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REVIEW

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Gut microbe–host interactions in post-COVID syndrome: a debilitating or restorative partnership?

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ABSTRACT

Post-COVID syndrome (PCS) patients have reported a wide range of symptoms, including fatigue, shortness of breath, and diarrhea. Particularly, the presence of gastrointestinal symptoms has led to the hypothesis that the gut microbiome is involved in the development and severity of PCS. The objective of this review is to provide an overview of the role of the gut microbiome in PCS by describing the microbial composition and microbial metabolites in COVID-19 and PCS. Moreover, host–microbe interactions via the microbiota-gut-brain (MGB) and the microbiota-gut-lung (MGL) axes are described. Furthermore, we explore the potential of therapeutically targeting the gut microbiome to support the recovery of PCS by reviewing preclinical model systems and clinical studies. Overall, current studies provide evidence that the gut microbiota is affected in PCS; however, diversity in symptoms and highly individual microbiota compositions suggest the need for personalized medicine. Gut-targeted therapies, including treatments with pre- and probiotics, have the potential to improve the quality of life of affected individuals.

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Introduction

People infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) developed coronavirus disease 2019 (COVID-19) with variable degrees of severity and duration. Some individuals developed long-lasting symptoms, which is a condition called post-COVID syndrome (PCS), long COVID, or post-acute sequelae of COVID-19 (PASC). Definitions of PCS vary between countries and institutes, with the one from the World Health Organization (WHO) being most widely accepted. It is stated as the continuation or development of new symptoms 3 months after the onset of COVID-19 by a SARS-CoV-2 infection with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis.[1,2] About 10% of the patients regress to PCS after a SARS-CoV-2 infection, which is estimated to affect 65 million patients worldwide, underscoring the significant global health challenge in this field.[3] PCS patients have reported highly diverse symptoms including, among many others, fatigue, shortness of breath, post-exertional malaise (PEM), postural orthostatic tachycardia syndrome (POTS), mast cell activation syndrome (MCAS), and various manifestations in the gastrointestinal tract such as abdominal pain and diarrhea (Figure 1a).[4] It has also been shown that PCS may lead to prolonged cognitive impairments.[5,6] The pathogenesis of PCS is still elusive, and fundamental studies are in their early stages, with multiple, potentially overlapping mechanisms identified for this disease.[3]

Gastrointestinal symptoms are highly prevalent in both COVID-19 and PCS. A meta-analysis reported that 12% of individuals with COVID-19 experienced gastrointestinal symptoms, whereas 22% experienced such symptoms in the case of PCS.[7] These findings suggest that the gut microbiota might be involved in PCS,[3,8] in parallel to its involvement in functional gastrointestinal disorders[9,10] (Figure 1b). Indeed, a recent study showed that the gut microbiota composition was associated with PCS symptoms.[11] The gut microbiome is a collection of various types of microorganisms living in the (human) intestine.[12] Most research focused on bacterial interactions in this ecosystem, however, it became evident that viruses, including the bacteriophages, and fungi,

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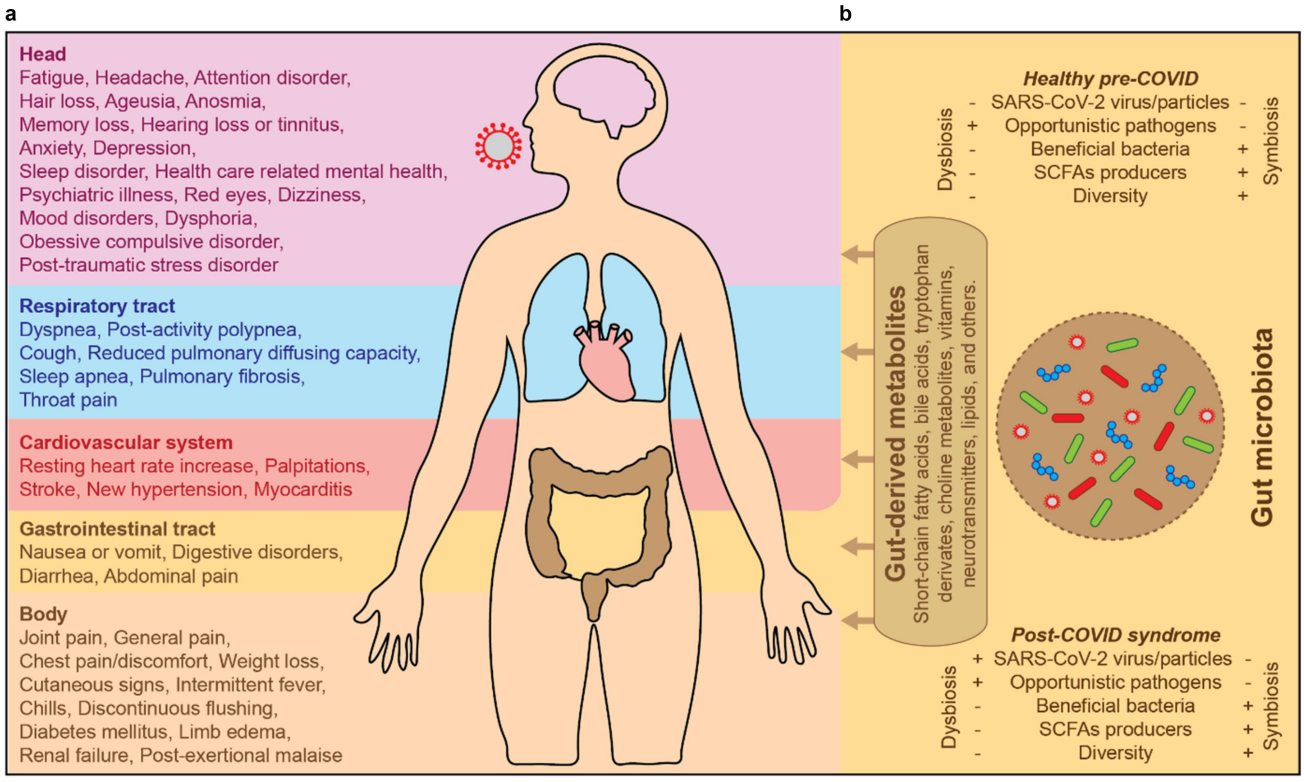

