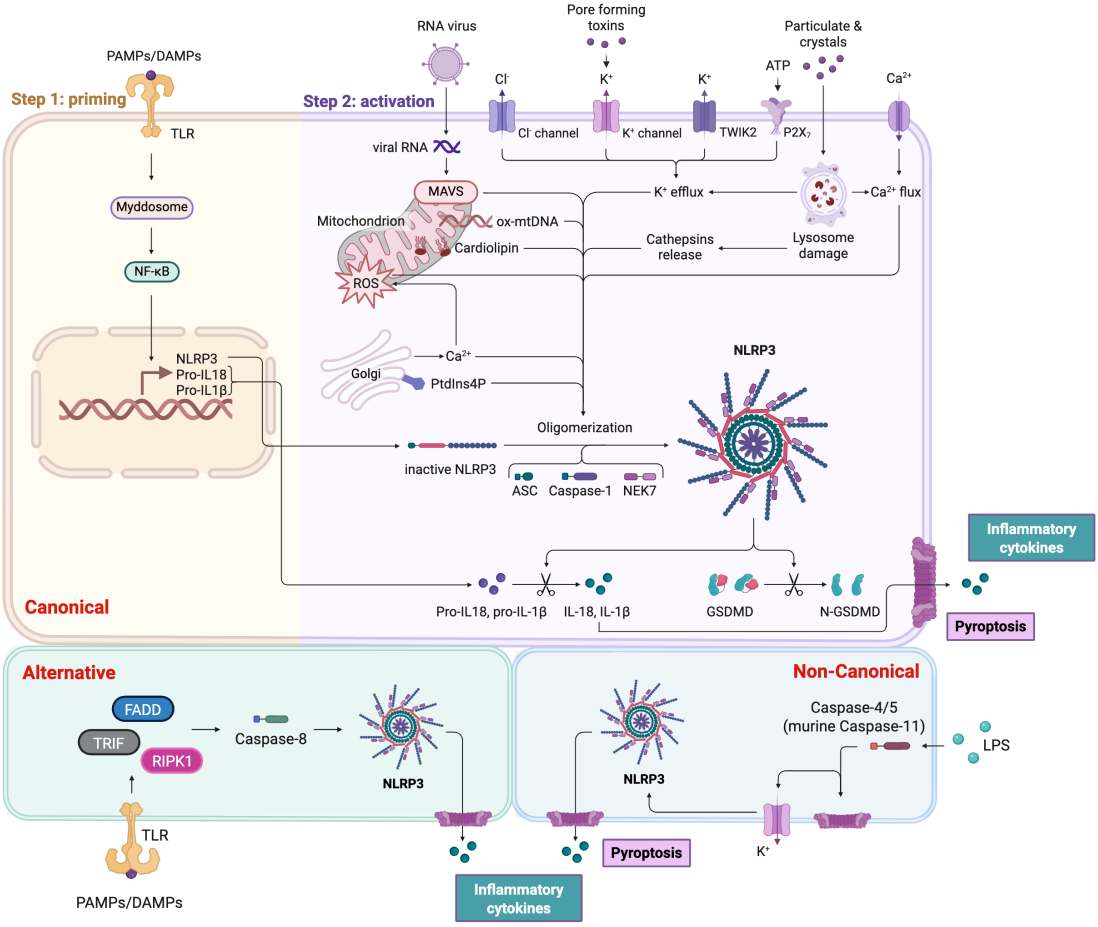

Figure 3 Mechanisms of canonical, non-canonical and alternative NLRP3 inflammasome activation. Canonical nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain containing 3 (NLRP3) inflammasome activation requires two consequential steps: priming and activation. On pathogens-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) recognition by toll-like receptors (TLRs), the priming step (or signal 1, highlighted in yellow on the left) engages a complex of intermediate proteins named Myddosome, which, in turn, signal to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and incite the expression of NLRP3, pro-interleukin (IL)-1β and IL-18 (pro-IL-1β and pro-IL-18). The activation step (signal 2, highlighted in purple on the right) is provided by a wide range of PAMPs/DAMPs, accompanied by a number of cellular events, such as ion fluxes, which ensure the proper inflammasome nucleation and culminates in the self-cleavage and activation of pro-caspase-1. Active caspase-1 induces the maturation of gasdermin D into the active form (N-GSDMD) and the release of IL-1β and IL-18 in the extracellular milieu, concomitantly with the inflammatory form of cell death known as pyroptosis. The non-canonical pathway is initiated on recognition of internalised cytosolic lipopolysaccharide (LPS) by caspase-4/5 (or murine caspase-11) via direct binding, resulting in caspases autoproteolysis and activation. Afterwards, active caspase-4/5/11 leads to the cleavage of GSDMD and the formation of plasma membrane pores, which, in turn, trigger the K^+ efflux and the subsequent initiation of the NLRP3 inflammasome through the canonical pathway as a feed-forward loop. The alternative pathway requires a single signal and the involvement of TIR-domain-containing adapter-inducing interferon-β (TRIF), receptor-interacting protein kinase 1 (RIPK1), FAS-associated death domain (FADD)-caspase-8 complex, but not apoptosis-associated speck-like protein containing a CARD (ASC) speck formation or K^+ efflux and does not culminate in pyroptosis.

been shown that the NLRP12 inflammasome controls IL-1β and IL-18 production after *Yersinia pestis* infection, dampening the infection.[49] Furthermore, *Salmonella typhimurium* and *Burkholderia pseudomallei* trigger the NLRP12, which performs negative regulation of pro-inflammatory cytokines in an inflammasome-independent fashion.[50 51] The inflammasome NLRC4 senses flagellin protein from Gram-negative bacteria, such as *S. typhimurium*,[52] *Pseudomonas aeruginosa*,[53] *S. flexneri*[54] and *Chromobacterium violaceum*.[55] While hyperactivation of the NLRC4 inflammasome has been reported in a rare disease known as autoinflammation with infantile enterocolitis,[56] a balanced activation is considered beneficial. On pathogen infection, IL-1β secreted via NLRC4 engagement by intestinal mononuclear phagocytes—including macrophages and dendritic cells—neutralises insults by preserving tolerance to non-responder commensal microbes. Intriguingly, IL-1β stimulates endothelial cells to express adhesion molecules, such as the vascular cell adhesion protein-1 (VCAM-1), intercellular adhesion molecule-2 or the selectin family proteins to recruit neutrophils at the intestinal mucosa to activate pathogen clearance.[57] Sellin *et al* reported that NLRC4 inflammasome activation can potentially drive the clearance of *Salmonella*-infected enterocytes.[58] AIM2 inflammasome recognises *L. monocytogenes*, following decreased levels of *A. muciniphila* and *Anaeroplasma*, while increasing those of *Anaerostipes*, *Bifidobacterium*, *Flexispira*, *Prevotella* and *Paraprevotella* species, thus causing reduced susceptibility to colitis and protection against CRC.[59] The pyrin-based inflammasome is an innate immune sensor developed by

host organisms to recognise Rho guanosine triphosphatase-inactivating bacterial toxins released in infections by diarrhoeal pathogen *Clostridioides difficile*,[60] or by the opportunistic pathogen *Burkholderia cenocepacia*.[61] On activation, the pyrin inflammasome processes IL-18, thus promoting intestinal barrier integrity and preventing colon inflammation and tumourigenesis.[62] Conversely, pyrin inflammasome activation through *Y. pestis* virulent factor outer protein M inhibited the secretion of IL-1β and IL-18, ultimately altering the intestinal barrier integrity and promoting inflammation and tumourigenesis.[63] The dysbiosis-driven inflammatory environment, characterised by high levels of immune mediators such as IL-1β and IL-18 within the gut, holds the potential to modulate the so-called 'second brain' activities by altering neurotransmission. Indeed, it has been described that aberrant NLRP3 inflammasome activity contributes to the onset of obesity, mainly due to a reshaping of enteric tachykinin motor pathways.[64] Additionally, the enteric neuroimmune circuit is orchestrated by the presence of enteric neurons capable of activating the inflammasome complex and releasing cytokines in the presence of certain microbes regulating pathogen clearance in a pro-inflammatory *scenario*.[65] It is now well established that GI function, both within the digestive system and in relation to distal organs, depends on a balanced ratio of symbiotic and dysbiotic microbial metabolites. The inflammasome may serve as a key intermediary platform linking the host to its intestinal microbiota.[66] An imbalance in metabolites may coincide with uncontrolled inflammasome activation in the intestinal lumen, fostering a pro-inflammatory environment. On the other hand, excessive inhibition of the inflammasome can promote the expansion of pathobionts, as its proper activation depends on a finely tuned, multistep regulatory process that remains only partially understood. Consequently, there is a growing interest in developing inflammasome-targeted therapies as part of personalised clinical management.

