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**Enterochromaffin Cells–Gut Microbiota Crosstalk: Underpinning the Symptoms, Pathogenesis, and Pharmacotherapy in Disorders of Gut-Brain Interaction**

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## **Abstract**

Disorders of gut-brain interaction (DGBIs) are common conditions in community and clinical practice. As specialized enteroendocrine cells, enterochromaffin (EC) cells produce up to 95% of total body serotonin and coordinate luminal and basolateral communication in the gastrointestinal (GI) tract. EC cells affect a broad range of gut physiological processes, such as motility, absorption, secretion, chemo/mechanosensation, and pathologies, including visceral hypersensitivity, immune dysfunction, and impaired gastrointestinal barrier function. We aim to review EC cell and serotonin-mediated physiology and pathophysiology with particular emphasis on DGBIs. We explored the knowledge gap and attempted to suggest new perspectives of physiological and pathophysiological insights of DGBIs, such as (1) functional heterogeneity of regionally distributed EC cells throughout the entire GI tract; (2) potential pathophysiological mechanisms mediated by EC cell defect in DGBIs; (3) cellular and molecular mechanisms characterizing EC cells and gut microbiota bidirectional communication; (4) differential modulation of EC cells through GI segment-specific gut microbiota; (5) uncover whether crosstalk between EC cells and (i) luminal contents; (ii) enteric nervous system; and (iii) central nervous system are core mechanisms modulating gut-brain homeostasis; and (6) explore the therapeutic modalities for physiological and pathophysiological mechanisms mediated through EC cells. Insights discussed in this review will fuel the conception and realization of pathophysiological mechanisms and therapeutic clues to improve the management and clinical care of DGBIs.

## **Key Words**

Enterochromaffin cells; Gastrointestinal microbiome; Gut barrier dysfunction; Gastroparesis; Irritable bowel syndrome

## Introduction

Functional gastrointestinal disorders (FGIDs) are currently defined as disorders of gut-brain interactions (DGBIs) to underscore the term "functional" as it has a connotation of being primarily psychologically in origin.<sup>1</sup> Moreover, in place of brain-gut interaction, which overestimates the importance of cerebral dysfunction over abnormalities in the gut, in the new Rome classification, these are called DGBIs, overemphasizing the importance of gut over the brain.<sup>1</sup> The board of directors of the Rome foundation, with the feedback and final approval from the chairs and co-chairs of the Rome IV committees, created the new definition for DGBI and defined it as a group of disorders classified by gastrointestinal (GI) symptoms related to any combination of the following: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered central nervous system (CNS) processing.<sup>2</sup> DGBIs, for instance, irritable bowel syndrome (IBS) and functional dyspepsia (FD), are common conditions needing referral to gastroenterology clinics and are also highly prevalent in the community.<sup>3</sup> DGBIs are heterogeneous conditions with intertwined pathophysiological mechanisms, such as altered gut motility, impaired GI barrier function, gut immune dysregulation, gut microbial dysbiosis including small intestinal bacterial overgrowth, low-grade inflammation, visceral hypersensitivity, and dysregulated gut-brain axis.<sup>4,5</sup> A recent global epidemiological study of 73 076 participants in 33 countries reported that more than 40% of participants met the Rome IV criteria for at least one DGBIs.<sup>6</sup> This study indicated FD and IBS were the top rankers amongst the 22 DGBIs on the pooled prevalence rate.<sup>6</sup> Furthermore, DGBIs and specific GI motility disorders often present significant clinical overlaps, for instance, gastroparesis and FD-postprandial distress syndrome (90%); gastroparesis and slow-transit constipation (60%); and FD and IBS (15-42%).<sup>7-9</sup> The rising prevalence of DGBIs established a

substantial burden and challenge on the patients, families, and the healthcare system.<sup>3</sup> Despite extensive research, the underlying pathophysiology of DGBIs remains enigmatic providing the impetus for future research to elucidate the precise pathologies and treatment to optimize patient care.

Serotonin (5-hydroxytryptamine, 5-HT) affects a wide range of gut and brain physiological functions such as gut motility, secretion, visceral sensation, immune regulation, gastrointestinal epithelial integrity, sleep, and mood.<sup>10,11</sup> The vast majority of serotonin in our body is produced and secreted from enterochromaffin (EC) cells.<sup>11</sup> Serving as a communication circuit between the apical and basolateral interface of the gut epithelium, EC cells sense chemical-mechanical stimuli, transduce signals to enteric nervous system (ENS) and CNS, and neighboring cells; these functions reinforce EC cells as a front-line player in modulating the pathophysiology of DGBIs.<sup>12</sup> However, the potential mechanistic link between EC cells/serotonin metabolism and the DGBIs is largely elusive. In this review, we uncovered the most recent discoveries of EC cells and/or serotonin-mediated physiology and pathophysiology in DGBIs. Decoding complex mechanisms will strengthen the understanding of DGBIs, facilitate potential diagnostic tools, and therapeutic modalities to refine the management of these disorders.

## **Current Challenges of Disorders of Gut-Brain Interactions**

To broaden our horizons on pathophysiology of DGBIs, current challenges that need to be addressed include: (1) symptoms may not always reflect the pathophysiological mechanisms. Without knowing the cellular and molecular pathologies, the medications may be ineffective and results in higher healthcare expenditure and poor quality of life.<sup>3,13,14</sup> Better pathophysiology-

directed therapeutic strategies rather than symptomatic treatment are warranted. (2) The interplay between EC cells/serotonin signaling and gut microbiota and/or its derived metabolites in the pathogenesis of DGBIs are enigmatic. The association between altered serotonin signaling and symptom severity among patients with DGBIs is scanty.<sup>11,15,16</sup> (3) Despite gut microbiota having an essential role in gut health, its precise functions in DGBI are cloudy.<sup>4</sup> Given the fact that the gut microbiome varies between person, race, ethnicity, nationality, and even neighboring communities, what constitutes a healthy microbiome? What is gut microbial dysbiosis?<sup>17,18</sup> (4) The mechanism of EC cells contributing to the intestinal barrier integrity and the fundamental impact of gut microbiota and its derived molecules on EC cell's function are limited.<sup>19</sup> Large-scale and longitudinal studies utilizing multi-omics analysis are needed to dissect the exact contribution of gut microbiota and serotonin signaling alterations in the progression/regression of DGBIs. (5) Research on the role of the vagus nerve innervation in ENS, which connects the gut to the brain, concerning EC cell in DGBI pathophysiology is enigmatic?<sup>20</sup> How does the interaction/interplay between EC cells, sensory afferent neurons, and gut immune cells modulate visceral hypersensitivity in DGBIs?<sup>21</sup> Further, how does the gut-brain-neuroendocrine axis involve psychological comorbidity or early adverse life events that lead to DGBIs?<sup>21</sup> (6) The explicit understanding of the central players, such as EC cells and serotonin, and how they coordinate the gut functions and crosstalk with neighboring cells and luminal content is elusive. New drugs targeting EC cells may offer hope for novel therapeutic approaches in the clinical care of DGBIs. (7) The mode of administration and the pharmacokinetics of therapeutic agents are critical features for clinical outcomes.<sup>22,23</sup> The therapeutic modalities of enhancing the selectivity and specificity of the drugs for the gut cells are another approach to achieve better target efficacy.<sup>24</sup> A further challenge for achieving the prokinetic effect is still incomplete, which