Figure 1. The gut microbiota and symptoms of the post-covid syndrome.

influence the host's health and are important for the microbiota composition. As such, the gut microbiome can be regarded as an organ on its own due to its complexity and involvement in various body functions. The microbiota exhibits great interindividual variations and differences between age groups,[13] adding to its complexity for analyses.[14]

In physiological conditions, the gut microbiome orchestrates metabolism, immune functions, and creating resilience against opportunistic infections.[15] In general, a high gut microbiome diversity is associated with a healthy lifestyle and a resilient microbiota that can protect against invading pathogens.[16] Lower bacterial diversity is considered a hallmark of gut microbiota dysbiosis, a term that is not well defined but is frequently used in the field of microbiota research. Factors discussed to induce gut microbiota dysbiosis include antibiotic use, exposure to pesticides, chronic stress, and strict hygiene. Moreover, associations have been discovered between diet and gut microbiome signatures linked to general health. Diet factors linked to an altered gut microbiome associated with decreased general health were low levels of fibers, high carbohydrate intake, and high glycemic load.[17–21]

A low bacterial diversity is negatively associated with multiple clinical markers such as blood glucose, inflammation, and liver dysfunction.[22] A reduced bacterial diversity has been observed in various chronic inflammatory diseases, such as obesity, diabetes, and inflammatory bowel disease. Notably, Mendelian randomization has demonstrated causal relationships between the gut microbiome composition and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), a disorder exhibiting manifold symptomatic overlaps with PCS.[23] Yet, it is unclear how the gut microbiome is affected in PCS.

The objective of this review is to provide an overview of the role of the gut microbiome in PCS by describing host–microbe interactions and the influence of microbial metabolites in the body via the microbiota-gut-brain (MGB) and microbiota-gut-lung (MGL) axes. Furthermore, we aim to explore gut microbiome targeted therapies to support the recovery of

PCS by reviewing both preclinical model systems and clinical studies.

The gastrointestinal tract and the gut microbiome during COVID-19

Although the lung was considered as the main target organ of SARS-CoV-2 since infection is primarily inducing severe respiratory complications, various reports suggested that other organs are involved as well.[24] The gastrointestinal tract has been hypothesized to act as an alternative route for viral transmission.[25,26] This is based on the high expression levels of angiotensin-converting enzyme 2 (ACE2) in the gastrointestinal tract, the presence of gastrointestinal symptoms in COVID-19 patients, and the lasting detection of virus particles in feces. Infection with SARS-CoV-2 is mediated by ACE2 and transmembrane protease, serine 2 (TMPRSS2) for viral entry and replication.[27] ACE2 is localized at the cell membrane or can adopt a soluble form to enter the circulation. The function of ACE2 is to convert angiotensin II to angiotensin (1–7). ACE2 expression has been identified in various organs, including the respiratory and gastrointestinal tract with the highest levels of protein expression in the small intestine and in the colon[29,30] Consequently, ACE2-mediated SARS-CoV-2 entry into the host enteric cells has been detected, underscoring viral transmission in the gastrointestinal tract.[31] The viral infection leads to epithelial barrier damage, which contributes to inflammatory processes that may lead to symptoms such as diarrhea. However, it is rather unclear whether the virus can actively replicate in the human intestine.[32,33]

The immune system plays a crucial role in clearing SARS-CoV-2 particles from the body. Following the initial infection with the virus, the innate immune system launches a response toward virally infected cells via pathogen recognition receptors (PRRs). Recognition of viral particles triggers an interferon response, leading to an inflammatory cascade, and, in turn, controlled cell death of infected tissues.[34,35] This is followed by the adaptive immune response, involving the production of highly specific antibodies capable of efficiently binding to viruses and aiding in the clearance of viral particles from the body.[36] Intestinal immunoglobulin A (IgA) is the primary effector molecule that can prevent, among other antiviral molecules, the accumulation of viral particles in the intestine as part of the mucosal immune system. It is produced by B cells in the intestine and secreted into the lumen, where it can bind and limit the entry of microorganisms into the host. In stool samples of severe COVID-19 patients, elevated IgA reactive to SARS-CoV-2 has been detected.[37]

The interaction between the gut microbiome and the gastrointestinal system, covering the epithelium, immune system, and mucosal barrier, is dynamic and adaptive via continuous feedback mechanisms[38–41] In healthy situations, these interactions occur bidirectionally forming a symbiosis and gut microbes are well tolerated by the host's immune system. Altered host-microbe interactions in the gastrointestinal tract can be developed in diseased states, for example during viral infection, fungal overgrowth, or upon higher prevalence of opportunistic bacteria, leading to activated immune response mediated by lymphocytes resident in the epithelium.

As a result of SARS-CoV-2 infection in the human gastrointestinal tract, differences in the gut microbiome have been detected[42] based on the analysis of fecal material of individuals during or shortly after the infection. In adults, during or shortly after a SARS-CoV-2 infection, there has been a reduction in bacterial diversity, a decrease in short-chain fatty acids (SCFAs) producing bacteria, and an increase in opportunistic pathogens compared to people without acute SARS-CoV-2 infection, as extensively reviewed by Zhang et al.[42] Several mechanisms have been postulated to grasp SARS-CoV-2-induced gut dysbiosis in COVID-19, including the presence of intestinal inflammation, dysregulation of ACE2, and direct viral infection of bacteria.[25] Gut dysbiosis prior to infection might lead to more severe COVID-19 outcomes.[43] Gut dysbiosis has been observed in several diseases, such as obesity and diabetes,[44] both diseases related to a reduced immune response[45] and a worse outcome of COVID-19.[46] Thus, gut dysbiosis might play an important role in COVID-19. In children, comparable alterations in the gut microbiome have been detected, although studies were limited by smaller sample sizes.[42,47] Moreover, the gut mycobiome and

virome showed compositional changes during COVID-19, with an increased presence of *Candida albicans, Candida auris, Aspergillus flavus*, and depletion of multiple bacteriophages.[42]