THE MICROBIOTA-INFLAMMASOME AXIS ERA: AN UNEXPECTED GAME-CHANGER IN CANCER DEVELOPMENT AND SUPPRESSION

It is well known that the tumour microenvironment (TME), also named tumour immune microenvironment (TIME), offers an attractive niche for microbial growth and immune cell migration and proliferation. However, their dynamic interaction and influence on tumour physiology, therapy response and antitumour immunity are still incompletely appreciated, probably due to technological limitations. The interplay between microbial dysbiosis and the inflammatory milieu is believed to be a critical factor in promoting several types of cancer. Documented evidence supports several mechanisms through which a dysbiotic phenotype favours inflammasome-mediated carcinogenesis, particularly in the context of GC, CRC and PDA, as focused below. Conversely, in other circumstances, the microbiota-inflammasome axis displays tumour suppression performances, thus emphasising the diverse roles in tumourigenesis. Microbial biomass can, indeed, directly interfere with cancer onset and development by altering various aspects of the host genome through the release of genotoxins (eg, colibactin, cytolethal distending toxin and enteropathogenic Escherichia coli secreted protein F (EspF)) or metabolites (eg, lithocholic and deoxycholic acid, acetate, butyrate) that enhance the epithelial-mesenchymal transition and/or cancer cell proliferation.[67] Additionally, altered microbe composition can indirectly contribute to all stages of carcinogenesis, including proliferation, immunosuppression, angiogenesis and metastasis, by influencing the immune landscape and altering the local cytokine profile within the TIME, particularly IL-1β and IL-18, via inflammasome activation.[45 68] Acute and chronic inflammation, a status often linked with dysbiosis, leads to the release of ASC-dependent mature IL-18, which inhibits the caspase-8-mediated apoptosis pathway while enhancing the survival and proliferation of GC cells.[69] The abundance of IL-1β within the TIME of GC is instrumental in recruiting myeloid-derived suppressor cells, a key component of the TME with several immunosuppressive activities.[70 71] In the same way, IL-18 suppresses the tumouricidal function of natural killer (NK) cells through the expression of immuno-suppressive co-stimulatory protein programmed cell death 1 on the cell surface.[72] Moreover, IL-18 contributes to the downregulation of the cluster of differentiation 70 in GC cells, known to enhance the cytotoxicity of NK cells and to induce tumour-specific T cell memory, thus displaying immune escape features.[73] Additionally, it has been shown that NLRP3-dependent IL-1β drives immuno-suppressive $CD4^+$ T cell polarisation to the TIME of PDA and instructs them to secrete IL-22, a molecule associated with invasive growth in numerous malignancies, including those involving the GI tract.[74] Other studies have identified further mechanisms of inflammasome-mediated immune suppression, such as AIM2-dependent release of alarmin IL-1α, which facilitates tumourigenesis.[75] In addition, pro-inflammatory cytokines confer migration abilities to several cancer cells. IL-1β and IL-18 facilitate the transmigration of malignant cells into the bloodstream and intensify the surface expression of adhesion molecules (such as VCAM-1) on endothelial as well as cancerous cells, allowing dissemination and infiltration into remote niches.[76 77] Invasion and metastasis are processes driven by shifts from the epithelial to mesenchymal phenotype in a programme named epithelial-to-mesenchymal transition (EMT).[78] In this context, several studies reported a functional role of IL-1β and IL-18 in the EMT. IL-1 downregulates epithelial cadherin expression while supporting the expression of zinc finger protein SNAIL (also known as SNAI1), both EMT signatures in GC cells.[79 80] However, the inflammasome has also been shown to suppress tumourigenesis as well, a role mostly characterised in CRC. In particular, bioavailable IL-18 cytokine promotes epithelial barrier regeneration during colitis-associated cancer[81 82] and coordinates NK cell-mediated and T cell-mediated antitumour immune responses.[83] IL-18 also inhibits the expression of IL-22, which, if copiously available within the CRC microenvironment, promotes tumourigenesis over time.[84] Novel insightful observations indicate inflammasomes as a primer in cancer development and, conversely, suppression.[27] Below, we outline the involvement of different inflammasomes-microbiota axis in GI tumourigenesis (figure 4).

NLRP3 inflammasome

A few studies have investigated the interplay between NLRP3, microbiota and CRC. MCC950-driven NLRP3 inhibition increases the abundance of Firmicutes, while reducing that of *Bacteroidetes* (whose presence is a recognised potential signature in subjects with CRC), thus ascribing this compound in the list of potential therapeutic tools against CRC. It was shown that the NLRP3 influence on the microbiota composition is mediated by altering the level of oxidation indicators. Administration of *Bacillus cereus* restored the proper microbiota configuration by restraining the toll-like receptor 4 (TLR4)-NF-κB-NLRP3 inflammasome signalling pathway.[85] Moreover, certain microbial species, including *Enterococcus faecalis* and *Fusobacterium nucleatum*, can trigger the non-canonical NLRP3 inflammasome

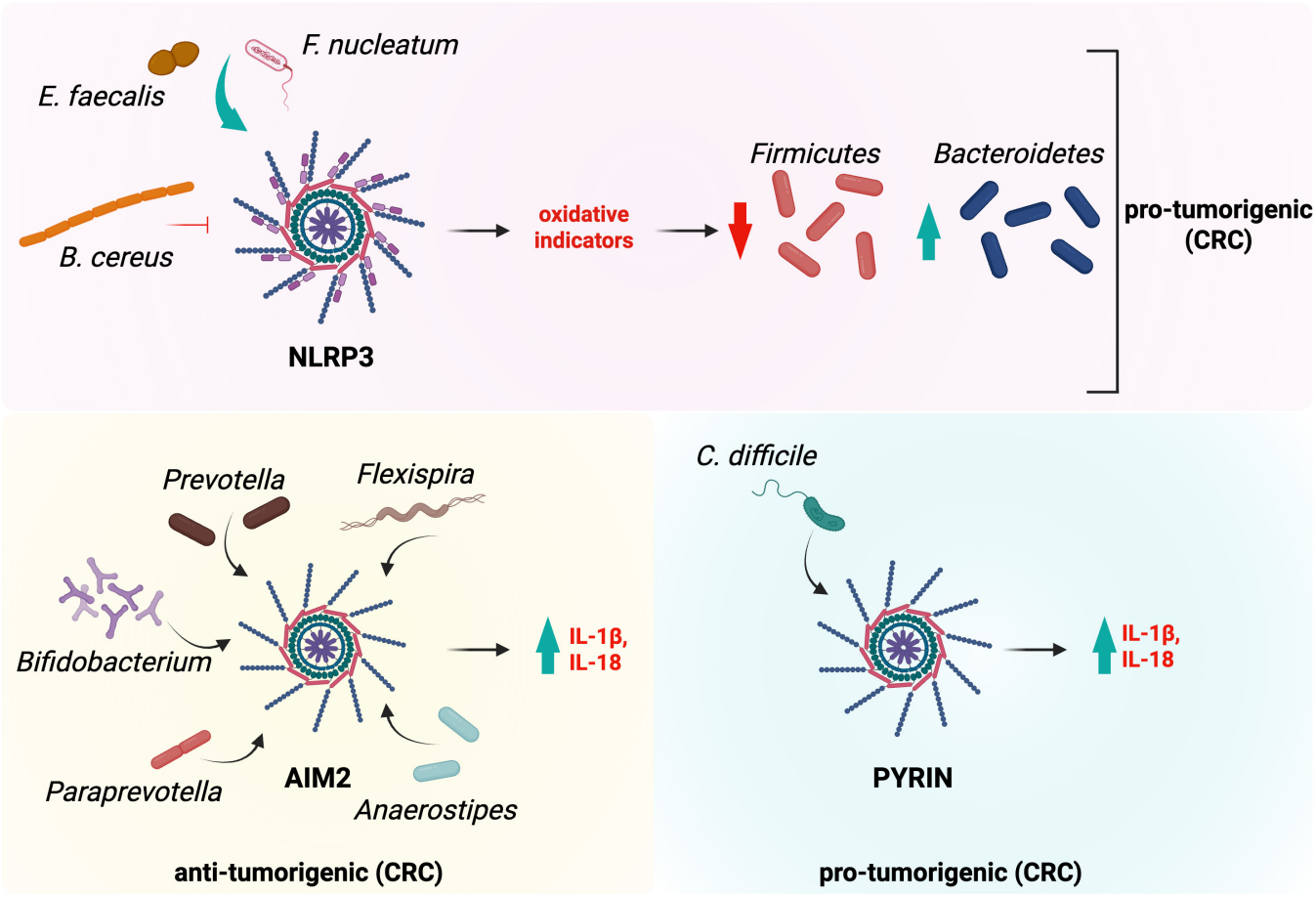

Figure 4 The inflammasome-microbiota axis in GI tumourigenesis. While *Bacillus cereus* colonisation prevents nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain containing 3 (NLRP3) activation, *Enterococcus faecalis* and *Fusobacterium nucleatum* sustain its activation, which reflects an altered Firmicutes/*Bacteroidetes* ratio leading, ultimately, to colorectal carcinogenesis (CRC, upper panel). Absent in melanoma 2 (AIM2) anti-tumourigenic properties are indirectly triggered by several Gram-positive and negative bacteria (lower left panel). *Clostridioides difficile* mediates pro-tumourigenic tasks of the pyrin inflammasome (lower right panel).

activation, leading to an increased production of IL-1β, colitis and ultimately to CRC.[86] Thus, a mutual regulation between the NLRP3 inflammasome and microbial strains guarantees the appropriate gut homeostasis (figure 4, upper panel).

AIM2 inflammasome

Elevated concentrations of *Anaerostipes*, *Bifidobacterium*, *Flexispira*, *Prevotella* and *Paraprevotella* encourage AIM2's antitumoral tasks. AIM2-depleted mice developed colitis, essentially due to IL-18 shortage, as a common prerequisite in the early stages of CRC. Interestingly, transferring gut bacteria from wild-type to AIM2-deficient mice reversed the phenotype, thus implying the involvement of the microbiota-AIM2 axis in regulating gut homeostasis.[87] In addition, the presence of a markedly dysbiotic profile exacerbated the susceptibility of AIM2-deficient mice to CRC, pointing to a significant contribution of AIM2 in modulating microbial imbalances, controlling intestinal inflammation and preventing tumour formation[59] (figure 4, lower left panel).

Pyrin inflammasome

Gut dysbiosis can produce inflammatory molecules that activate the pyrin inflammasome. Certain pathogenic microorganisms, like *C. difficile*, often isolated from colorectal cancerous lesions, trigger pyrin inflammasome activation, contributing to chronic inflammation. This persistent inflammation may promote a pro-tumourigenic environment, increasing the risk of CRC development[88] (figure 4, lower right panel).