requires activation of both luminal and myenteric circuitry 5-HT receptor 4 (5-HTR4).<sup>24</sup> Furthermore, 5-HTR expression is abundant in the GI cells throughout the gut, but differential expression of their subtypes specific to the GI cells is elusive, which is critical for the on-target and off-target side effects associated with 5-HTR agonists and antagonists. (8) To discriminate FD from gastroparesis, which has a similar underlying clinicopathological spectrum, is a significant challenge in clinical practice and research, given that these disorders are indistinguishable by gastric emptying assessment.<sup>25</sup> Precise biomarkers for identifying the pathologies are warranted besides the gastric emptying test, which does not correlate with symptoms and their severity.<sup>25</sup> (9) The clinical presentation of DGBIs significantly overlaps. For instance, 60-70% of patients with gastroparesis have slow transit constipation (STC), and the overlap between IBS and FD is 15-42%.<sup>3,8</sup> These clinical findings raise questions about the causal effect and the origin of the different subtypes DGBIs. Significant clinical overlaps among DGBIs suggest the sharing pathophysiological mechanisms and therapeutic targeting of these cellular and molecular pathologies might provide a better treatment approach.

## **Gastrointestinal Cells Dynamics in Gut Health and Disorders of Gut-Brain Interactions**

### **1. Gastrointestinal Cells Dynamics in Gut Physiology**

The GI tract is a highly dynamic tubular organ that processes ingested food, assimilates water and nutrients, and eliminates waste to maintain our health.<sup>26</sup> A recent human intestinal single-cell resolution spatiotemporal analysis has cataloged 101 cell types and 9 intestinal

compartments clusters, including immune, epithelium, muscularis, endothelium, neural, fibroblast, myofibroblast, pericyte, and mesothelium.<sup>27</sup> The staggering complexity of intercellular interactions coordinates the directional movement of luminal contents from ingestion to expulsion.<sup>28</sup> The pacemaker activity of interstitial cells of Cajal (ICCs), platelet-derived growth factor-alpha cells, smooth muscle cells, enteroendocrine cells, glia cells, and excitatory and nitrergic neurons of the ENS maintains the regular GI motility pattern (Figure).<sup>29</sup> The cellular and molecular remodeling in these cells are responsible mechanisms for GI function alterations in FD, gastroparesis, and IBS.<sup>30</sup> However, the inconsistent association between altered gut physiology and symptoms warrants further investigations of differential innervating patterns and complexity of enteric and vagal neurons, pacemaker cells, immune and enteroendocrine cells in region-specific GI segments.

As the second-largest epithelium in our body, the GI tract constitutes a surface area of > 30 m<sup>2</sup>.<sup>31</sup> A large surface area of GI epithelium in direct contact with luminal contents is responsible not only for nutrient absorption but also protection against environmental pathogens.<sup>32</sup> The gut epithelium functions as a protective barrier that withstands extreme pH variations, mechanical abrasion, and bacteria colonization. Enteroendocrine cells (< 1% of gut epithelium) are primary secretory lineages that serve vital functions that rely on hormones and neurotransmitters to integrate signals arising in the gut to the brain.<sup>33</sup> EC cell is a unique subset of enteroendocrine cells, scattered among epithelium throughout the GI tract, synthesize and secrete 90-95% of the serotonin in the human body (Figure).<sup>16</sup> As a specialized intestinal epithelium, EC cells closely coordinate with neighboring cells to adopt various strategies balancing the intestinal barrier functions with secretion, chemo-and mechanosensation, nutrient absorption, and crosstalk with gut microbiota.<sup>34</sup> Serotonin is a versatile biogenic amide,

neurotransmitter, and hormone, which is primarily responsible for diarrhea associated with carcinoid tumors.<sup>35</sup> It regulates pivotal peripheral and central effects, including secretion, visceral sensation, gut motility, energy hemostasis, hematopoiesis, inflammation, sleep, food intake, aggression, depression, anxiety, and psychosis critical for gut health and alternations responsible for DGBIs.<sup>11</sup>

## **2. Gastrointestinal Cells Dynamics in Disorders of Gut-Brain Interactions Pathophysiology**

State-of-the-art studies suggest impaired GI barrier function as a central pathological mechanism that regulates gut-brain interactions due to immune dysregulation via microbial alterations and food antigens.<sup>36,37</sup> Gut immune dysregulation drives a breach in the intestinal barrier and functional deficits in GI pacemaker cells, leading to alerted visceral sensation, secretion, and gut motility.<sup>38</sup> As a result, the activated mast cells and eosinophils induce tissue damage and interferes with enteric nerve function. In addition, dysregulation of the neuroimmune crosstalk has been proposed as the leading cause of visceral hypersensitivity responsible for abdominal pain in subsets of DGBIs.<sup>39</sup> Studies suggest EC cells serve as a communication circuit for the gut-brain interactions. The interplay between host and gut microbiota modulates serotonin signaling/metabolism, affecting gut neurohormonal-immunoregulation, intestinal barrier function, and visceral sensation.<sup>40</sup>

### **Enterochromaffin Cells Mediating Gut Physiology**

Owing to their extensive heterogeneity and variability, the functional roles of EC cells and serotonin are still in large part undetermined concerning the scope of its contribution to gut-brain homeostasis.

## **1. Functional Heterogeneity**

EC cells secrete cholecystokinin (CCK), secretin, and peptide YY (PYY), even though serotonin is the primary hormone synthesized and secreted by these cells.<sup>41</sup> Twenty-five percent of serotonin-containing cells also secrete PYY in the human colon, whereas CCK and serotonin colocalize in the murine small intestine.<sup>42</sup> More intriguingly, researchers identified serotonin, CCK, and PYY inside one single vesicle of EC cells (Figure).<sup>43</sup> The dynamic functions of EC cells are not restricted to secretion; spontaneously, the tasting/sensing function is due to the expression of nutrient receptors, and transporters differ considerably between EC cells in gut region-specific manner.<sup>44</sup> Small intestine EC cells are specialized for sensing nutrients and colonic EC cells for gut microbiota and its derived molecules.<sup>44</sup> Small intestine EC cells are devoid of nutrient metabolites sensing receptors such as G protein-coupled bile acid receptor 1 (GPBAR1), G protein-coupled receptor 119 (GPR119), free fatty acid receptor 1 (FFAR 1), calcium-sensing receptor, and GPR142 (Figure).<sup>44</sup> Colonic EC cells express GPCR sensors of microbial molecules, comprising of receptors for short chain fatty acids (SCFAs), for instance, FFAR2, olfactory receptor 558 (OLF558), OLF78; aromatic acids receptors, GPR35; secondary bile acids receptors, GPBAR1; and acyl-amides and lactate, GPR132 (Figure).<sup>44</sup>