Recovery from acute infection is associated with the restoration of the gut microbiome, although the process may be more protracted than anticipated and may not be fully restored in all recovered patients.[48] Interestingly, the gut microbiota of persons recovered from acute SARS-CoV-2 infection, that did not show any symptoms of PCS, was indistinguishable from that of uninfected controls at 6 months.[49] Antibiotics were commonly prescribed to patients with COVID-19, even though they are ineffective against virus infections. Yet, a meta-analysis by Nandi et al. [50] documented an overuse of antibiotics in patients with COVID-19. Antibiotics have a profound impact on the composition of the gut microbiome,[51,52] although additional potential mechanisms are a negative effect on the epithelial barrier and a spread of resistant micro-organisms.[53] The administration of antibiotics during SARS-CoV-2 infection or prior use of antibiotics within 3 years before COVID-19 negatively affected resilience to symptoms, leading to more severe clinical outcomes, including hospital admission and 30-day mortality.[54] Nevertheless, an association between antibiotic use and the development of PCS has not been observed.[55] More research on the relationship between the use of antibiotics, the gut microbiome composition, resilience to severe acute infection, and the development of PCS is warranted.

Gut microbiome composition during PCS

To evaluate the gut microbiome in PCS, we summarized the findings of human studies reporting 16S or shotgun metagenomic sequencing data on the gut microbiome composition (Table 1). Since the definition of the PCS is not well aligned, we followed the definitions as indicated per reported study, although these can vary, and uniformity is desired. Five out of six studies reported reduced bacterial diversity in PCS. The abundance of opportunistic pathogens was positively correlated with persistent PCS symptoms, while beneficial bacteria exhibited the most significant inverse correlations with PCS symptoms. Yet, no longitudinal studies exist that compared samples from the same individual before and after infection. Current studies compared the gut microbiota of patients with PCS to that of SARS-CoV-2 infected controls or uninfected controls. As not all studies have found a significant association between the gut microbiota and PCS,[58] the highly diverse symptoms of PCS and individual microbiota suggest the need for more homogeneous study cohorts. Therefore, only associations can be drawn between the gut microbiota and the severity of PCS. No causative explanations can be given at this stage.

Nevertheless, the outcomes of the first studies indicate reduced bacterial diversity in the gut microbiome of individuals with PCS.[11,49,56–59] Importantly, it resembles various characteristics of a dysbiotic gut ecosystem, including an increase in opportunistic pathogens and a decrease in beneficial commensals. Persistent PCS symptoms were positively correlated with pathogens like *Streptococcus anginosus*, *Streptococcus vestibularis*, *Streptococcus gordonii*, and *Clostridium disporicum* in the fecal microbiome. In addition, *Clostridium innocuum* and *Actinomyces naeslundii* correlated with fatigue and neuropsychiatric symptoms.[49] Beneficial bacteria, such as *Faecalibacterium prausnitzii*, serve as important sources of the SCFA butyrate, which is produced by the fermentation of dietary fibers in the large intestine.[60] Butyrate has been shown to improve the host's metabolism and immune response. The abundance of *F. prausnitzii*, as well as other beneficial bacteria like *Bifidobacterium pseudocatenulatum*, *Roseburia inulinivorans*, and *Roseburia hominis*, were negatively correlated with PCS at 6 months.[49,61]

Profound insight on the relation between inter-individual PCS symptoms and the gut microbiome has been obtained by Su et al.[11] They revealed that the gut microbiome was the top factor explaining variance in PCS symptoms, followed by COVID-19 vaccination, COVID-19 history, and demographics. Furthermore, three types of gut microbiota (enterotypes) in PCS were identified, all different from that of healthy volunteers. The symptoms of insomnia, fatigue, and difficulty concentrating showed the most significant difference between PCS enterotypes. This further highlights the role of a dysbiotic gut microbiome in the manifestation of PCS.

Table 1. Summary of human studies assessing the gut microbiome in post-COVID syndrome.

Type	Study design	Study PCS definition	Methodology	Sample timing	Findings	Reference
Prospective study	81 PCS patients, 68 non-infected controls, Hong Kong SAR, China.	At least one persistent symptom 4 weeks after clearance of the virus.	Shotgun metagenomic sequencing.	6 months after hospital admission.	Abundance of opportunistic pathogens positively correlated with severity of symptoms (e.g. *Streptococcus anginosus, Streptococcus vestibularis, Streptococcus gordonii, Clostridium disporicum*) Depletion of beneficial commensals (e.g. *Collinsella aerofaciens, F. prausnitzii, Blautia obeum*). Negative correlation of typical SCFAs producers and symptom scores. Reduced bacterial diversity in PCS individuals.	Liu, Mak et al. (2022)[49]
Prospective follow-up study	55 PCS patients, 75 COVID-19 recovered, 32 non-infected controls, Wuhan, China.	One or more PCS symptoms one-year after discharge.	16S rRNA sequencing.	1-year after discharge.	Enriched opportunistic pathogens (e.g. *Veillonella*). Depletion of beneficial commensals (e.g. *Eubacterium hallii, Subdoligranulum, Ruminococcus, Dorea, Coprococcus, Eubacterium ventriosum*). Reduced bacterial diversity in PCS individuals.	Zhang, Zhou et al. (2023)[56]
Prospective study	45 PCS patients, 25 healthy controls, Hong Kong SAR, China.	At least one persistent GI symptom, including decreased appetite, diarrhea, abdominal pain, taste disorder, emaciation, and xerostomia, which could not be explained by an alternative diagnosis 3 months after clearance of SARS-CoV-2.	Shotgun metagenomic sequencing.	3 months follow-up.	Reduced bacterial diversity in PCS individuals.	Zhang, Weng et al. (2023)[57]
Retrospective study	20 PCS patients, 52 COVID-19, 85 non-COVID illness, United Kingdom.	Signs and symptoms that have developed during or after an infection consistent with COVID-19 and not explained by an alternative diagnosis	Shotgun metagenomic sequencing.	Positive swab test with symptoms lasting ≥84 days	No differences in bacterial diversity.	Österdahl, Whinston et al. (2023)[58]
Prospective study	32 PCS patients, 39 controls, Sweden.	Persisting symptoms ≥12 weeks after the infection.	Shotgun metagenomic sequencing.	Sampling time after infection varies.	Only two groups were significantly different between PCS and controls (decreased abundance of Firmicutes and Ascomycota). Reduced bacterial diversity in PCS individuals.	Hamrefors, Kahn et al. (2024)[59]
Prospective and retrospective study	707 PCS patients, 201 COVID-19 recovered, 653 healthy controls, Hong Kong SAR, China.	Having at least one persistent symptom that cannot be explained by an alternative diagnosis four weeks after recovery from COVID-19.	Shotgun metagenomic sequencing	Average 5 months follow-up.	Enriched opportunistic pathogens (e.g. *Clostridium bolteae, Flavonifractor plautii*). Depletion of beneficial commensals (e.g. *Bifidobacterium adolescentis* and *Roseburia hominis*). Reduced bacterial diversity and richness in PCS individuals.	Su, Lau *et al.* (2024)[11]