THE MICROBIOTA IN THE GUT-BRAIN AXIS: A BIDIRECTIONAL CROSSTALK

The microbiota's influence on brain function: a link to neurological diseases

The microbiota in the GBA has gained significant attention due to its role in bidirectional communication between the gut microbiome and brain.[89–91] It regulates immune, enteric and neuroendocrine systems by producing neuroactive substances, metabolites and hormones. GBA components include the enteric, central and peripheral nervous systems, neuroendocrine connections and humoral pathways. It involves neuropeptides, signalling molecules from the gut microbiota or enterochromaffin cells, and active cytokines, such as IL-1β, secreted in response to inflammasome activation induced by microbiota-derived metabolites, thereby signalling to the enteric neurons and vagus nerve (figure 5).[92] Bidirectional signalling occurs through inflammatory mediators, metabolic signalling, oxidative stress markers, stress modulators, neuroendocrine factors and vagus nerve communication.[93] Altered gut microbiota is

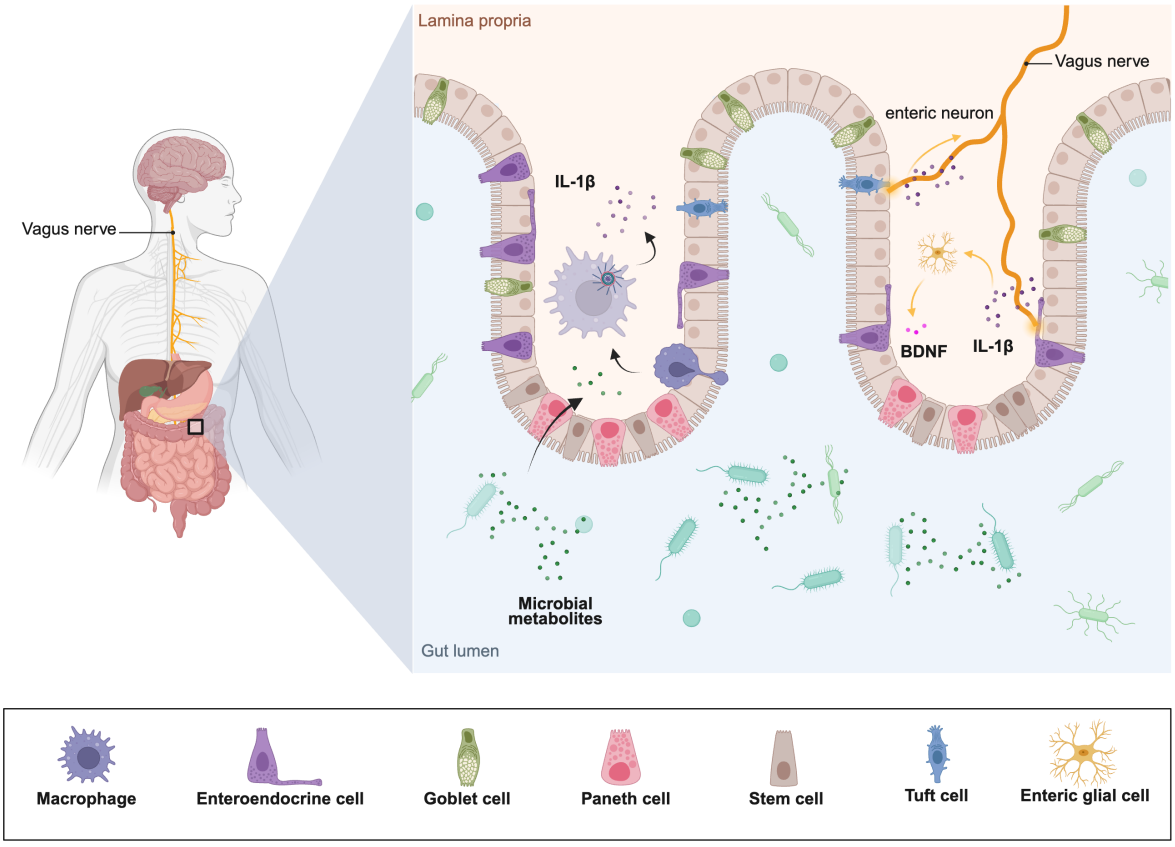

Figure 5 The microbiota-gut-brain axis and the role of the vagus nerve. This schema illustrates the vagus nerve's role in mediating communication between the central nervous system (CNS) and the GI tract, which is crucial for regulating various physiological processes, including inflammation. In the intestinal lamina propria, macrophages respond to microbiota-derived metabolites by activating the inflammasome, leading to the release of mature interleukin-1β (IL-1β), which signals to the enteric neuron and vagus nerve. Moreover, IL-1β induces the expression of the brain-derived neurotrophic factor (BDNF) from glial cells, which contribute to the sensation of pain. This pro-inflammatory cytokine is part of the innate immune response to infection or injury. The vagus nerve is essential for the 'inflammatory reflex', orchestrating innate immune responses and reactions to infections and tissue injuries through bidirectional communication between the brain and the immune system.

linked to neurodevelopmental, mood and neurodegenerative disorders. The microbiota can stimulate serotonin,[94] dopamine[95] and gamma-aminobutyric acid (GABA)[96] production, with *Bacteroides*, *Lactobacillus* and *Bifidobacterium* identified as key GABA producers.[97 98] A crucial GBA regulator is the intestinal barrier, comprising the mucus layer, epithelial barrier and gut vascular barrier. The mucus layer, rich in mucins produced by goblet cells, regulates microbiota-host interactions and immune responses. Among well-established roles of the intestinal barrier, the protection against external hazards and homeostasis maintenance stands out. Structural and functional alterations of this barrier are features typically of IBS, characterised by visceral hypersensitivity and chronic low-grade inflammation. Changes in intestinal permeability facilitate the passage of the microorganisms and derived metabolites from the gut lumen to the intestinal lamina propria where they act as DAMPs, triggering inflammasome activation in resident macrophages and, consequently, the release of IL-1β.[99 100] Active IL-1β induces the expression of the brain-derived neurotrophic factor (BDNF) from the enteric glial cells, which further contribute to the sensation of pain.[101] It has been shown that commensal bacteria, including *Bacteroides*, Firmicutes and *Lactobacillus*, produce IgA proteases and protease inhibitors, which preserve the EIB integrity and prevent pathogen colonisation.[102] Subjects suffering from IBS display a well-characterised microbial composition. Among other differences, individuals show an increased abundance of *Akkermansia*, *Methanobrevibacter*, *Clostridiales*, *Veillonella*, *Faecalitalea* and *Prevotella*, while a reduction in *Ruminococcaceae_UCG-003*, *Lactobacillus*, *Turicibacter*, *Enterococcus*, *Weissella*, *Oxalobacter* and *Bacteroides* has been observed compared with healthy individuals.[103] Molecularly, IBS is characterised by the downregulation of tight junction-associated proteins, such as occludin, zonula occludens-1 (ZO-1) and claudin-1, in the colon tissue, reflecting a loss of barrier functions and immune activation.[104] Studies on germ-free (GF) mice showed similar outcomes, highlighting microbiota's role in EIB integrity, immune system and brain physiology, from early development to behaviour.[105 106] Microbiota also affects blood-brain barrier (BBB) permeability, as GF mice show reduced tight junction protein expression, leading to increased permeability.[107] In addition, these mice display abnormal HPA axis development, with elevated plasma adrenocorticotropic hormone (ACTH) and corticosterone levels and reduced BDNF expression in response to stress. Probiotic *Bifidobacterium infantis*, but not *Escherichia coli*, reversed these

effects when administered early.[108] It has been found that intestinal barrier impairments and gut microbiome alterations are linked to systemic neurological and neuropsychiatric manifestations, such as mild cognitive impairment (MCI), multiple system atrophy (MSA), AD, PD, MS, ASD, anxiety and depression. Two independent research groups noticed a decreased expression of occludin and altered distribution of ZO-1 in colonic biopsies from subjects affected by PD and MSA compared with age-matched healthy individuals.[109 110] Additionally, increased intestinal permeability correlates with elevated level of α-synuclein in early PD.[111] Beside PD, increased serum levels of C-type lectin-like receptor 2 and zonulin, also markers of intestinal barrier alterations, were observed in patients with MCI and AD compared with healthy subjects.[112] Similarly, high concentrations of zonulin, fatty acid-binding protein 2 and LPS in the plasma of subjects affected by MS, anxiety, depression and ASD have been diagnosed.[113–115] Population screening led by Patel *et al* linked IBS onset with anxiety and depressive episodes.[116] In particular, 30%–50% of patients with IBS have been found to suffer from anxiety, depression and mood disorder.[117] Another large-scale analysis on patients with IBD reported stroke, dementia and MS-related symptoms.[118] Lastly, CRC onset rarely leads to neurological disorders, with few manifestations of vasculitis, sensory neuropathy and encephalomyelitis.[119–121] Recent research performed on animal models of PD,[122] AD[123] and MS[124] exhibited analogous outcomes. Restoring barrier integrity may offer a therapeutic strategy to prevent or slow neurodegeneration. Leblhuber *et al* were able to effectively decrease the level of faecal haptoglobin in subjects affected by AD through the daily administration of a probiotic mix consisting of *Lactobacillus* and *Bifidobacterium*, suggesting an improvement in intestinal permeability.[125] These findings highlight microbiota's critical role in brain function and its potential as a therapeutic target for neurological and neuropsychiatric disorders.