## **2. Life Cycle of Enterochromaffin Cells: Self-renewal and Mature Enterochromaffin Cells**

The turnover rate of the gut epithelial cell is 3-5 days.<sup>45</sup> The epithelium starts its journey from the crypt base and migrates to the villus until it reaches the top edge and has been “squeezed out” while undergoing apoptosis. During its short but magnificent life, the epithelial cells not only “witness” a great war between host-pathogenic microbes, and the harmonious mutualistic relationship between host and commensal microbiota, but also deliver its substantial

duties as the major subtype of enteroendocrine cells. EC cells have different cell morphologies and heterogeneous distribution throughout the entire GI tract (Figure).<sup>42,46</sup> The typical “wine-glass” type of EC cells are located in the small intestine, whereas the “axon-like” EC cells are predominantly found in the colon.<sup>47</sup> The “wine-glass” shaped EC cells are believed to be “open type” and directly taste luminal contents. The “axon-like” EC cells tend to function as “closed type” enteroendocrine cells. A recent murine study demonstrated 2 subpopulations of EC cells. This study clarified their positions, with the mature cells positioned in the apical surface and the self-renewal cells (expressing leucine-rich repeat-containing G-protein coupled receptor 5 or Ki67) may serve as precursor cells to keep up EC cell populations in the basolateral surface (Figure).<sup>16</sup> This discovery challenges the stereotype that the epithelium has a short life and showed EC cells could sustain their cell features for a long time, and some even last a lifetime.

### **3. Apical and Basolateral Interface of Enterochromaffin Cells**

EC cells interact with neighboring enterocytes, other enteroendocrine cells, enteric neurons, gut microbiota, dietary contents, and chemical- and mechanical-stimuli, as autocrine, paracrine, synaptic, and endocrine moderators (Figure).<sup>33,48</sup> Activation of EC cell sensory receptors by food antigens, acidity, invading pathogens, and mechanical stimuli triggers serotonin release.<sup>12</sup> Although there was recently a breakthrough in understanding EC cell sensory pathway, it is well-accepted that the luminal sensing signaling involves a complex of autocrine, neurocrine, and paracrine actions.<sup>40</sup> For instance, EC cells are mechanosensitive; upon activation, Piezo2 channels generate an ionic current essential for the intracellular  $\text{Ca}^{2+}$  resulting in serotonin release spontaneously.<sup>49</sup> Meanwhile, EC cells are chemosensitive and recruit transient receptor potential ankyrin 1 channel (TRPA1) to detect irritants leading to the activation of voltage-gated  $\text{Ca}^{2+}$  channel-dependent serotonin release.<sup>50,51</sup> In addition, neighboring

enteroendocrine L-cells express nutrient metabolite receptors, which trigger glucagon-like peptide secretion to stimulate serotonin secretion from EC cells suggesting that EC cells sense nutrients indirectly via the L-cell and relay the signal luminal nutrient metabolites to basolateral vagal afferent neurons.<sup>44</sup> Once serotonin is released, the signals travel to the local ENS and distant organs, including the pancreas and the brain, thus regulating food intake, digestive enzyme secretion, and bowel movement.<sup>52</sup>

Not long ago, researchers thought EC cells interact with nerves indirectly through paracrine or endocrine signals.<sup>53</sup> Using transgenic and advanced optical tools, EC cells received a better description as having several anatomic features observed in neurons, including dendritic-like spines and axon-like processes, suggesting EC cells may communicate with ENS through synaptic structure, known as neuropod.<sup>48,54</sup> Through neuropod, serotonin released from EC cells acts on extrinsic primary afferent neurons (EPANs) and intrinsic primary afferent neurons in a paracrine and synaptic manner.<sup>40</sup> EPANs stimulated via serotonin modulate nausea and abdominal discomfort. We believe that the mature EC cells positioned in the luminal surface serve to act as paracrine modulators; self-renewal EC cells are primarily positioned in the basolateral crypt base with neuropod sever to connect the gut-brain axis.

Taken together, EC cells not only communicate with neighboring epithelium and enteric neurons as paracrine and synaptic modulators, as well as “tasting” and “feeling” gut microbiota and food particles via chemo- and mechanosensing receptors.<sup>55</sup> Thus, as a flexible and resourceful communication circuit, the EC cell is crucial in GI health and diseases.

# Enterochromaffin Cells Mediating Pathophysiology Mechanisms of Disorders of Gut-Brain Interactions

The regulation of GI functions entails complex coordinated interactions between gut and brain, which necessitates the proper functioning of GI pacemaking cells, epithelial cells, and immune cells.<sup>16,29,30</sup> Any functional defects in these cells will potentially hinder gut function and generate symptoms of DGBIs.<sup>30</sup>

## 1. Apical Interface of Enterochromaffin Cell

### (1) Gut microbiota dysbiosis

Gut microbiota dysbiosis is a notable pathophysiological mechanism of DGBIs.<sup>17, 36, 56</sup> The fecal microbiota profile of IBS patients differs significantly from that of healthy subjects and influences gut physiology, for instance, motility, GI barrier function, visceral sensation, and gut immune function.<sup>4,57</sup> In a recent meta-analysis, IBS patients had a reduced number of commensal bacteria of genera *Bifidobacterium* and *Lactobacillus* and an increased number of pathogenic bacteria of genera *Enterobacter* as compared to the healthy subjects.<sup>58</sup> Studies reported overrepresentation of *Methanobrevibacter smithii* in stool samples from patients with FD, functional constipation, and constipation-predominant IBS (IBS-C).<sup>59,60</sup> A microbiome signature linked with symptom severity of IBS has been suggested with reduced diversity of commensal bacteria and the presence of pathogenic bacteria such as methanogenic or Clostridiales species.<sup>59</sup> Fecal microbiota transplantation (FMT) may be beneficial; however, a meta-analysis of randomized-controlled trials in IBS patients reported no significant improvement in symptoms after FMT compared to placebo treatment.<sup>61</sup> On the contrary, a double-blind, randomized

controlled trial in IBS patients suggested FMT was beneficial with a significant improvement in symptoms compared with placebo.<sup>62</sup> Still, the clinical outcome of gut microbiota intervention with FMT is hazy, particularly due to the unclearness of the healthy microbiome constitution as well as the interplay between host and gut microbiota.

Recent findings highlighted the crosstalk between host and microbiota in gut homeostasis.<sup>34 63</sup> One study demonstrated the critical role of indigenous spore-forming bacteria promote serotonin biosynthesis from colonic EC cells modulating GI motility.<sup>34</sup> On the other hand, elevating luminal serotonin increases the number of *Turicibacter sanguinis*, a spore-forming bacteria, which expresses a neurotransmitter sodium symporter protein with structural and sequence homology to mammalian serotonin reuptake transporter (SERT).<sup>63</sup> Treating *T. sanguinis* with serotonin or fluoxetine enhanced its colonization in the murine gut.<sup>63</sup> Furthermore, one study showed serotonin was capable of decreasing virulence gene expression of *Citrobacter rodentium* and enterohemorrhagic *Echerichia coli* by inactivating bacterial serotonin receptor transcription.<sup>64</sup> Together, these findings suggested that the selective indigenous gut bacteria crosstalk with the host serotonergic pathways to boost their colonization in the gut, also sparked interest in whether modulating EC cell and gut microbiota interaction may be an effective treatment strategy for DGBIs.