Bacteria are, next to enteric viruses, the most abundant microorganism in the gut microbiota and thus, have been much in focus in recent years. However, alterations in the mycobiome have been related to several host conditions, such as irritable bowel syndrome and neurological disorders.[61,62] In the fecal mycobiome, an increase of fungal pathogens from the genera *Candida* and *Aspergillus* has been detected.[63] This is regarded as mycobiota dysbiosis, which induces systemic immunogenic activation of pathways related to antifungal immunity.[64] This has been observed in hospitalized patients with severe COVID-19, whereas the long-lasting effects for people with PCS have not yet been fully understood.[65] Fungal infections are related to neurodegenerative diseases suggesting a role also in neurological symptoms of PCS, however, causative relationship is not drawn.[66]

Importantly, correlation alone does not provide enough evidence for causation. A causative role of a gut dysbiosis has been made in mice, where fecal transplants from stressed mice induced similar symptoms in the recipient mouse via a neuroinflammatory pathway.[67] The transfer of gut microbiota from individuals with PCS into germ-free mice induced lung inflammation, worsened the outcomes of a challenge with *Klebsiella pneumoniae*, and reduced the cognitive performance of the mice.[68] The donor material exhibited a higher abundance of *Enterobacteriaceae* strains with antibiotic resistance, which is a family of various opportunistic bacteria, and lower levels of SCFAs in feces compared to healthy controls. Treatment with the probiotic *Bifidobacterium longum* 5^{1A} resulted in reduced memory impairment and weight loss induced by viral infection, suggesting potential gut-targeting treatments for PCS. These animal studies established causative relationships between the gut microbiota and the development of neurocognitive symptoms as seen in PCS. Importantly, this interaction is bilateral: neurological impairment, including depression, can cause dysbiosis in the gut, whereas gut dysbiosis can promote depression.

Debilitating symptoms in PCS could be a result of continuous infections due to a persistent viral reservoir.[69] Natarajan et al. [70] analyzed the fecal RNA shedding up to 10 months after COVID-19 diagnosis in 113 individuals. Fecal SARS-CoV-2 RNA was detected in 49.2% of individuals within the first week after diagnosis. Furthermore, 12.7% and 3.8% shed virus RNA in feces after 4 and 7 months, respectively, suggesting that the virus can survive the harsh conditions, including the influence of bile acids, in the intestine. Viral RNA correlated with intestinal symptoms, such as abdominal pain, nausea, and vomiting, partly explaining some of the lasting symptoms of PCS.[70] However, there was no significant correlation between fecal or respiratory viral load and PCS development.[28,71] Some individuals with PCS may not fully clear infectious material after an acute infection. Instead, viral material persisted in intestinal tissues as a 'reservoir.' Viral protein expression from this reservoir could modulate the host immune response and contribute to the pathology of PCS.[69] Viral persistence might result from inhibiting effects of SARS-CoV-2 on the interferon cascade, a crucial antiviral response, potentially leading to virus production in epithelial cells.[72] A stable gut ecosystem, which usually comes along a high microbiota diversity, provides a strong barrier to invading pathogens, including viral pathogens.[15,73] Interestingly, both the primary bile acid chenodeoxycholinic acid (CDCA) and the secondary bile acid ursodeoxycholic acid (UDCA) appear to play a role in the presence of virus particles in feces. These can modulate the binding of the virus to receptors and thereby affect entry into the host.[74] Dysbiosis in the gut microbiome affects the mucosal barrier and increases the production of pro-inflammatory cytokines during acute infections, which may be related to the development and severity of PCS.[75–77]

The assessment of anti-viral responses in patients with PCS is challenging and has not been reported. For example, the intestinal antibody response exhibits a high interindividual variation,[78,79] potential due to specific eating patterns, stool consistency, and gut transit time. Thus, measurements of fecal antibodies need a great number of human subjects to present meaningful results. Furthermore, in this patient group, only fecal measurements are feasible. Reaching other sides in the intestine, for example, via endoscopy,

might be too invasive for vulnerable patients. These measurements are easier to assess *in vitro* or in animal studies. However, in both, a PCS model that captures all most symptoms, particularly neurological ones, does not exist yet.