The hypothalamic-pituitary-adrenal axis: the role of gut microbiota in health and disease

The bidirectional communication between the gut and the brain involves multiple pathways, including neuroanatomical routes (such as the vagus nerve, autonomic nervous system and enteric nervous system), the HPA axis, the immune and lymphatic systems as well as microbial-derived metabolic products.[126 127] Among these pathways, the HPA axis has historically been one of the most extensively studied. HPA axis activation, mediated by glucocorticoids, is a key regulatory pathway. Threats trigger immune responses, releasing pro-inflammatory cytokines like tumour necrosis factor (TNF)-α, IL-1β, IL-18, IL-6 and interferon (IFN)α/β, which are part of the acute immune response and originate from microglia, vascular and endothelial cells. The adaptive immune response involves IL-2 and IFNγ, crucial for viral defence. Cytokines stimulate the HPA, leading to glucocorticoid release, which suppresses further cytokine production via negative feedback, protecting against excessive inflammation. Glucocorticoids regulate immunity, shifting from T helper (Th)1 (inflammatory) to Th2 (anti-inflammatory) responses, inhibiting TNF-α and IL-2 while promoting IL-4, IL-10 and IL-13.[128 129] Thus, glucocorticoids act as immunosuppressors, modulating cytokine activity.[130] Cytokines induce the release of glucocorticoid hormones, either directly or through the corticotropin-releasing hormone (CRH). CRH stimulates ACTH release from the adenohypophysis, which, in turn, promotes the release of glucocorticoids, dehydroepiandrosterone, aldosterone and its derivatives.[131] Cytokine receptors have been found along the HPA at all levels. Hence, every segment of this axis provides a functional bridge between hormones and cytokines. Peripheral administration of low doses of endotoxin stimulates macrophages to release IL-1β, which enters the bloodstream and behaves as a hormone, activating HPA and CRH release (figure 6). However, IL-1β may also activate the production and release of other cytokines and prostaglandins.[132] Conversely, prolonged exposure to stress-related conditions and/or subsequent hypercortisolaemia can trigger inflammatory responses through chronic activation of the HPA axis, leading to the dysregulation of the inflammatory feedback.[133] For example, increased IL-1β secretion can create a positive feedback loop that enhances HPA activity, promoting further glucocorticoid release. High doses of endotoxin can directly impact the production of cytokines and prostaglandins, influencing the HPA axis and triggering elevated levels of IL-1β.[132] Microglia, the brain's primary immune surveillance cells, can also be activated by chronic psychosocial and/or physical stress and corresponding HPA activation. Activated microglia secrete various pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6, while exhibiting a reduced phagocytic potential.[133] Alternatively, microglia can take on an anti-inflammatory phenotype, secreting cytokines like IL-4 and IL-10 and transforming growth factor-β (TGF-β), associated with heightened phagocytic activity. In response to stress and age-related neurodegeneration, microglia are typically found in a pro-inflammatory state, influenced by interactions between HPA activation and microglial signalling. Chronic stress can 'prime' microglia, making them more responsive to subsequent stimuli, which is why stress is particularly harmful in AD development. Glucocorticoid release through the HPA axis intricately modulates the expression of immune-related genes in microglia.[134 135] Stress could also activate microglia through another mechanism, namely via monoamine neurotransmitters like norepinephrine released by sympathetic neurons located in the locus coeruleus. Sympathetic activation by repeated social defeat stress in mice leads to increased microglial gene expression, including IL-1β and TNF-α at an early stage, IL-6 later on, and CD14 and C-X3-C motif chemokine receptor 1 throughout the stress period.[136 137] Increased caspase-1 activity cleaves glucocorticoid receptors, contributing to glucocorticoid resistance, hypercortisolaemia in chronic stress and the positive feedback between stress-induced HPA activation and neuroinflammation.[138] The gut microbiota regulates the HPA axis and mediates the interaction between stress and inflammation. Chronic psychosocial stress disrupts the gut microbiota and immune responses, further activating the HPA, affecting the colon, mesenteric lymph nodes and pituitary gland, and influencing gut epithelial integrity. While HPA hyperactivation independently promotes neuroinflammation, microbiota and its metabolites modulate pro-inflammatory responses via GBA signalling, building resilience against stress-induced changes linked to AD. Several studies suggest the association between the gut microbiota and the HPA axis. Stress-induced catecholamines support the growth of Gram-negative bacteria, including *E. coli*, *Yersinia enterocolitica* and *P. aeruginosa*,[139] thus ultimately triggering LPS-mediated amyloid-like plaque formation and AD onset.[140] HPA dysregulation reflects alterations in major depression, bipolar disorder and schizophrenia as well. Cheung

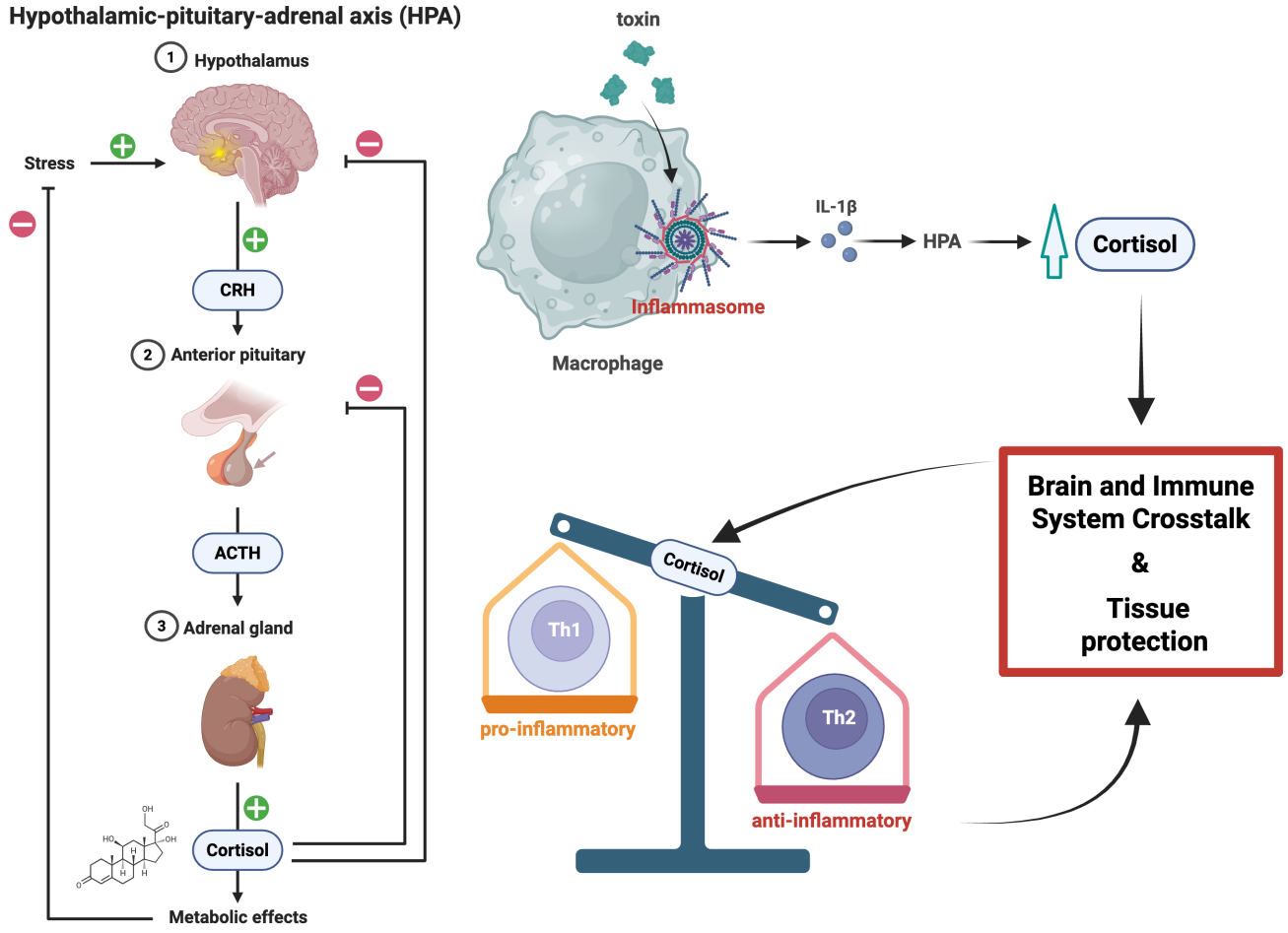

Figure 6 The immune system and brain crosstalk: involvement of the hypothalamic-pituitary-adrenal (HPA) axis. This schema illustrates the intricate interplay between the immune system and the brain, emphasising the roles of critical components such as the HPA axis, cortisol, inflammasome and interleukin-1β (IL-1β). The HPA axis regulates the body's stress response and maintains equilibrium. Stress triggers the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which in turn stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH prompts the adrenal glands to produce cortisol, a steroid hormone, which regulates metabolism, immunity and stress. As stress diminishes, cortisol levels return to normal, reinstating the balance. The HPA can also be activated by cytokines like IL-1β, released during inflammation. For instance, on encountering toxins, macrophages undergo activation, initiating the inflammasome cascade and prompting the release of IL-1β. This cytokine can subsequently activate the HPA axis, increasing cortisol production. Furthermore, cortisol suppresses the immune system by inhibiting pro-inflammatory mediators such as cytokines, chemokines and prostaglandins, thereby mitigating inflammation. Notably, heightened cortisol levels facilitate the shift from a cellular T helper 1 (Th1)/pro-inflammatory to a humoral T helper 2 (Th2)/anti-inflammatory immune response, thus promoting an anti-inflammatory milieu and safeguarding tissue integrity.

et al reported higher abundance of *Anaerostipes*, *Blautia*, *Clostridiales*, *Klebsiella*, Lachnospiraceae *incertae sedis*, *Parabacterioides*, *Parasutterella*, *Phascolarctobacterium* and *Streptococcus* species in patients affected by major depression, compared with healthy individuals.[141] Bipolar disorder has been associated with increased levels of Coriobacteriaceae and *Flavonifractor* and a lower abundance of *Faecalibacterium* and Ruminococcaceae.[142–144] Lastly, lower abundance of Firmicutes has been demonstrated in subjects affected by schizophrenia.[145] Thus, although the detailed mechanisms of bidirectional communication remain to be fully elucidated, the role of the gut microbiota in immune modulation and HPA axis dysfunction is emerging as highly relevant for the prevention and treatment of neurodegenerative diseases, particularly dementia and other neurological disorders. In addition to this, recent evidence indicates that sleep deprivation induces gut dysbiosis and intestinal barrier disruption, leading to microglial activation and cognitive decline. Persistent sleep deprivation (<6 hours per night) during midlife is associated with a 30% increased risk of dementia. Furthermore, chronic sleep deprivation alters gut microbiota, reducing mucus thickness and tight junction protein levels in mouse colons via NLRP3 inflammasome activation, which impairs BBB integrity, activates NLRP3 in the brain and leads to microglial activation and cognitive impairment.[146] Studies have shown that compared with control mice, faecal microbiota transplantation from sleep-deprived mice replicates these pathological changes.[147] A similar cognitive decline associated with sleep deprivation has been observed in humans, where sleep deprivation reduces short-chain fatty acid-producing species and induces systemic inflammation through the TLR4/NF-κB

signalling pathway.[148 149] Future research will elucidate the impact of the gut microbiome on brain circuits that regulate sleep and circadian rhythms.[150–153] This should help to develop pharmacological tools that more effectively target sleep and circadian disturbances.