## **(2) Gut Microbial-derived molecules**

One of the fundamental regulations of serotonin biogenesis is host-microbiota crosstalk.<sup>65</sup> Gut microbial-derived molecules communicate signals to modulate the targeted cells' function, eventually affect the physiological and pathophysiological mechanisms, influencing health status; still, the precise molecular mechanisms in DGBIs are elusive.<sup>66</sup>

**Bile acids.** Gut microbiota dysbiosis induces alternations in the bile acid (BA) pool.<sup>67,68</sup> On the contrary, reduction in BA levels influences gut microbial diversity leading to gut microbiota dysbiosis.<sup>69</sup> A higher amount of BAs results in increased intestinal fluid secretion.<sup>10</sup> Excess fecal BAs in diarrhea-predominant IBS (IBS-D) lead to altered gut microbiota profile enriched in Clostridia.<sup>70</sup> Higher amounts of BA signatures, chenodeoxycholic acid and cholic acid, were reported in stool samples from patients with IBS-D and lower amounts in patients with IBS-C.<sup>71</sup> The authors also found a positive correlation between a higher amount of BAs and abdominal pain severity. Furthermore, BAs can activate Takeda G-protein-coupled receptor 5 (TGR5) localized on EC cells and promote gut motility.<sup>72</sup> TGR5-deficient mice displayed prolonged colonic transit and reduced fecal pellet frequency compared to control mice advocating TGR5-dependent improvement in gut motility mediated by EC cell serotonin secretion.<sup>73</sup> The aforementioned findings suggest that the interplay between BAs, EC cells, and gut microbiota modulates DGBIs pathogenesis.

**Short chain fatty acids.** SCFAs such as butyrate, propionate, and acetate are the prime fermentation metabolites produced from gut microbiota-mediated degradation of dietary fiber.<sup>74</sup> SCFAs are essential to sustain EC cell and serotonin production via activation of GPCRs (FFAR2).<sup>44</sup> Compared to the germ-free (GF) mice, conventionally raised and humanized mice had higher colonic tryptophan hydroxylase 1 (TPH1) mRNAs due to butyrate-related increase in EC cell TPH1 expression and serotonin production.<sup>75</sup> Furthermore, butyrate alleviates impaired barrier function through increasing claudin-2 expression. *Bifidobacterium dentium*-derived acetate stimulate serotonin release from EC cells.<sup>76</sup> Meanwhile, this bacterium colonized mice had upregulated expression of 5-HTR2A in the hippocampus leading to normalized anxiolytic behavior.<sup>76</sup> Moreover, isovalerate, a volatile fatty acid metabolite generated by gut microbiota,

evoked serotonin release from EC cells, through voltage-gated  $\text{Ca}^{2+}$  channel modulated 5HTR3 sensory neurons.<sup>12</sup> These studies showed that gut homeostasis relies on microbiota-derived SCFA, which plays an important role in GI motility, visceral sensation, secretion, and intestinal barrier function regulation, further reinforcing EC cells as central modulators in the gut-brain axis.

***Aryl hydrocarbon receptor and serotonin signaling.*** The AhR has a fundamental role in host defense against bacteria in the intestine and functions as a biosensor connecting gut microbiota to program ENS and immune cells affecting gut homeostasis.<sup>77,78</sup> Microbiota-directed AhR expression in neurons of the colon tissue enables these neurons to communicate with luminal contents and to trigger the expression of neuron-specific effector mechanisms.<sup>79</sup> Depleting neuron-specific AhR, or overexpression of AhR negative feedback regulator cytochrome P450 1A1 (CYP1A1) causes colonic dysmotility in mice.<sup>79</sup> Intriguingly, serotonin serves as a ligand/activator of AhR in intestinal epithelial cells through increasing CYP1A1 expression of the canonical gene target of AhR.<sup>80,81</sup> Furthermore, this serotonin-AhR-CYP1A1 pathway was confirmed in SERT knockout mice.<sup>80</sup> These studies demonstrated a novel mechanistic link between serotonergic and AhR signaling, which have implications in modulating gut immune function and motility.

***Tryptophan metabolites.*** Tryptophan (Trp) metabolism is crucial in host-microbial communication to fine-tune gut physiology.<sup>10,57,71,82</sup> Trp metabolism comprises 3 critical pathways in modulating gut functions: (1) indole pathway (direct transformation into metabolites by the gut microbiota, such as AhR and indole derivatives ligands); (2) kynurenine pathway (transformation into kynurenine and its derivatives by immune and epithelial cells); and (3)

serotonin pathway (transformation into serotonin and its derivatives by EC cells). Impaired Trp metabolism contributes to DGBIs pathogenesis, particularly IBS.<sup>10,82</sup>

**Tryptamine.** Tryptamine is a monoamine metabolite product of tryptophan metabolism, and the gut bacteria involved in this process are *Clostridium sporogenes* and *Ruminococcus gnavus*.<sup>83</sup> Tryptamine relay its signal in the gut primarily via the 5-HTR4 pathway.<sup>84</sup> Tryptamine participates in regulating intestinal secretion. Tryptamine generated in both GF and humanized mice showed increased epithelium fluid secretions in colonoids. Furthermore, gut motility improved in GF mice colonized with *Bacteroides thetaiotaomicron*, a tryptamine-producing engineered bacteria.<sup>84</sup> Tryptamine also activates 5-HTR4 on goblet cells to release mucus.<sup>19</sup> In a murine inflammatory bowel disease model, GF mice colonized with *B. thetaiotaomicron* exhibited increased mucus release with improved intestinal barrier function and reduced aggressive phenotypes.<sup>19</sup> Tryptamine levels were elevated in the stools of IBS-D patients and reduced in IBS-C patients.<sup>71</sup> Tryptamine can modulate gut motility by triggering the release of serotonin by enteric neurons or acting as an AhR ligand.<sup>79</sup> A recent study showed gut microbiota induces AhR expression in enteric neurons, enabling them more responsive/excitable to microbiota-derived AhR ligands, such as tryptamine, and contributing to gut motility.<sup>79</sup>

**Kynurenine.** Patients with IBS often have altered kynurenine pathway with elevated kynurenine levels in the serum.<sup>85,86</sup> Indoleamine 2,3-dioxygenase, a key metabolite in the kynurenine pathway, positively correlated with IBS symptoms severity.

**Serotonin.** Alterations in the serotonin pathway are one of the crucial features in DGBIs.<sup>10</sup> Whereas the patients with IBS-C have reduced serotonin activity, those with IBS-D have increased serotonin expression. Studies have shown serotonin metabolisms in the physiology and pathophysiology of IBS, FD, and gastroparesis.<sup>10,16</sup> Clostridiales species trigger EC cells to

produce serotonin by inducing TPH1 expression.<sup>34</sup> This effect is sustained by gut microbiota-derived metabolites (propionate, butyrate, tyramine, cholate, and deoxycholate).<sup>34</sup> One recent study showed the role of TRPA1 in the regulation of serotonin secretion by EC cells through Trp metabolites.<sup>51</sup> Indoles are metabolite products of the tryptophan metabolism of *Edwardsiella tarda*. Furthermore, authors showed indoles activate TRPA1 in EC cells to release serotonin that excites enteric neurons to accelerate gut motility and stimulate vagal afferents for CNS modulation.<sup>51</sup>

Sleep disturbances are also a major contributing factor in IBS development and positively correlated with worsening IBS symptoms.<sup>87,88</sup> Monoamine oxidase A converts serotonin to 5-hydroxyindoleacetic acid in the brain and gut.<sup>89</sup> In the brain, serotonin is converted into melatonin, the primary hormone of the body's sleep-wake cycle.<sup>89</sup> Indoleamine 2,3-dioxygenase might shunt Trp to kynurenine in the brain, leading to sleep disturbances.<sup>57</sup> Thus, these findings demonstrated that Trp metabolites are crucially important contributing to different facets of gut physiology and brain function.