Altered profile of gut-derived metabolites in PCS

Host–microbe interactions are orchestrated by secretion, interaction, and response to signal molecules, which can serve as biomarkers for a systems biology approach. Characteristically, microbial metabolites are linked to the maintenance of core functions in the body such as digestion and immune system development.[80] In contrast, an imbalanced production of metabolites due to microbial dysbiosis or affected uptake of metabolites can lead to various diseases.[81] Typical classes of metabolites of the gut microbiota include but are not limited to SCFAs,[82] bile acids, gases, tryptophan and indole derivatives, choline metabolites, vitamins, neurotransmitters, and other lipids.[80,81]

The serum metabolome of individuals with PCS differed from that of healthy controls 2 years after infection.[83] The metabolomics approach identified 53 molecules that were significantly different between acute COVID-19 patients and healthy controls. Of those, 27 remained different in a PCS patient group analyzed 2 years after initial infection. Focusing on the SCFAs propionic and butyric acids, these were found significantly increased during COVID-19, potentially due to a leaky gut as observed by Lunjani et al.,[84] and in turn, a leakage of SCFAs into the blood circulation from the intestines. Butyrate-producing bacteria, including *Bifidobacterium pseudocatenulatum* and *Faecalibacterium prausnitzii* showed the largest inverse correlations with PACS at 6 months.[49,85] Of interest, there was no difference for these SCFAs between healthy controls and PCS patients after 2 years in the study by López-Hernández et al.,[83] suggesting that the leaky gut had resolved after 2 years as these SCFA levels were normalized in their study population. Pathway analysis on the altered metabolic serum profiles between controls and PCS patients suggested differences in the biosynthesis of phospholipids, gluconeogenesis, the glucose-alanine cycle, the Warburg effect, and taurine and hypotaurine metabolism.

Targeted measurements of gut microbiome-derived metabolites in the serum of patients with PCS, which were compared to healthy volunteers, are reported in an explorative study by Sadlier et al.[86] A disruption in metabolites with immunomodulatory properties (S1P, 12-HETE), energy metabolism (mannose, glutamate, succinate), and tryptophan pathway (serotonin, quinolinate) was observed, which indicates that the functionality of the gut microbiome is affected in PCS. All these metabolites are involved in specific pathways of the host, thus, a change in these (gut-derived) metabolites might influence the host's function. Yano et al.[87] did show that indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis[87–89] They suggest that altering the microbiota could improve serotonin-related disease symptoms. Conversely, dysbiosis observed in COVID-19 and PCS could cause serotonin-related disease symptoms including psychological symptoms such as anxiety, depressed mood, insomnia, cognitive decline, and physical symptoms such as chronic fatigue, disturbed sleep, loss of appetite, headaches, as well as stomach pains. These symptoms have also been reported by PCS patients.

The fecal metabolome, that includes all metabolites produced by the gut microbiota, has not yet been explored in PCS; however, studies in COVID-19 patients suggest disruption during infection. Analysis of fecal metabolites showed significantly lower fecal concentrations of SCFAs and L-isoleucine in patients with COVID-19 before and after disease resolution.[90] Moreover, Lv et al. [91] compared the fecal metabolites of 56 COVID-19 patients with matched healthy controls and found increased levels of nutrients, such as sucrose, which should have been metabolized and absorbed in the higher intestinal tract. These metabolites might provide mechanistic insights into disturbed digestion, leading to symptoms such as diarrhea. Furthermore, harmful metabolites like oxalate were increased in COVID-19 patients. Fecal oxalate metabolism has been linked to cardiovascular diseases,[92]

offering initial mechanistic insights into how the microbiota can influence cardiovascular symptoms in PCS.

Disrupted host–microbe interactions in the gut during PCS

It has been demonstrated that the gut microbiota plays a pivotal role in influencing almost all of the host's body functions, albeit to varying degrees.[93] Host–microbe interactions in the gut are affected in PCS as observed by the alterations in the composition of the gut microbiome. Specifically, the altered gut microbe composition influences host processes mediated by microbial metabolites. These fuel local and systemic signaling pathways and act as immunomodulatory components. The bacteria in the gut are a major source of immune-modulatory molecules such as lipopolysaccharide (LPS), SCFAs, and the anti-inflammatory molecule desaminotyrosine,[94] thereby it is capable of shaping the immune response in the gut.[39] This was demonstrated in germ-free animals and microbiota-depleted mice (via antibiotic treatment), which, as a result, were more susceptible to infections, attributed not only to the absence of the microbiota but also to deficiencies in the immune response.[95,96]

Giron et al. [97] suggested that fungal translocation from the gut and lung partly contributes to the inflammation found in PCS. First, they observed higher levels of zonulin, an important tight junction protein between cells, in the blood circulation of individuals with PCS compared to infected controls without lasting symptoms, indicating increased gut permeability in PCS.[98] Second, they found elevated beta-glucan, a membrane compound of fungi, levels in PCS, correlating with various inflammatory markers such as IL-6 and the number of symptoms. Third, they noted that the inflammation induced by beta-glucans interfered with the tryptophan catabolism pathway,[99] some metabolites of this pathway have established neurotoxic properties. Moreover, Kusakabe et al. [64] found that gut fungal pathobionts contribute to immune activation during inflammatory diseases, offering potential mycobiota-immune therapeutic strategies for PCS. Overall, these studies elegantly demonstrated that the mycobiome likely influences the development of PCS and provided insights into new treatment strategies.[97]

A SARS-CoV-2 infection is associated with a so-called 'cytokine storm', characterized by the uncontrolled release of high levels of pro-inflammatory cytokines such as IL-6, which negatively affects the function of several tissues.[100] Cytokine expression and immune cell activation remained elevated 8 months after infection in individuals with PCS, whereas they were lower in infected individuals without lasting symptoms.[101] Translocation of pro-inflammatory compounds from the intestine into the blood circulation is a plausible mechanism to explain chronic (low-grade) inflammation,[102,103] however, it has been challenging to prove due lack of sensitive techniques.[104]

Signaling through the microbiome-gut-brain and microbiome-gut-lung axes in PCS

The relationship of the microbiome in the GI tract and the communication through circulating metabolites with the brain is termed the microbiota-gut-brain (MGB) axis. In essence, the MGB axis is an interorgan communication pathway that functions in both directions and involves immune and neural systems.[105] The MGB axis is connected to the neuronal system through the central nervous system, enteric nervous system, and autonomic nervous system.[106] This has been reported to link the gut microbiota via its metabolites to the cognitive and emotional centers of the brain.[107] In addition, microbial metabolites play a critical role in maintaining the homeostasis of both gut and systemic immunity.[108]