DECIPHERING THE MICROBIAL ECOSYSTEM: THE REVOLUTION OF ARTIFICIAL INTELLIGENCE IN MICROBIOTA ANALYSIS AND THERAPEUTIC APPROACH

Given the complex interplay within the microbiota and the vast amount of data generated by high-throughput technologies (eg, metabolomics, transcriptomics, metagenomics), there is a growing reliance on computational modelling and AI to bridge the gap between current knowledge and the development of personalised, targeted interventions. The development of various omics technologies has been widely applied to explore the connections between microorganisms and host metabolism. However, single-omics analyses offer only a fragmented view of the complex biological processes underlying gut microbiota dynamics and human disease. Today, a holistic, multi-omics approach is increasingly essential to unravel this intricate network. Thus, a key advantage of AI in microbiology is represented by its ability to efficiently process and decipher large heterogeneous data. Machine learning (ML) and deep learning, the two main algorithms of AI, have emerged as the hallmark 21st century modernisation in all the medical contexts, including microbiota-applied strategies. Recently, Oh *et al* successfully developed an AI model, called DeepMicro, for the accurate identification of bacterial species from metagenomic sequencing (DNA analysis), enabling the simultaneous detection of multiple pathogens difficult to culture and therefore predicting disease associations.[154] Intriguingly, research led by Pasolli *et al* developed an algorithm able to predict host characteristics from microbiota composition with high accuracy.[155] ML algorithms can determine the most effective probiotic combination[156] and personalise nutrition plans for IBS-affected patients.[157] Moreover, the integration of large-scale metagenomics with AI makes it possible to predict antibiotic resistance.[158] Thus, identifying patterns in microbial composition related to a variety of pathophysiological outcomes, including GI and neurological disorders, has greatly enhanced our understanding of the complex interplay between the microbiome and human health. However, current AI applications need to overcome several challenges. First, high-dimensional data with a relatively small sample size may hinder the development of accurate prediction models.[159] Another issue is the integration of various omics when elucidating molecular interactions underpinning diseases.[160] An additional key obstacle is the highly individualised microbial community due to factors like diet, geography, genetics and lifestyle. Thus, developing AI models that are both accurate and generalisable across diverse populations remains a complex task.[161] Below, we outline emerging AI-based approaches linking microbial composition to IBD diagnosis, CRC onset and neurological diseases, such as PD, AD, ASD and depression.

AI application in IBD diagnosis

IBD is often misdiagnosed despite the abundance of diagnostic tests. A pilot study conducted by Franzosa *et al* showed that ML-assisted microbiota analysis could improve the diagnosis of IBD. The authors performed faecal 16S metagenomic analysis on nearly 700 patients with IBD, compared with healthy individuals, revealing 50 differential bacterial taxa between the two groups and substantial discrepancies between CD and UC, thus ensuring more accurate IBD diagnoses.[162]

AI-assisted microbiota signature in CRC

AI-assisted microbiota profiling linked to GI cancer diagnosis is still in its early stages. Only recently, in fact, has AI software been employed to validate well-documented patterns of microbiota composition in subjects affected by CRC. For instance, CRC-derived faecal samples displayed distinct microbiological markers compared with healthy individuals, including abundance of *Porphyromonas*, *Peptostreptococcus*, *Parvimonas* and *Fusobacterium*, responsible for altering proliferation, progression, to promote angiogenesis, metastasis dissemination and chemoresistance. Moreover, lower levels of the Lachnospiraceae family, which trigger immune surveillance function of $CD8^+$ T cells, linked therefore to antitumourigenic properties, have been observed in patients with CRC. Similarly, AI-based approach CRC samples display reduced levels of the *Clostridiales* order, including *Eubacterium eligens*, *Eubacterium ventriosum* and *Anaerostipes*.[163] In addition, several predictive microbiome signatures for CRC have been disclosed by AI, with high accuracy. No less importantly, models predicting patients with CRC responsive to therapy by using the gut microbiota repertoire have also been developed.[164]

AI application in neurological disorders

AI is currently being used to understand the mechanisms connecting relationships between gut microbiota and brain functions. Several AI models are now able to associate microbial signatures with patterns attributable to mental health conditions. Notably linked to decreased Lachnospiraceae and increased Verrucomicrobiaceae levels, studies on dysbiosis-driven PD onset are increasingly growing. Pietrucci *et al* tested three different ML algorithms to analyse 16S rRNA sequences from 472 patients with PD compared with 374 healthy individuals, revealing 22 bacterial families linked to PD prediction.[165] Numerous differences were revealed through AI-assisted metagenomics analysis between ASD and normal samples. Genera belonging to the Actinobacteria phylum, such as *Bifidobacterium* and *Collinsella*, and members of the Firmicutes phylum, including Erysipelatoclostridiaceae, *Murdochiella*, *Butyricicoccus*, *Clostridium*, Lachnospiraceae *UCG-004* and *E. eligens,* were found to be decreased in ASD cases compared with controls. In contrast, genera belonging to the Bacteroidota phylum, including Prevotellaceae and *Parabacteroides*, those from the Enterobacteriaceae family, *Sarcina*, *Anaerosporobacter* and *Oscillospira* showed significant increase.[166] A similar approach has been recently employed to identify bacterial biomarkers for depressive disorder. ML identified eight altered species between 36 subjects affected by depressive disorders vs 36 healthy individuals. The level of *Alistipes*, *Dysosmobacter*, *Actinomyces*, *Ruthenibacterium* and *Thomasclavelia* was significantly increased, whereas *Faecalibacterium*, *Pseudobutyrivibrio* and *Roseburia* were reduced.[167] Lastly, another group led by Mohta and Oudah showed, through an ML-based approach, a prevalence of *Streptococcus* and *Dorea* in patients affected by MS compared with healthy cases, rather characterised by a prevalence of *Bacteroides uniformis*.[168]

CONCLUSIONS AND PERSPECTIVES

Notoriously, the analysis of the human gut microbiome and the association between bacterial species and the host phenotype might result in cleaning the Augean stables labour. The intricate circuit between the GI microbiota and the inflammasome,

the main component of the innate immune system, is pivotal in maintaining gut health and systemic homeostasis throughout the body. Unbalanced dynamics between those two systems might reflect significant pathological outcomes. A dysbiotic phenotype, bridged to chronic inflammasome activation, increases the body's vulnerability to critical illness, exacerbating tissue damage and the onset and progression of inflammatory conditions, such as IBD, cancer and neurological disorders. Gaining a deeper understanding of these relationships is, in fact, essential to promote pioneering targeted therapies and preventive strategies for these diseases. As we live in the era of AI integration in every aspect of the medical field, it will surely assist in unravelling the complexities of intestinal ecosystem-inflammasome interactions, thus contributing to novel microbiota-based therapeutic strategies. Emerging AI-based models can analyse large datasets and detect patterns within complex biological systems, providing new insights into how microbial communities and immune responses are interconnected at the molecular level. In addition, intensive scientific research armed with AI could effectively identify novel biomarkers in dysbiosis or abnormal inflammasome activation, which are invaluable for disease prevention, early diagnosis and tailoring personalised therapies based on each individual's unique microbiota composition, thus making it possible to achieve.

Author affiliations
[1]Department of Neurology, Beth Israel Deaconess Medical Center and Division of Sleep Medicine, Harvard Medical School, Boston, MA, USA
[2]Personalized Medicine Laboratory, National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte (BA), 70013, Italy
[3]Department of Medicine, Beth Israel Deaconess Medical Center and Division of Endocrinology, Harvard Medical School, Boston, MA, USA
[4]Department of Sciences, University "Roma Tre", Rome, Italy
[5]Scientific Direction, National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte (BA), 70013, Italy
[6]Department of Experimental Medicine, Sapienza Università di Roma, Rome, Italy
[7]Department of Clinical and Molecular Medicine, Sapienza Università di Roma, Rome, Italy

Acknowledgements The authors would like to thank BioRender.com (https://www.biorender.com/) for providing the tools used to create figures 1–6 and online supplemental table 1 in this review.

Contributors RDL, VA, EA and RN conceptualised and authored the manuscript. SN, SI, GG and PT edited the document, offering valuable insights and suggestions. All authors contributed to the article and approved the submitted version.