Gut microbial metabolites are key molecules relaying signals to fill a specific niche and maintain bidirectional gut and brain interactions. Taken together, gut microbiota and their host may share a “common language” and harmonies as a mutualistic system. Deciphering this “common language” will offer therapeutic perspectives in DGBIs.

### **(3) Intestinal barrier dysfunction**

In susceptible conditions, GI infection or intake of particular foods weakens the intestinal barrier integrity through damage to the tight junctions, resulting from proteasome-mediated degradation activated by inflammatory molecules including proteases, histamine, and

eicosanoids.<sup>37,90</sup> Because of impaired barrier function, food antigens and pathogens cross the gut epithelium and activate a T helper 2 cell response that results in immune activation mediated via mast cells and eosinophils.<sup>91, 92</sup> In patients with IBS, an elevated number of colonic mast cells correlates with impaired intestinal barrier function and visceral hypersensitivity.<sup>93,94</sup> Duodenal mucosa of patients with IBS challenged with food antigens have increased intervillous spaces and epithelial breaks compared to healthy controls.<sup>95</sup> The cell-to-cell adhesion protein, zonula occludens-1, and occludin expression are significantly reduced in duodenal tissues from patients with DGBIs compared to healthy subjects.<sup>96,97</sup> Duodenal biopsy samples from patients with DGBIs more often have decreased transepithelial electrical resistance resulting in intestinal barrier dysfunction with immune cell infiltration.<sup>36,37,98</sup> One study examined whether serotonergic signaling alterations may commence a chain of events leading to changes in epithelial integrity in healthy individuals and patients with IBS.<sup>85</sup> The authors found increased serotonergic metabolic activity, which happened after intake of the serotonin precursor, 5-hydroxytryptophan, and reinforced intestinal mucosal barrier function through redistribution of zonula occludens-1 in healthy individuals; such regulation was absent in IBS patients.<sup>85</sup> A commensal bacterium, *B. dentium*, stimulates EC cells to release serotonin, which interacts with 5-HTR4 on goblet cells to promote Mucin 2 and Trefoil factor 3 release, resulting in the activation of C-X-C motif chemokine receptor 4 to trigger actin cytoskeleton rearrangement and restitution mediating intestinal epithelial repair.<sup>99</sup> Overall, these studies point to the broad implications of a potential protective mechanism involving serotonin signaling for impaired intestinal barrier function.

#### **(4) Visceral hypersensitivity**

The modulators for visceral sensation comprise transient receptor potential vanilloid subtype 1 (TRPV1), protease-activated receptors, histamine, serotonin, tachykinin, cannabinoid, acid-sensing ion channels, voltage-gated sodium and calcium channels, and TRPA1.<sup>12,-100</sup> The TRPV1 activation by capsaicin, thermal stimulus, acidic pH, inflammatory mediators, prostaglandins, nerve growth factors, and microbes trigger the release of neuropeptides, such as substance P and calcitonin gene-related peptide-1.<sup>100</sup> These neuropeptides are responsible for enhanced visceral sensitivity and induce painful and/or non-painful sensations.<sup>101</sup> The hypersensitivity mediated through chemicals such as elevated visceral sensation to acid associates with nausea. The increased TRPV1 expression in colonic tissues often correlates with abdominal pain in patients with IBS-D.<sup>102,-103</sup> Visceral hypersensitivity in patients with gastroparesis associates with abdominal pain, anorexia, and early satiety.<sup>104</sup>

The expression level of TRPA1 in EC cell-enriched fraction is 16 times higher than that in the whole mucosal layer.<sup>105</sup> Upon TRPA1 receptor stimulation, Ca<sup>2+</sup> channel-dependent serotonin release is activated in EC cell.<sup>12</sup> These data suggest that TRPA1 receptors on EC cells may serve as the primary detector of luminal contents prior to mucosal damage and a potential therapeutic target for the management of IBS.<sup>12</sup> Furthermore, serotonin signaling mediates visceral hypersensitivity by activation of 5-HTR3 on extrinsic sensory neurons in IBS patients.<sup>106</sup> Activation of 5-HTR4 via tegaserod and naronapride attenuates colonic hypersensitivity.<sup>107</sup> In contrast, acute oral administration of 5-hydroxytryptophan~~5-HTP~~ elevates visceral nociceptive responses and abdominal distension, resulting in increased pain sensation in a subset of IBS patients.<sup>108</sup>

Taken together, serotonergic modulation through selective serotonin reuptake inhibitor (SSRI) and 5-HT<sub>2</sub> agonists/antagonists of visceral sensation appear promising to [the](#) development-of novel therapeutic modalities for DGBIs.

## 2. Basolateral Interface of Enterochromaffin Cell

### (1) Altered gut motility

Functional defects of particular GI cells, for instance, ICCs, [smooth muscle cells](#)SMCs, EC cells, immune cells, and enteric neurons, have the potential to hamper gut motility.<sup>13</sup> The normal antral motility, essential for triturating solid food and maintaining the gastric emptying rhythm, is mediated by intrinsic nitrergic and cholinergic neurons and extrinsic vagal innervation that facilitate pyloric motor functions and intragastric contractions.<sup>109</sup> Abnormalities of the proximal stomach's intrinsic inhibitory innervation (nitrergic pathway), inhibition of cholinergic (5-HT<sub>1A</sub> on cholinergic neurons) and 5-HT<sub>2B</sub> pathways, and an impaired antro-fundic reflex are involved in defective gastric accommodation and delayed gastric emptying.<sup>13,109,110</sup> 5-HT<sub>2B</sub> antagonist normalized the impaired gastric accommodation; in contrast, 5-HT<sub>2B</sub> agonist exacerbated the inhibition of gastric accommodation.<sup>111</sup> A recent study reports reduced EC cell numbers in the antral biopsy in patients with idiopathic gastroparesis.<sup>16</sup> Furthermore, depletion of EC cells in a murine model of gastroparesis and STC led to delayed gastric emptying and colonic motility. This study provided possible pathogenesis of alteration in EC cell numbers and mucosal serotonin in gastroparesis and STC.<sup>16</sup> Further studies are warranted to underpin how and to what extent alternations of each cell type contribute to symptom generation and severity. Furthermore, to elucidate underlying neuromuscular and neuroimmune mechanisms mediated via EC cells of DGBIs are currently unclear [and](#) warrants further research.