The metabolism of the essential amino acid tryptophan is a central pathway integrating microbial metabolism to that of the host. In the gut, tryptophan metabolism can be categorized into three distinct routes, which are the serotonin, kynurenine, and indole pathways.[109] The diverse tryptophan metabolites, either catabolized by eukaryotic or prokaryotic cells in the gut, act on various signaling pathways which upon dysregulation contribute to diseases of the gastrointestinal, immune, or neuronal system. This indicates the importance of tryptophan metabolism in physiological conditions in a symbiotic gut microbe–host interaction network. The uptake of gut-derived metabolite tryptophan,[110] as a precursor

for serotonin, has been reduced and associated with PCS symptoms.[111,112]

Interestingly, deletion of ACE2 in mice reduced tryptophan uptake, suggesting an influence of ACE activity and amino acid uptake,[113] which in turn affected serotonin levels in the brain of mice.[114] Particularly, the expression of ACE2 in the small intestine might be important for amino acid uptake and, simultaneously, an important route for SARS-CoV-2 to enter the host.[115] Other mechanisms where the gut microbiome has been shown to be involved are in the development of depression, a major symptom of PCS,[116,117] through the synthesis of glutamate, butyrate, serotonin, and gamma amino butyric acid (GABA); all of those key neurotransmitters linked to depressive disorders.[118] Interestingly, other viral infections, such as Epstein–Barr virus (EBV), also affect the MGB axis highlighting the importance of viral infections in the development of neurological disorders.[119] The mechanisms in the MGB axis in PCS are currently poorly understood. Gut-derived substances such as toxins, produced by opportunistic pathogens, have been suggested to influence and damage cerebral blood vessels.[120] The fungal pathogen *Candida albicans* is associated with chronic brain diseases such as Alzheimer's disease,[121] although, causative evidence is lacking.

Another systemic effect initiated by the gut microbiome involves the respiratory system and is termed the microbiota-gut-lung (MGL) axis.[122] In healthy persons, the lungs are colonized by transient microbiota that are rapidly cleared by the immune system. Interestingly, a disturbed tryptophan metabolism has been noted in chronic inflammatory lung diseases.[123] It appears that tryptophan is needed to mount an inflammatory response in the lung.[124] A functional imbalance of ACE2 expression in the (small) intestine, reduced tryptophan uptake and, in turn, reduced the immune response in the lung. This mechanism might also apply to the development of PCS, however, it has not been tested yet. The airway system is continuously exposed to the external environment which relies on effective clearance by the immune system.[125] Modulation of the immune system through gut-derived metabolites is a key component of the MGL axis. Examples of some mechanisms by which the gut microbiota arms the lung to combat viral respiratory infections are described by Sencio et al.[126] They showed how the gut microbiota affects the lung's synthesis of type I interferons (IFNs), which are widely recognized for their ability to regulate viral infections like SARS-CoV-2. Both SCFAs, which are the byproduct of the fermentation of dietary fiber by commensal bacteria, and desaminotyrosine, which is produced from the metabolism of flavonoids and amino acids, affected the IFN response in the MGL. It is plausible that any alteration in the microbiota composition and function can alter the beneficial crosstalk between the gut and the lungs. Other proposed mechanisms have been described as components of the MGL axis. First, the interconnection of mucosal tissues via immune cell migration, second, the paracrine communication via secreted cytokines and growth factors, and, third, distant exposure to microbial-associated molecular patterns (MAMPs) via initial absorption in the gut epithelium;[127] all three potentially involved in PCS but not tested yet. Other viral infections, such as influenza, were related to the MGL axis, highlighting the importance of immune training via the gut.[126]

A functional relationship between the (gut) microbiota and the lung has been indicated, as respiratory symptoms during acute SARS-CoV-2 infection have been associated with altered gut microbiota.[128] The lung harbors its own low but diverse microbial biomass.[129] Changes in the lung microbiome, such as an enrichment of fungal cultures like *Candida*, have been linked to COVID-19 severity.[130] Similarly, *Candida* species were increased in the gut microbiome,[64] suggesting a disturbed anti-fungal response of the immune system or a connection between the gut and lung. Moreover, a decreased abundance of butyrate-producing bacteria might influence symptoms related to lung function. Animal studies have shown that (oral) butyrate administration in mice reduced the severity of lung diseases, mainly by regulating inflammation.[131] While a direct interaction between the gut and the lung is plausible[132] in PCS, given the literature on other respiratory diseases, it has not been tested how the MGL axis relates to persistent symptoms seen in PCS. Due to the complexity of interactions within the MGB and MGL axes, and other interorgan

communication pathways such as the microbiota-gut-liver axis,[133] exact mechanisms remain unclear regarding physiology and pathology during PCS which warrants more research.

Targeting the gut microbiome for alleviation of PCS

A dysbiotic microbiota has been related to various diseases, including PCS. Although causal roles of the gut microbiota have only been assigned to specific diseases such as recurrent *Clostridium difficile* infection, targeting a dysbiotic microbiota may help to partially prevent or alleviate the severity of diseases such as PCS. Preclinical models, including various *in vitro* platforms and animal studies, are powerful tools to screen the application of novel treatments. In a personalized approach, this could unlock the possibility of finding the right intervention for the right person. In this section, we provide an overview of preclinical model systems and tested interventions, followed by an overview of clinical studies effectively modulating the gut microbiome in PCS.

Overview of preclinical model systems and interventions in PCS

A wide variety of preclinical model systems for the gut microbiome exist, ranging from basic models based on microbiomes derived from fecal samples to advanced host–microbe interaction models and *in vivo* animal models.[134] Specifically for PCS, no *in vitro* models are available to test interventions in this patient group. PCS is a complex disease that manifests in multiple organs and appears to be intertwined between these organs. *In vitro* systems may only grasp small parts of these intertwined systems, such as gut microbiota dysbiosis and leaky gut syndrome, but have the advantage of high throughput screening of multiple compounds or development of personalized therapies (Figure 2).