Funding PT is supported by a grant from LILT, Italy (2021U0001642), and a PRIN 2022 grant from the Italian Ministry of University and Research (no.: 2022RY8549). EA is supported by the Sapienza grant (no.: RM123188E8E9A26E). RN is supported by the Italian Ministry of Health, Ricerca Corrente 2025 (no.: DDG 799/24).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Stefano Nardone http://orcid.org/0000-0002-9317-4784
Roberto Negro http://orcid.org/0000-0002-4319-5923

REFERENCES

1 Ruan W, Engevik MA, Spinler JK, *et al*. Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration. *Dig Dis Sci* 2020;65:695–705.
2 Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021;19:55–71.
3 Cryan JF, O'Riordan KJ, Cowan CSM, *et al*. The Microbiota-Gut-Brain Axis. *Physiol Rev* 2019;99:1877–2013.
4 Penders J, Thijs C, van den Brandt PA, *et al*. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2007;56:661–7.
5 Kalliomäki M, Collado MC, Salminen S, *et al*. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008;87:534–8.
6 The TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes* 2007;8:286–98.
7 Kostic AD, Gevers D, Siljander H, *et al*. The Dynamics of the Human Infant Gut Microbiome in Development and in Progression toward Type 1 Diabetes. *Cell Host & Microbe* 2015;17:260–73.
8 Örtqvist AK, Lundholm C, Halfvarson J, *et al*. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study. *Gut* 2019;68:218–25.
9 Butler MI, Cryan JF, Dinan TG. Man and the Microbiome: A New Theory of Everything. *Annu Rev Clin Psychol* 2019;15:371–98.
10 Sarkar A, Yoo JY, Valeria Ozorio Dutra S, *et al*. The Association between Early-Life Gut Microbiota and Long-Term Health and Diseases. *J Clin Med* 2021;10:459.
11 Claesson MJ, Cusack S, O'Sullivan O, *et al*. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci USA* 2011;108:4586–91.
12 Zhang W, Lyu M, Bessman NJ, *et al*. Gut-innervating nociceptors regulate the intestinal microbiota to promote tissue protection. *Cell* 2022;185:4170–89.
13 Appleton J. The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health. *Integr Med (Encinitas)* 2018;17:28–32.
14 Pavlov VA, Chavan SS, Tracey KJ. Molecular and Functional Neuroscience in Immunity. *Annu Rev Immunol* 2018;36:783–812.
15 Gil-Cruz C, Perez-Shibayama C, De Martin A, *et al*. Microbiota-derived peptide mimics drive lethal inflammatory cardiomyopathy. *Science* 2019;366:881–6.
16 Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med* 2016;8:51.
17 Aburto MR, Cryan JF. Gastrointestinal and brain barriers: unlocking gates of communication across the microbiota–gut–brain axis. *Nat Rev Gastroenterol Hepatol* 2024;21:222–47.
18 Hou K, Wu Z-X, Chen X-Y, *et al*. Microbiota in health and diseases. *Signal Transduct Target Ther* 2022;7:135.
19 Celano G, Calabrese FM, Riezzo G, *et al*. A Multi-Omics Approach to Disclose Metabolic Pathways Impacting Intestinal Permeability in Obese Patients Undergoing Very Low Calorie Ketogenic Diet. *Nutrients* 2024;16:2079.
20 Kang Y, Cai Y, Pan W. Change in gut microbiota for eczema: Implications for novel therapeutic strategies. *Allergol Immunopathol (Madr)* 2018;46:281–90.
21 Grant MB, Bernstein PS, Boesze-Battaglia K, *et al*. Inside out: Relations between the microbiome, nutrition, and eye health. *Exp Eye Res* 2022;224:109216.
22 Behary J, Amorim N, Jiang X-T, *et al*. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun* 2021;12:20422.
23 Zhang Q, Ma C, Duan Y, *et al*. Gut Microbiome Directs Hepatocytes to Recruit MDSCs and Promote Cholangiocarcinoma. *Cancer Discov* 2021;11:1248–67.
24 Moravejolahkami AR, Chitsaz A, Hassanzadeh A, *et al*. Effects of anti-Inflammatory-antioxidant-rich diet and co-supplemented synbiotics intervention in patients with progressive forms of multiple sclerosis: a single-center, single-blind randomized clinical trial. *Nutr Neurosci* 2023;26:1078–89.
25 Cui B, Luo H, He B, *et al*. Gut dysbiosis conveys psychological stress to activate LRP5/β-catenin pathway promoting cancer stemness. *Signal Transduct Target Ther* 2025;10:79.
26 Wang R, Liu G, Chen H, *et al*. Linking oxytocin to nicotine dependence: An experimental study of the brain, behavior, and gut microbiota in rats. *Neurosci Lett* 2025;852:138198.
27 Yao J, Sterling K, Wang Z, *et al*. The role of inflammasomes in human diseases and their potential as therapeutic targets. *Signal Transduct Target Ther* 2024;9:10.
28 Amaan A, Prekshi G, Prachi S. Microbiome-Gut-Brain Axis: AI Insights. *Insights Biol Med* 2024;8:001–10.
29 Magupalli VG, Negro R, Tian Y, *et al*. HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. *Science* 2020;369:eaas8995.
30 Arrè V, Negro R, Giannelli G. The role of inflammasomes in hepatocellular carcinoma: Mechanisms and therapeutic insights. *Ann Hepatol* 2025;30:101772.