## (2) Gut immune dysfunction

The increased recruitment of mast cells and eosinophils to gut inflammation induces tissue damage, leading to impaired gut homeostasis amongst DGBIs patients.<sup>91,112</sup> Breach of intestinal barrier allows infiltration of luminal contents, further cascading the immune events and contributing to the development of symptoms in DGBIs.<sup>113</sup> FD patients show elevated proinflammatory cytokine production and enhanced numbers of  $\alpha 4\beta 7$  integrin CCR9 T lymphocytes.<sup>114</sup> Macrophage-driven immune dysregulation is the primary factor associated with gastroparesis in a recent transcriptomic analysis of gastric body biopsies.<sup>115</sup> Genes associated with specific macrophages [are enriched](#) in gastric tissues from gastroparesis patients compared with controls.<sup>115</sup> A reduction of CD206+ macrophages in the gastric antrum of patients with diabetic gastroparesis correlates with the loss of ICCs.<sup>116</sup>

A wide range of immune cells, including macrophages, mast cells, dendritic cells, and T cells, have extensive 5-HTR expression.<sup>117</sup> 5-HTR1A mediates the chemotactic influence of serotonin on mast cells, 5-HTR2A on eosinophils, 5-HTR1B, and 5-HTR2A on dendritic cells, and 5-HTR2B and 5-HTR7 on macrophages.<sup>117</sup> In a murine postoperative ileus model, 5-HTR4 agonist mosapride accelerated acetylcholine release from myenteric cholinergic neurons, subsequently activated  $\alpha 7$ -nicotinic acetylcholine receptor on macrophages to inhibit their inflammatory reactions and improved the intestinal motility.<sup>118</sup> Increased serotonin release mediating gut immune dysfunction contributes to the development of IBS symptoms.<sup>119</sup> Spontaneous release of serotonin correlates with the severity of abdominal pain and increased mast cells in patients with IBS.<sup>119</sup> These findings suggest a possible new paradigm for the development of DGBIs initiated through gut immune dysregulation via serotonin metabolisms.

### **(3) Neuroimmune dysfunction**

Immune cells interact with ENS to modulate gut function.<sup>120</sup> Intestinal mast cell infiltration and its close proximity to submucosal plexus neurons leads to altered neuronal responsiveness in patients with DGBIs.<sup>121-123</sup> Further, the close proximity of activated mast cells to colonic nerves correlated with abdominal pain in patients with IBS.<sup>94,124</sup> Sprouting of fine nerve fibers was more often noted in the duodenal tissues of patients with IBS compared to healthy controls.<sup>123,125</sup> The grade of fine nerve fiber sprouting was positively correlated with the degree of gut immune alterations in patients with IBS.<sup>125</sup> Moreover, the mucosal mediators from IBS patients trigger the activation of visceral pain pathways.

Not only 5-HTRs are enriched in gut immune cells, but also EC cells are capable of “sensing” immune regulators through specific receptors.<sup>126</sup> One state-of-the-art study demonstrated neuroimmune interaction mediating through EC cells.<sup>50</sup> IL-33 sensed by EC cells induces release of serotonin, which mediates activation of enteric neurons resulting in accelerated gut motility.<sup>50</sup> Furthermore, histamine and serotonin regulate enteric neuron functions in patients with IBS.<sup>119</sup> These studies revealed an immune-neuroendocrine axis in calibrating serotonin release for intestinal homeostasis.

### **(4) Dysregulated gut-brain interaction**

Dysregulation of the gut-brain axis is a critical pathophysiological mechanism of DGBIs.<sup>127</sup> Brain modulates gut physiology, for instance, motility, visceral sensations mediating symptoms of DGBIs.<sup>128</sup> Similarly, alterations in gut physiology feedback to the brain sustain psychological health.<sup>128</sup> In a meta-analysis, the prevalence of anxiety and depressive disorders is

23% in IBS patients.<sup>129</sup> The presence of depression and anxiety symptoms are overrepresented in IBS patients with a frequency of 39% and 29%, respectively. Psychological comorbidity and corticotropin-releasing hormone pathways contribute to the development of impaired gut barrier function.<sup>130</sup> In response to stress, eosinophils release corticotropin-releasing hormone and substance P, which lead to activation of mast cells, ultimately resulting in impaired gut barrier function.<sup>91</sup> Functional brain MRI studies in patients with DGIBIs demonstrate abnormalities of structural and functional networks in areas of the brain responsible for processing information of vasovagal reflux and visceral motor system.<sup>131</sup> Gut microbiota dysbiosis alters the function of neurotransmitters, such as serotonin, acetylcholine, dopamine, and  $\gamma$ -aminobutyric acid, either by the production or consumption of these substances, leading to changes in behavior and emotional state.<sup>21</sup> The identification of BAs and their receptors in the brain reinforces the gut-brain axis.<sup>132</sup> In addition, EC cells throughout the entire gut axis sense luminal contents and generate electrical signals bridging the mucosal barrier through interface with the CNS via extrinsic afferent neurons and the ENS via intrinsic afferent neurons.<sup>50,51</sup> Thus, EC cells play a fundamental role in linking the gut connectome to the brain connectome.

As the primary source of body serotonin, EC cell-derived serotonin impacts its circulating levels and has the power to modulate brain function directly or indirectly. The information about luminal microenvironment is broadcasted to the brain by EC cells through the super-highway “vagus nerve.”<sup>21</sup> Vagal afferents nerve terminals express 5-HTR3 and locate in proximity to the EC cells.<sup>11</sup> A direct interaction of EC cell signaling to vagal afferents in response to gut luminal chemical contents with the vagal neurocircuits is involved in reflexes, such as, vomiting.<sup>11</sup> Probiotic intervention with *Bifidobacterium* spp. increases serotonin levels in the brain leading to improved behavior in the depression murine model.<sup>133</sup> Oral administration

of an SSRI elevates the gut serotonin bioavailability, alleviates depressive-like behavior in mice, possibly through vagus nerve activation.<sup>133</sup> Overall, these findings suggest that EC cells and their derived serotonin have the potential to relay signals to the brain. Further, identifying and characterizing the specific gut microbiota and their metabolites for enhancing gut-brain physiological functions via EC cells/serotonin signaling may offer a treatment strategy for extrinsic neuron modulation.

### **3. Post-infection Disorders of Gut-Brain Interactions and Serotonin Signaling**

#### **(1) Post-infection disorders of gut-brain interactions**

Post-infection DGBIs (PI-DGBIs) develops in about 10% of patients after a bout of gut infections such as bacterial, protozoal, or viral gastroenteritis.<sup>68,134</sup> Transient non-specific and specific GI inflammation are often associated with long-lasting DGBIs symptoms, for instance, bacterial, parasitic, viral gastroenteritis, and after resolution of a flareup of inflammatory bowel disease (IBD).<sup>68</sup> The pathophysiological mechanism of PI-DGBIs include gut dysmotility, microbial dysbiosis, intestinal barrier dysfunction, visceral hypersensitivity, altered serotonin metabolism, bile acid malabsorption, increased density of EC cells, and T lymphocytes.<sup>10,68</sup> A meta-analysis of 45 studies including 21 421 participants with acute gastroenteritis showed a pooled prevalence of 10.1% of PI-DGBIs at 12 months after the episode.<sup>135</sup> A subgroup of patients with gastroparesis has an onset of symptoms after prior gastroenteritis.<sup>13</sup> Various viral agents such as Epstein-Barr virus, enterovirus, and cytomegalovirus could cause PI-gastroparesis.<sup>13</sup> Clinical presentation of patients with PI-gastroparesis is similar to patients with gastroparesis without prior gastroenteritis but is associated with an acute onset and severe symptoms. Acute gastroenteritis also leads to the onset of FD, known as PI-FD.<sup>68</sup> Furthermore,