To test interventions on gut microbiome dysbiosis, fecal material from patients with PCS can be used and cultured *ex vivo* or dysbiosis can be induced experimentally via antibiotic treatment of the fecal material. *In vitro* fermentation models can be used to test whether a gut-targeted treatment, such as dietary fibers, can influence the abundance

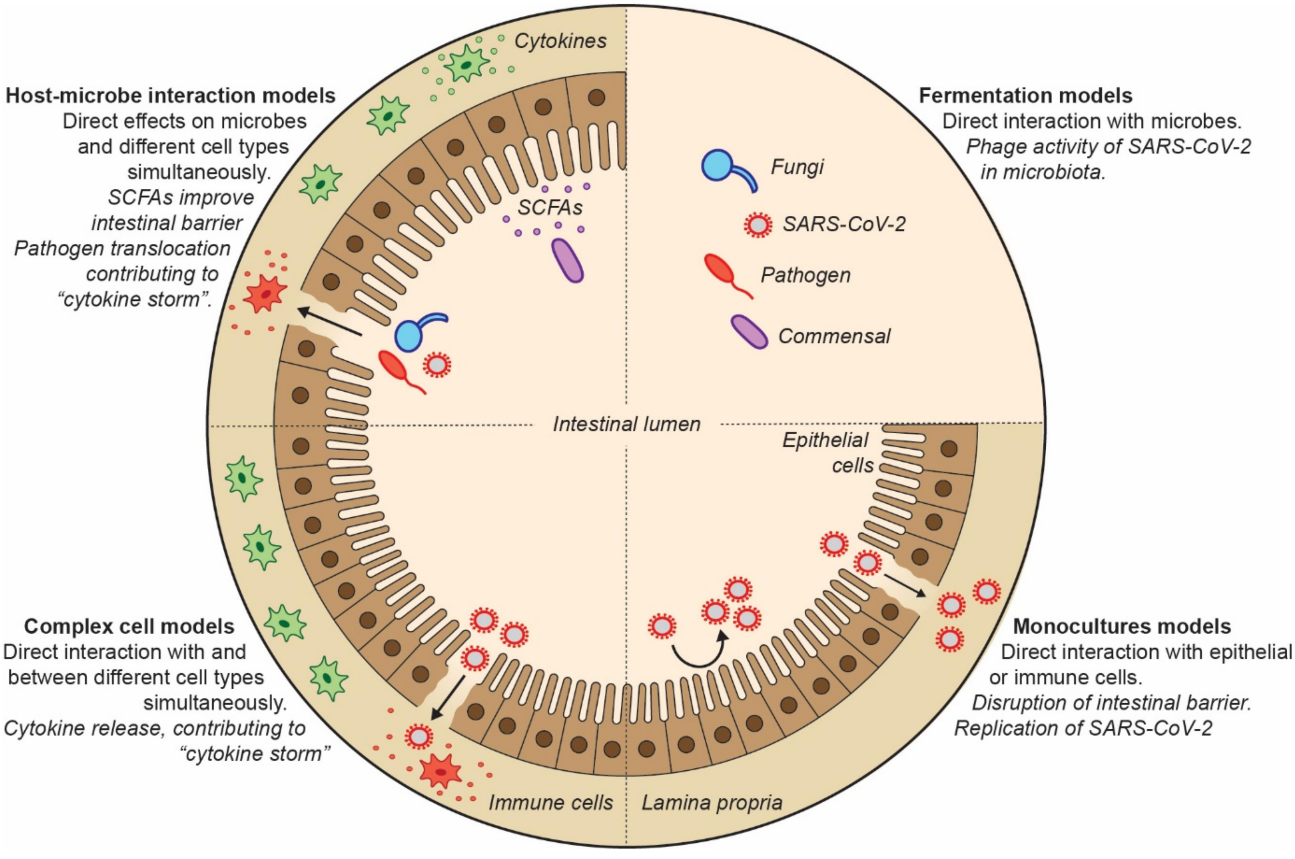

Figure 2. Intestinal *in vitro* models applicable to screen for interventions in PCS.

of specific pathogens, including bacteria and viruses. For example, 2'-fucosyllactose inhibited the growth of *Clostridioides difficile* in a complex microbiota used in a fermentation model.[135] The interaction of SARS-CoV-2 with the gut microbiota has not been explored yet. It has been suggested that the virus may act as a bacteriophage, a virus that targets bacteria; however, evidence for this concept is limited.[136] To this stage, no COVID-19 treatments have been reported in an *in vitro* fermentation model of human-derived gut microbiota.

In vitro mono-cell cultures with Caco-2 cells, a well-studied epithelial cell line, combined with SARS-CoV-2 show that the virus rapidly infects these cells due to its expression of various entry proteins.[137,138] Infection with the virus effectively disrupted the epithelial barrier *in vitro* and enabled the virus to enter the host's blood circulation.[139] Blocking these entry sides with pharmacological treatments[140] may prevent recurrent SARS-CoV-2 infection originating from the intestinal virus reservoir. Mono-cell cultures are readily available, easy to grow, and therefore, provide an option for high-throughput screening of novel drugs. These models save time and resources as well as reduce the use of experimental animals, making these *in vitro* cultures important tools for emerging pathogens. Natural ingredients, such as curcumin, have been shown to reduce cytokine secretion *in vitro* after SARS-CoV-2 infection,[141] providing evidence to reduce the severity of the cytokine release through natural compounds.

Complex cultures with different cell types provide more intricate and physiologically relevant insights. However, this complexity increases the variability of the results and logistical readiness of the model, reducing its usage within high-throughput screening. Interestingly, infection of a complex gut-on-a-chip model with different cell types showed that SARS-CoV-2 efficiently infected epithelial cells with a high viral load and inflammatory response but did not infect endothelial cells that grew behind the epithelial barrier. However, prolonged infection damaged both cell types, leading to the invasion of the virus into the host.[139] These models provide mechanistic insight into the "cytokine storm" induced by SARS-CoV-2.