31 Trakman GL, Fehily S, Basnayake C, *et al*. Diet and gut microbiome in gastrointestinal disease. *J Gastroenterol Hepatol* 2022;37:237–45.
32 Al Bataineh MT, Künstner A, Dash NR, *et al*. Uncovering the relationship between gut microbial dysbiosis, metabolomics, and dietary intake in type 2 diabetes mellitus and in healthy volunteers: a multi-omics analysis. *Sci Rep* 2023;13:17943.
33 Boyden ED, Dietrich WF. Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat Genet* 2006;38:240–4.
34 Sandstrom A, Mitchell PS, Goers L, *et al*. Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. *Science* 2019;364:eaau1330.
35 Ewald SE, Chavarria-Smith J, Boothroyd JC. NLRP1 is an inflammasome sensor for Toxoplasma gondii. *Infect Immun* 2014;82:460–8.
36 Neiman-Zenevich J, Stuart S, Abdel-Nour M, *et al*. Listeria monocytogenes and Shigella flexneri Activate the NLRP1B Inflammasome. *Infect Immun* 2017;85:e00338-17.
37 Williams TM, Leeth RA, Rothschild DE, *et al*. The NLRP1 Inflammasome Attenuates Colitis and Colitis-Associated Tumorigenesis. *J Immunol* 2015;194:3369–80.
38 Tye H, Yu C-H, Simms LA, *et al*. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. *Nat Commun* 2018;9:3728.
39 Van Immerseel F, Ducatelle R, De Vos M, *et al*. Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *J Med Microbiol* 2010;59:141–3.
40 Seo S-U, Kamada N, Muñoz-Planillo R, *et al*. Distinct Commensals Induce Interleukin-1β via NLRP3 Inflammasome in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury. *Immunity* 2015;42:744–55.
41 McCoy AJ, Koizumi Y, Toma C, *et al*. Cytotoxins of the human pathogen Aeromonas hydrophila trigger, via the NLRP3 inflammasome, caspase-1 activation in macrophages. *Eur J Immunol* 2010;40:2797–803.
42 Su Y-C, Wang C-C, Chen Y-W, *et al*. Haemolysin Ahh1 secreted from Aeromonas dhakensis activates the NLRP3 inflammasome in macrophages and mediates severe soft tissue infection. *Int Immunopharmacol* 2024;128:111478.
43 Kim S, Bauernfeind F, Ablasser A, *et al*. Listeria monocytogenes is sensed by the NLRP3 and AIM2 inflammasome. *Eur J Immunol* 2010;40:1545–51.
44 Kitamoto S, Nagao-Kitamoto H, Jiao Y, *et al*. The Intermucosal Connection between the Mouth and Gut in Commensal Pathobiont-Driven Colitis. *Cell* 2020;182:447–62.
45 Levy M, Thaiss CA, Zeevi D, *et al*. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell* 2015;163:1428–43.
46 Shen C, Li R, Negro R, *et al*. Phase separation drives RNA virus-induced activation of the NLRP6 inflammasome. *Cell* 2021;184:5759–74.
47 Elinav E, Strowig T, Kau AL, *et al*. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 2011;145:745–57.
48 Nowarski R, Jackson R, Gagliani N, *et al*. Epithelial IL-18 Equilibrium Controls Barrier Function in Colitis. *Cell* 2015;163:1444–56.
49 Vladimer GI, Weng D, Paquette SWM, *et al*. The NLRP12 Inflammasome Recognizes Yersinia pestis. *Immunity* 2012;37:96–107.
50 Zaki MH, Man SM, Vogel P, *et al*. Salmonella exploits NLRP12-dependent innate immune signaling to suppress host defenses during infection. *Proc Natl Acad Sci U S A* 2014;111:385–90.
51 Pudla M, Onsoi P, Utaisincharoen P. NLRP12 attenuates tumor necrosis factor-α production in Burkholderia pseudomallei-infected RAW264.7 macrophages. *Asian Pac J Allergy Immunol* 2022.
52 Franchi L, Amer A, Body-Malapel M, *et al*. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol* 2006;7:576–82.
53 Sutterwala FS, Mijares LA, Li L, *et al*. Immune recognition of Pseudomonas aeruginosa mediated by the IPAF/NLRC4 inflammasome. *J Exp Med* 2007;204:3235–45.
54 Suzuki T, Franchi L, Toma C, *et al*. Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. *PLoS Pathog* 2007;3:e111.
55 Venkatramanan M, Nalini E. Regulation of virulence in *Chromobacterium violaceum* and strategies to combat it. *Front Microbiol* 2024;15:1303595.
56 Romberg N, Al Moussawi K, Nelson-Williams C, *et al*. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet* 2014;46:1135–9.
57 Franchi L, Kamada N, Nakamura Y, *et al*. NLRC4-driven production of IL-1β discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. *Nat Immunol* 2012;13:449–56.
58 Sellin ME, Müller AA, Felmy B, *et al*. Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict Salmonella replication in the intestinal mucosa. *Cell Host Microbe* 2014;16:237–48.
59 Man SM, Zhu Q, Zhu L, *et al*. Critical Role for the DNA Sensor AIM2 in Stem Cell Proliferation and Cancer. *Cell* 2015;162:45–58.
60 Xu H, Yang J, Gao W, *et al*. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature New Biol* 2014;513:237–41.
61 Gavrilin MA, Abdelaziz DHA, Mostafa M, *et al*. Activation of the pyrin inflammasome by intracellular Burkholderia cenocepacia. *J Immunol* 2012;188:3469–77.
62 Sharma D, Malik A, Guy CS, *et al*. Pyrin Inflammasome Regulates Tight Junction Integrity to Restrict Colitis and Tumorigenesis. *Gastroenterology* 2018;154:948–64.
63 Chung LK, Park YH, Zheng Y, *et al*. The Yersinia Virulence Factor YopM Hijacks Host Kinases to Inhibit Type III Effector-Triggered Activation of the Pyrin Inflammasome. *Cell Host & Microbe* 2016;20:296–306.
64 Pellegrini C, Fornai M, Benvenuti L, *et al*. NLRP3 at the crossroads between immune/inflammatory responses and enteric neuroplastic remodelling in a mouse model of diet-induced obesity. *Br J Pharmacol* 2021;178:3924–42.
65 Jacobson A, Yang D, Vella M, *et al*. The intestinal neuro-immune axis: crosstalk between neurons, immune cells, and microbes. *Mucosal Immunol* 2021;14:555–65.
66 Arrè V, Scialpi R, Centonze M, *et al*. The 'speck'-tacular oversight of the NLRP3-pyroptosis pathway on gastrointestinal inflammatory diseases and tumorigenesis. *J Biomed Sci* 2023;30:90.
67 Rossi T, Vergara D, Fanini F, *et al*. Microbiota-Derived Metabolites in Tumor Progression and Metastasis. *Int J Mol Sci* 2020;21:5786.
68 Kolb R, Phan L, Borcherding N, *et al*. Obesity-associated NLRC4 inflammasome activation drives breast cancer progression. *Nat Commun* 2016;7:13007.
69 Deswaerte V, Nguyen P, West A, *et al*. Inflammasome Adaptor ASC Suppresses Apoptosis of Gastric Cancer Cells by an IL18-Mediated Inflammation-Independent Mechanism. *Cancer Res* 2018;78:1293–307.
70 Tu S, Bhagat G, Cui G, *et al*. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 2008;14:408–19.
71 Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest* 2015;125:3356–64.
72 Terme M, Ullrich E, Aymeric L, *et al*. IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res* 2011;71:5393–9.
73 Kang JS, Y.Bae S, R.Kim H, *et al*. Interleukin-18 increases metastasis and immune escape of stomach cancer via the downregulation of CD70 and maintenance of CD44. *Carcinogenesis* 2009;30:1987–96.
74 Daley D, Mani VR, Mohan N, *et al*. NLRP3 signaling drives macrophage-induced adaptive immune suppression in pancreatic carcinoma. *J Exp Med* 2017;214:1711–24.
75 Sorrentino R, Terlizzi M, Di Crescenzo VG, *et al*. Human lung cancer-derived immunosuppressive plasmacytoid dendritic cells release IL-1α in an AIM2 inflammasome-dependent manner. *Am J Pathol* 2015;185:3115–24.
76 Vidal-Vanaclocha F, Amézaga C, Asumendi A, *et al*. Interleukin-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases. *Cancer Res* 1994;54:2667–72.
77 Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, *et al*. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* 2000;97:734–9.
78 Fu X-T, Dai Z, Song K, *et al*. Macrophage-secreted IL-8 induces epithelial-mesenchymal transition in hepatocellular carcinoma cells by activating the JAK2/STAT3/Snail pathway. *Int J Oncol* 2015;46:587–96.
79 Jee YS, Jang TJ, Jung KH. Prostaglandin E(2) and interleukin-1β reduce E-cadherin expression by enhancing snail expression in gastric cancer cells. *J Korean Med Sci* 2012;27:987–92.
80 Zaki MH, Boyd KL, Vogel P, *et al*. The NLRP3 Inflammasome Protects against Loss of Epithelial Integrity and Mortality during Experimental Colitis. *Immunity* 2010;32:379–91.
81 Dupaul-Chicoine J, Yeretssian G, Doiron K, *et al*. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 2010;32:367–78.
82 Salcedo R, Worschech A, Cardone M, *et al*. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J Exp Med* 2010;207:1625–36.
83 Fabbi M, Carbotti G, Ferrini S. Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP. *J Leukoc Biol* 2015;97:665–75.
84 Huber S, Gagliani N, Zenewicz LA, *et al*. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature New Biol* 2012;491:259–63.
85 Sheng K, Xu Y, Kong X, *et al*. Probiotic *Bacillus cereus* Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice through Improvement of the Intestinal Barrier Function, Anti-Inflammation, and Gut Microbiota Modulation. *J Agric Food Chem* 2021;69:14810–23.
86 Chung I-C, OuYang C-N, Yuan S-N, *et al*. Pretreatment with a Heat-Killed Probiotic Modulates the NLRP3 Inflammasome and Attenuates Colitis-Associated Colorectal Cancer in Mice. *Nutrients* 2019;11:516.
87 Cui JZ, Chew ZH, Lim LHK. New insights into nucleic acid sensor AIM2: The potential benefit in targeted therapy for cancer. *Pharmacol Res* 2024;200:107079.
88 Patel RK, Cardeiro M, Frankel L, *et al*. Incidence of Colorectal Cancer After Intestinal Infection Due to *Clostridioides difficile* *World J Oncol* 2024;15:279–86.
89 Jänig W. The autonomic nervous system. In: Smith RF, ed. *Fundamentals in neurophysiology*. 3rd edn. New York, Berlin, Heidelberg, Tokyo: Springer-Verlag, 1983.
90 Cannon WB. Organization for physiological homeostasis. *Physiol Rev* 1929;9:399–431.