approximately one-third of patients with IBD after resolution of a flareup of IBD experience symptoms of IBS.<sup>136</sup> Additionally, acute infective gastroenteritis leads to the onset of IBS, termed PI-IBS. A broad range of bacterial pathogens has been reported in PI-IBS, such as *Salmonella enterica*, *Clostridioides difficile*, *Campylobacter jejuni*, *Vibrio cholerae*, and *Escherichia coli*.<sup>137</sup> Reduced gene expression of SERT has been reported in patients with PI-IBS, suggesting increased bioavailability of luminal serotonin may trigger stimulation of 5-HTRs on afferent neurons leading to enhanced visceral sensation.<sup>68</sup> In colonic tissue of patients with *Shigella* and *Campylobacter* PI-IBS had increased EC cell density.<sup>138,139</sup> However, patients with post-*Giardia* IBS had decreased EC cell density in colonic tissue compared with controls.<sup>140</sup>

## **(2) Post-corona virus disease 19 disorders of gut-brain interactions**

Previous studies on GI symptoms (nausea, abdominal pain, diarrhea, anorexia, and vomiting) overlapping in patients with corona virus disease 19 (COVID-19) showed diarrhea symptoms as the most prevalent associated with the severity of COVID-19.<sup>134,141</sup> PI-IBS, including IBS-D, is associated with increased serotonergic activity.<sup>68</sup> These observations highlighted the importance of alterations in serotonin metabolism. The overrepresentation of GI symptoms in patients with COVID-19 suggests that post-SARS-CoV-2 infection may lead to persistent abnormal gut functions underpinning certain pathophysiological aspects in PI-DGBIs. Hence, regulating serotonin signaling might be a potential therapeutic modality for the clinical care in a subset of COVID-19 patients.

## **Pathophysiology-directed Therapeutic Approach for Disorders of Gut-Brain Interactions**

Pathophysiological mechanisms, both peripheral (intestinal barrier dysfunction, altered motility, visceral hypersensitivity, gut microbiota dysbiosis, gut immune dysfunction, bile acid malabsorption, and enteric neuroendocrine abnormality) and central (psychological comorbidity, cognitive dysfunctions, and neuroendocrine dysregulation), may be a better strategy to be targeted by therapeutic agents to treat patients with DGBIs (Refer to Tables 1, 2, and 3 with key references cited.). Based on the significant overlaps due to common pathogenesis between the different DGBIs, pathophysiology-directed therapeutic modalities may provide precise clinical outcomes in DGBIs. Furthermore, studies are warranted on personalized treatment of different DGBIs employing a robust study design incorporating longitudinal multicentric studies integrating multi-omics data of gut physiology and pathophysiology. Such comprehensive approaches may lead to novel therapeutic modalities, resulting in targeted restoration of abnormal physiological functions in DGBIs patients.

## **Conclusion and Further Directions**

Serotonin metabolism has a central role in gut physiology and pathophysiology. The aforementioned EC cell-mediated physiological and pathophysiological mechanisms are differentially affected in DGBIs but remain tightly interconnected.<sup>12,16, 50-52</sup> Moreover, serotonin metabolism is directly or indirectly modulated through the gut microbiota, reinforcing the essential roles of EC cells in the regulation of gut-brain axis.<sup>153-156</sup> Therefore, EC cell is a potential therapeutic target that may usher the emergence of pathophysiology-modifying therapies, using pharmacological agents targeting serotonin signaling or exploiting gut

microbiota interventions. The pathophysiological mechanisms for similar symptoms may differ between patients with DGBIs. Therefore, the pathophysiological basis may be more relevant than clinical symptom profiles in differentiating, characterizing, and defining different DGBIs. The current challenges of DGBIs warrant a holistic approach to better characterize the patients based on the multi-omics data on the transcriptome, host epigenome, dietary profiles, gut microbiome, and metabolome from longitudinal studies. However, owing to the heterogeneous nature of DGBIs, further research is warranted to gain an advanced understanding of pathophysiological mechanisms for better clinical care. To discover the precise biomarkers discriminating the disorders with overlapping symptoms profile and refining pathophysiology-directed therapeutics targets, may assist with the hope for the future to relieve symptoms and restore gut-brain homeostasis.

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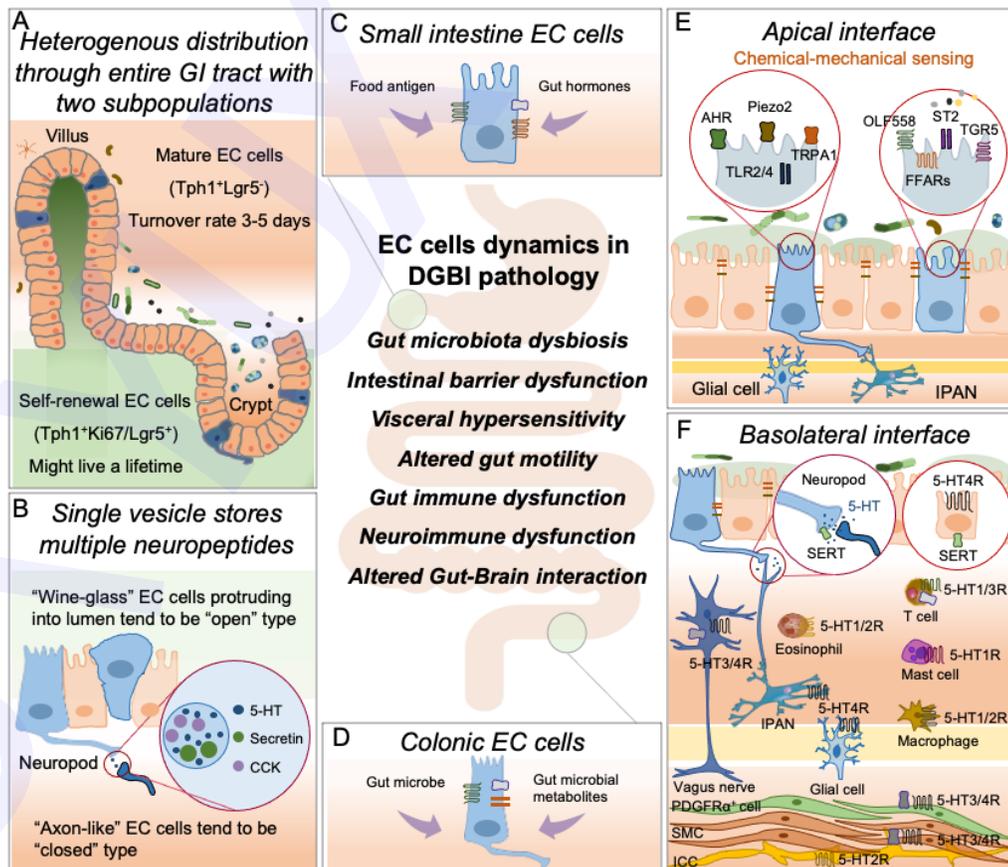
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## Figure Legend