The combination of *in vitro* fermentation models and cell culture models provides valuable insight regarding host–microbe interaction. Beneficial bacteria can produce the SCFAs formate, acetate, propionate, and butyrate[142] that collectively improve the epithelial barrier function[143] and therefore, may alleviate the impact of the SARS-CoV-2-induced barrier disruption. Particularly, treatment with butyrate-producing bacteria might reduce the severity of COVID-19 infections by improving barrier function, as butyrate is the preferred fuel of colonocytes and is a main regulator of epithelial tight junctions.[144] Indeed, direct application of butyrate in human derived intestinal tissue reduced cytokine expression and the number of infected cells.[145] Combining viable gut microbiota with epithelial tissues and SARS-CoV-2 virus has not been explored yet but would give valuable insights into the activity of the virus in the intestine.

Animal models are the most complex pre-clinical models that can be used to validate results from *in vitro* screenings and to test novel treatments before their use in humans. However, the gut microbiota of animals, particularly the widely used murine models, differs from that of humans and is not always translatable to humans.[146] Similarly, not all animal models are susceptible to SARS-CoV-2 infection.[147] The virus can bind to human ACE2, but not to murine ACE2. Genetically modifying mice increased their susceptibility to the virus. Despite these challenges, several insights were gained from animal studies.[148] It has been suggested that the gut microbiota regulates intestinal ACE2 expression. Germ-free mice had higher intestinal ACE2 expression than conventional mice,[149] potentially due to the lack of intestinal butyrate production of the gut microbiota. Butyrate can reduce ACE2 expression.[145] A reduction in butyrate-producing bacteria may directly be related to an overexpression and, therefore, a higher entry chance of the virus, although this has not been tested in humans yet.

ACE2 expressing probiotics are promising treatment options.[150] Mechanistically, they compete with the intestinal ACE2 binding sites, leading to the binding of SARS-CoV-2 to probiotics instead of intestinal tissue. Butyrate administration protected against SARS-CoV-2-induced lung tissue damage in

golden hamsters,[151] which expressed ACE2.[152] These findings suggest that the gut microbiota has a major influence on COVID-19. Fecal transplantation, an experimental procedure to exchange a healthy microbiota with a dysbiotic microbiota, in PCS might be an option to improve the condition of these patients,[153] however, care has to be taken with this vulnerable patient group. They are susceptible to (intestinal) infections.[3] Importantly, infections have been reported after fecal transplants.[154] Extensive screening of the donor material might be needed to avoid adverse effects.[155]

Overview of clinical studies modulating the gut microbiome in PCS

Clinical intervention studies are needed to unravel the relationship between the gut microbiota and PCS. Various clinical studies have been performed using probiotics, prebiotics, synbiotics, and postbiotics to modulate a dysbiotic gut microbiome[156–158] Specifically in PCS, the first randomized, double-blind, placebo-controlled trial was conducted by Lau et al.[159] Inclusion criteria were the presence of at least one of 14 PACS symptoms for 4 weeks or more after confirmed SARS-CoV-2 infection. Intervention was performed by oral administration of a synbiotic preparation SIM01 containing probiotic strains (e.g. *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*) combined with prebiotic compounds galactooligosaccharide, xylooligosaccharide, and resistant dextrin. After 6 months of treatment with the synbiotic preparation, alleviation of PCS symptoms was identified.[159] For example, gastrointestinal upset was significantly improved compared to the placebo group, suggesting that indeed the gut targeted treatment improved gastrointestinal symptoms. However, the quality of life was not significantly improved, suggesting that it can only alleviate parts of the disease. In other complex diseases such as diabetes, only parts of the disease improved after symbiotic treatment,[160] suggesting that these interventions can be used as support but do not replace other treatments. Adverse events included mainly gastrointestinal symptoms such as diarrhea and bloating, however, both were comparable between placebo and synbiotic. The placebo consisted of a low dose of vitamin C, which was not present in the symbiotic, and a starch filler, which may explain some of the gastrointestinal symptoms in the placebo group. Overall, synbiotics appear to be safe in patients with PCS and have beneficial effects.

Therapeutic mechanisms can only be derived from other studies that tested effects of synbiotics on gastrointestinal health. For example, different types of synbiotics improved symptom scores of patients with irritable bowel disease,[161] potentially via improving microbiota symbiosis with the host and beneficial effects of prebiotics on stool consistency. Although more research is warranted, the study by Lau et al. [159] demonstrated the potential and impact of interventions targeting the gut microbiome in PCS.

Conclusion and perspectives

The COVID-19 pandemic has had a profound impact on society, leaving some individuals with long-lasting symptoms. PCS is a heterogeneous condition that is not only challenging to diagnose but also difficult to treat due to various nonspecific symptoms. Gastrointestinal discomfort is a common symptom in PCS, suggesting that SARS-CoV-2 disturbs gut homeostasis. Gut health is an important factor that affects various aspects of the body, where dysbiosis can lead to debilitating effects in the human body. Experimental research uncovered alterations in the gut microbiome of patients with PCS, including differences in composition and functionality. This is suggested to affect systemic processes via metabolites, microbe translocation, and through the MGB and MGL axes.

Providing gut-targeted therapies might help alleviate some of the symptoms in PCS. However, caution must be taken since these interventions improve parts of the disease, but do not cure PCS. The first study with synbiotics demonstrated that this treatment improved symptom scores.[159] More studies are needed to unravel the role of the host–

microbe interactions in PCS with the aim to provide urgently needed personalized treatment strategies and to activate the restorative capabilities of the gut microbiome in PCS.

List of abbreviations

COVID-19	coronavirus disease 2019
PCS	post-COVID syndrome
PASC	post-acute sequelae of COVID-19
PEM	post exertional malaise
POTS	postural orthostatic tachycardia syndrome
MCAS	mast cell activation syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
ME/CFS	Myalgic encephalomyelitis/chronic fatigue syndrome
ACE2	angiotensin-converting enzyme 2
TMPRSS2	transmembrane protease, serine 2
SCFA	short-chain fatty acid
RNA	ribonucleic acid
MGB	microbiota-gut-brain
MGL	microbiota-gut-lung
MAMP	microbial-associated molecular pattern
LPS	lipopolysaccharide
IgA	immunoglobulin A

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