91 Unwin N. Nicotinic acetylcholine receptor and the structural basis of neuromuscular transmission: insights from Torpedo postsynaptic membranes. *Q Rev Biophys* 2013;46:283–322.
92 Ahmed H, Leyrolle Q, Koistinen V, *et al*. Microbiota-derived metabolites as drivers of gut-brain communication. *Gut Microbes* 2022;14:2102878.
93 Ohara TE, Hsiao EY. Microbiota-neuroepithelial signalling across the gut-brain axis. *Nat Rev Microbiol* 2025.
94 Yano JM, Yu K, Donaldson GP, *et al*. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 2015;161:264–76.
95 Wang Y, Tong Q, Ma S-R, *et al*. Oral berberine improves brain dopa/dopamine levels to ameliorate Parkinson's disease by regulating gut microbiota. *Sig Transduct Target Ther* 2021;6.
96 Strandwitz P, Kim KH, Terekhova D, *et al*. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol* 2019;4:396–403.
97 Bravo JA, Forsythe P, Chew MV, *et al*. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 2011;108:16050–5.
98 Duranti S, Ruiz L, Lugli GA, *et al*. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. *Sci Rep* 2020;10:14112.
99 Ligumsky M, Simon PL, Karmeli F, *et al*. Role of interleukin 1 in inflammatory bowel disease--enhanced production during active disease. *Gut* 1990;31:686–9.
100 Scuderi SA, Casili G, Lanza M, *et al*. Modulation of NLRP3 Inflammasome Attenuated Inflammatory Response Associated to Diarrhea-Predominant Irritable Bowel Syndrome. *Biomedicines* 2020;8:519.
101 Fukumoto M, Takeuchi T, Koubayashi E, *et al*. Induction of brain-derived neurotrophic factor in enteric glial cells stimulated by interleukin-1β via a c-Jun N-terminal kinase pathway. *J Clin Biochem Nutr* 2020;66:103–9.
102 Bansal T, Alaniz RC, Wood TK, *et al*. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc Natl Acad Sci U S A* 2010;107:228–33.
103 Chong PP, Chin VK, Looi CY, *et al*. The Microbiome and Irritable Bowel Syndrome – A Review on the Pathophysiology, Current Research and Future Therapy. *Front Microbiol* 2019;10:1136.
104 Maharshak N, Huh EY, Paiboonrungruang C, *et al*. Enterococcus faecalis Gelatinase Mediates Intestinal Permeability via Protease-Activated Receptor 2. *Infect Immun* 2015;83:2762–70.
105 Luczynski P, McVey Neufeld K-A, Oriach CS, *et al*. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. *Int J Neuropsychopharmacol* 2016;19:pyw020.
106 Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe* 2015;17:565–76.
107 Braniste V, Al-Asmakh M, Kowal C, *et al*. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014;6:263ra158.
108 Sudo N, Chida Y, Aiba Y, *et al*. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 2004;558:263–75.
109 Clairembault T, Leclair-Visonneau L, Coron E, *et al*. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathol Commun* 2015;3:12.
110 Engen PA, Dodiya HB, Naqib A, *et al*. The Potential Role of Gut-Derived Inflammation in Multiple System Atrophy. *J Parkinsons Dis* 2017;7:331–46.
111 Forsyth CB, Shannon KM, Kordower JH, *et al*. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS One* 2011;6:e28032.
112 Wang X, Liu G-J, Gao Q, *et al*. C-type lectin-like receptor 2 and zonulin are associated with mild cognitive impairment and Alzheimer's disease. *Acta Neurol Scand* 2020;141:250–5.
113 Camara-Lemarroy CR, Silva C, Greenfield J, *et al*. Biomarkers of intestinal barrier function in multiple sclerosis are associated with disease activity. *Mult Scler* 2020;26:1340–50.
114 Esnafoglu E, Cırrık S, Ayyıldız SN, *et al*. Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects. *J Pediatr* 2017;188:240–4.
115 Stevens BR, Goel R, Seungbum K, *et al*. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. *Gut* 2018;67:1555–7.
116 Patel P, Bercik P, Morgan DG, *et al*. Irritable bowel syndrome is significantly associated with somatisation in 840 patients, which may drive bloating. *Aliment Pharmacol Ther* 2015;41:449–58.
117 Hadjivasilis A, Tsioutis C, Michalinos A, *et al*. New insights into irritable bowel syndrome: from pathophysiology to treatment. *Ann Gastroenterol* 2019;32:554–64.
118 Zamani M, Ebrahimtabar F, Alizadeh-Tabari S, *et al*. Risk of Common Neurological Disorders in Adult Patients with Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Inflamm Bowel Dis* 2024;30:2195–204.
119 Pantalone D, Muscas GC, Tings T, *et al*. Peripheral paraneoplastic neuropathy, an uncommon clinical onset of sigmoid cancer. Case report and review of the literature. *Tumori* 2002;88:347–9.
120 Anderson NE, Barber PA. Limbic encephalitis - a review. *J Clin Neurosci* 2008;15:961–71.
121 Sio TT, Paredes M, Uzair C. Neurological manifestation of colonic adenocarcinoma. *Rare Tumors* 2012;4:e32.
122 Lai F, Jiang R, Xie W, *et al*. Intestinal Pathology and Gut Microbiota Alterations in a Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Mouse Model of Parkinson's Disease. *Neurochem Res* 2018;43:1986–99.
123 Kim M-S, Kim Y, Choi H, *et al*. Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model. *Gut* 2020;69:283–94.
124 Zhang Y-G, Wu S, Yi J, *et al*. Target Intestinal Microbiota to Alleviate Disease Progression in Amyotrophic Lateral Sclerosis. *Clin Ther* 2017;39:322–36.
125 Leblhuber F, Steiner K, Schuetz B, *et al*. Probiotic Supplementation in Patients with Alzheimer's Dementia - An Explorative Intervention Study. *Curr Alzheimer Res* 2018;15:1106–13.
126 Zhao L, Xiong Q, Stary CM, *et al*. Bidirectional gut-brain-microbiota axis as a potential link between inflammatory bowel disease and ischemic stroke. *J Neuroinflammation* 2018;15:339.
127 Zhuang M, Zhang X, Cai J. Microbiota-gut-brain axis: interplay between microbiota, barrier function and lymphatic system. *Gut Microbes* 2024;16:2387800.
128 Gustafsson JK, Johansson MEV. The role of goblet cells and mucus in intestinal homeostasis. *Nat Rev Gastroenterol Hepatol* 2022;19:785–803.
129 Pellegrini C, Fornai M, D'Antongiovanni V, *et al*. The intestinal barrier in disorders of the central nervous system. *Lancet Gastroenterol Hepatol* 2023;8:66–80.
130 Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* 1998;94:557–72.
131 Adcock IM, Ito K. Molecular mechanisms of corticosteroid actions. *Monaldi Arch Chest Dis* 2000;55:256–66.
132 Mastorakos G, Pavlatou M, Diamanti-Kandarakis E, *et al*. Exercise and the stress system. *Hormones (Athens)* 2005;4:73–89.
133 Herman JP, McKlveen JM, Ghosal S, *et al*. Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Compr Physiol* 2016;6:603–21.
134 Tilders FJH, DeRuk RH, Van Dam A-M, *et al*. Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: Routes and intermediate signals. *Psychoneuroendocrinology* 1994;19:209–32.
135 Hänsel A, Hong S, Cámara RJA, *et al*. Inflammation as a psychophysiological biomarker in chronic psychosocial stress. *Neurosci Biobehav Rev* 2010;35:115–21.
136 Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 2005;308:1314–8.
137 Santos LE, Beckman D, Ferreira ST. Microglial dysfunction connects depression and Alzheimer's disease. *Brain Behav Immun* 2016;55:151–65.
138 Sorrells SF, Caso JR, Munhoz CD, *et al*. The stressed CNS: when glucocorticoids aggravate inflammation. *Neuron* 2009;64:33–9.
139 Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. *Life Sci* 1992;50:203–12.
140 Zhan X, Stamova B, Jin L-W, *et al*. Gram-negative bacterial molecules associate with Alzheimer disease pathology. *Neurology (ECronicon)* 2016;87:2324–32.
141 Cheung SG, Goldenthal AR, Uhlemann A-C, *et al*. Systematic Review of Gut Microbiota and Major Depression. *Front Psychiatry* 2019;10:34.
142 Evans SJ, Bassis CM, Hein R, *et al*. The gut microbiome composition associates with bipolar disorder and illness severity. *J Psychiatr Res* 2017;87:23–9.
143 Painold A, Mörkl S, Kashofer K, *et al*. A step ahead: Exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disord* 2019;21:40–9.
144 Coello K, Hansen TH, Sørensen N, *et al*. Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives. *Brain Behav Immun* 2019;75:112–8.
145 Shen Y, Xu J, Li Z, *et al*. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: A cross-sectional study. *Schizophr Res* 2018;197:470–7.
146 Zhao N, Chen Q-G, Chen X, *et al*. Intestinal dysbiosis mediates cognitive impairment via the intestine and brain NLRP3 inflammasome activation in chronic sleep deprivation. *Brain Behav Immun* 2023;108:98–117.
147 Li N, Tan S, Wang Y, *et al*. *Akkermansia muciniphila* supplementation prevents cognitive impairment in sleep-deprived mice by modulating microglial engulfment of synapses. *Gut Microbes* 2023;15:2252764.
148 Sabia S, Fayosse A, Dumurgier J, *et al*. Association of sleep duration in middle and old age with incidence of dementia. *Nat Commun* 2021;12:2289.
149 Wang Z, Chen W-H, Li S-X, *et al*. Gut microbiota modulates the inflammatory response and cognitive impairment induced by sleep deprivation. *Mol Psychiatry* 2021;26:6277–92.
150 De Luca R, Nardone S, Grace KP, *et al*. Orexin neurons inhibit sleep to promote arousal. *Nat Commun* 2022;13:4163.
151 Ramirez-Plascencia OD, Luca R, Machado NLS, *et al*. A hypothalamic circuit for circadian regulation of corticosterone secretion. *Res Sq* 2024.
152 Todd WD, Venner A, Anaclet C, *et al*. Suprachiasmatic VIP neurons are required for normal circadian rhythmicity and comprised of molecularly distinct subpopulations. *Nat Commun* 2020;11.
153 Venner A, De Luca R, Sohn LT, *et al*. An Inhibitory Lateral Hypothalamic-Preoptic Circuit Mediates Rapid Arousals from Sleep. *Curr Biol* 2019;29:4155–68.

154 Oh M, Zhang L. DeepMicro: deep representation learning for disease prediction based on microbiome data. *Sci Rep* 2020;10.
155 Pasolli E, Truong DT, Malik F, *et al*. Machine Learning Meta-analysis of Large Metagenomic Datasets: Tools and Biological Insights. *PLoS Comput Biol* 2016;12:e1004977.
156 Zeevi D, Korem T, Zmora N, *et al*. Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 2015;163:1079–94.
157 Karakan T, Gundogdu A, Alagözlü H, *et al*. Artificial intelligence-based personalized diet: A pilot clinical study for irritable bowel syndrome. *Gut Microbes* 2022;14:2138672.
158 Arango-Argoty G, Garner E, Pruden A, *et al*. DeepARG: a deep learning approach for predicting antibiotic resistance genes from metagenomic data. *Microbiome* 2018;6:23.
159 Ditzler G, Morrison JC, Lan Y, *et al*. Fizzy: feature subset selection for metagenomics. *BMC Bioinformatics* 2015;16:358.
160 Singh A, Shannon CP, Gautier B, *et al*. DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics* 2019;35:3055–62.
161 Wilmanski T, Diener C, Rappaport N, *et al*. Gut microbiome pattern reflects healthy ageing and predicts survival in humans. *Nat Metab* 2021;3:274–86.
162 Manandhar I, Alimadadi A, Aryal S, *et al*. Gut microbiome-based supervised machine learning for clinical diagnosis of inflammatory bowel diseases. *Am J Physiol Gastrointest Liver Physiol* 2021;320:G328–37.
163 Novielli P, Romano D, Magarelli M, *et al*. Explainable artificial intelligence for microbiome data analysis in colorectal cancer biomarker identification. *Front Microbiol* 2024;15:1348974.
164 Zeng T, Yu X, Chen Z. Applying artificial intelligence in the microbiome for gastrointestinal diseases: A review. *J of Gastro and Hepatol* 2021;36:832–40.
165 Pietrucci D, Teofani A, Unida V, *et al*. Can Gut Microbiota Be a Good Predictor for Parkinson's Disease? A Machine Learning Approach. *Brain Sci* 2020;10:242.
166 Peralta-Marzal LN, Rojas-Velazquez D, Rigters D, *et al*. A robust microbiome signature for autism spectrum disorder across different studies using machine learning. *Sci Rep* 2024;14:814.
167 Wang X, Cao D, Zhang H, *et al*. Utilizing metagenomic profiling and machine learning model to identify bacterial biomarkers for major depressive disorder. *Front Psychiatry* 2025;16:1539596.
168 Mohta B, Oudah M. Early screening for multiple sclerosis using gut microbiome and machine learning. 2024 IEEE 24th International Conference on Bioinformatics and Bioengineering (BIBE); 2024;Kragujevac, Serbia.