**Figure.** Enterochromaffin (EC) cells dynamics in gut physiology and disorders of gut-brain interactions. (A) Heterogenous distribution of EC cells. (B-D) Functional heterogeneity of EC cells. Small intestine EC cells are specialized for sensing nutrients and colonic EC cells for gut microbiota and its derived molecules. (E, F) Apical and basolateral interface of EC cells. EC cells coordinate bilateral communication through crosstalk between dietary, microbial, and inflammatory factors to which their apical surface is exposed and to enteric neurons with afferent endings close to their basolateral surface, coordinating gut-brain communication. GI, gastrointestinal; Tph1, tryptophan hydroxylase 1; Lgr5, leucine-rich repeat-containing G-protein coupled receptor 5; 5-HT, 5-hydroxytryptamine; CCK, cholecystokinin; DGBI, disorders of gut-brain interaction; AHR, aryl hydrocarbon receptor; TRPA1, transient receptor potential ankyrin 1; TLR, toll-like receptor; OLF558, olfactory receptor 558; ST2, suppressing the tumorigenicity 2 receptor; TGR5, Takeda G protein-coupled receptor 5; FFARs, free fatty acid receptor genes; SERT, serotonin reuptake transporter; 5-HTR, 5-hydroxytryptamine receptor; IPAN, intrinsic primary afferent neuron; PDGFR $\alpha$ , platelet-derived growth factor receptor  $\alpha$ ; SMC, smooth muscle cell; ICC, interstitial cells of Cajal.



## Tables

**Table 1.** Summary of Pathophysiology-directed Therapeutic Approach for Disorders for Gut-Brain Interactions

Drug name		Function/pathway	Pathophysiological mechanisms	Clinical outcome	DGBI types
Prokinetics	Prucalopride	5-HT <sub>4</sub> R agonist	Altered gut motility	Improved GI symptoms as assessed by the GCSI. Improved solid gastric emptying.	Gastroparesis, FD, chronic constipation
	Felcisetrag	Selective 5-HT <sub>4</sub> R agonist	Altered gut motility	Accelerated transit of solids throughout the gut in patients with idiopathic or diabetic gastroparesis. Stimulate motility and secretion through enhanced release of acetylcholine from excitatory motor neurons and interneurons.	Gastroparesis
	Alosetron Ondasetron Ramosetron Cilansetron	5-HT <sub>3</sub> R antagonist	Altered gut motility	Improved stool consistency and bowel movements.	IBS-D
	Buspirone Tandospirone	5-HT <sub>1</sub> R agonist	Altered gut motility	Fundus relaxation, improve gastric accommodation.	FD
	Relamorelin	Ghrelin receptor agonist	Altered gut motility	Stimulates nodose afferents and DMV neurons and accelerates gastric emptying.	Gastroparesis
	Metoclopramide  Domperidone  Itopride	Dopamine-2 receptor antagonists	Altered gut motility	Improved gut motility.	FD, gastroparesis
	Acotiamide	Muscarinic receptor antagonists	Altered gut motility	Inhibits acetylcholinesterase and antagonizes the presynaptic muscarinic receptors, present on cholinergic nerve endings, which leads to an increase in acetylcholine levels in the synaptic	FD

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Antibiotics	Neomycin Rifaximin		Modulating gut microbiota profile	Gut microbiota dysbiosis	Shifting the microbial community composition. Improvement in constipation, SIBO and dyspeptics symptoms.	IBS
Probiotics	<i>Bifidobacterium animalis</i> DN-173010154, <i>Bifidobacterium lactis</i> DN-173 <i>Lactobacillus gasseri</i>		Modulating gut microbiota profile	Gut microbiota dysbiosis	Shifting the microbial community composition. Improved symptoms and gut transit.	IBS
Bile acid sequestrants	Obeticholic acid Tropifexor Cholestyramine		FXR agonist	Bile acid alterations	Stimulate the synthesis and subsequent release of FGF-19 from ileal epithelial cells and inhibit bile acid synthesis by hepatocytes. Improve stool form and symptoms of diarrhea.	IBS-D
Bile acid transporter inhibitor	Elobixibat Cholestipol		IBAT antagonist	Bile acid alterations	Efficacious treatment for constipation, improving gut transit and symptoms via increasing colonic bile acids.	IBS-C
Anti-inflammatory agents	Mesalazine		Gut immune homeostasis	Low grade inflammation Visceral hypersensitivity	Sustained therapy response and benefits for a subgroup of patients with IBS.	IBS
	Ketotifen		Mast cell stabilizer	Visceral hypersensitivity	Increased the threshold for discomfort in patients with IBS with visceral hypersensitivity, and reduced IBS symptoms.	IBS
	Ebastine		Histamine receptor-1 antagonist	Visceral hypersensitivity	Reduced visceral hypersensitivity and abdominal pain in patients with IBS.	IBS

Acid suppressive therapy	Pantoprazole		Proton pump inhibitors	Gut immune dysfunction Epithelial barrier dysfunction Visceral hypersensitivity	Reduced duodenal eosinophilia, mast cells, and intestinal permeability in patients with FD.	FD
Neuromodulators	Amitriptyline		TCA	Gut-brain dysregulation	Affect gastrointestinal motility through anticholinergic and serotonergic mechanisms. TCAs reduce visceral hypersensitivity. Antidepressant therapy might lead to neurogenesis.	IBS, FD
	Mirtazapine		Tetracyclic antidepressants	Gut-brain dysregulation	Upregulates the levels of orexigenic hormones and downregulated the levels of anorexigenic hormones	FD
	Escitalopram		SSRI	Gut-brain dysregulation	Treating depressive symptoms with these molecules modulates the severity of GI symptoms indirectly via a positive effect on depression	IBS
	Venlafaxine Duloxetine		SNRI			
Intestinal Secretagogues	Lubiprostone		CCl2 agonist	Abnormal secretion	Increased fecal water content. Creates an ion gradient that promotes water and sodium secretion into the intestinal lumen.	IBS-C, chronic constipation
	Linaclotide		Guanylate cyclase-C receptor agonist	Abnormal secretion	Increase water secretion via targeting cGMP leads to the secretion of chloride and bicarbonate into the intestinal lumen	IBS-C
Visceral Analgesics	Oliceridine		Biased $\mu$ -Opioid receptor ligands	Visceral hypersensitivity	Management of moderate to severe acute pain in adults for whom alternative treatments other than opioids had failed.	IBS

	Olorinab		Cannabinoid type 2 receptor agonist	Visceral hypersensitivity	Potential analgesic effects in patients with IBS	IBS
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DGBIs, disorders of gut-brain interactions; 5-HTR, 5-hydroxytryptamine receptor; GI, gastrointestinal; GCSI, gastroparesis cardinal symptom index; FD, functional dyspepsia; DMV, dorsal motor nucleus of the vagus; IBS-D, diarrhea-predominant irritable bowel syndrome; SIBO, small intestinal bacterial overgrowth; IBS, irritable bowel syndrome; FXR, farnesoid X receptor; FGF-19, fibroblast growth factor 19; IBAT, ileal bile acid transporter; IBS-C, constipation-predominant irritable bowel syndrome; TCA, tricyclic antidepressant; SSRI, serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; CC12, type 2 chloride channel; cGMP, cyclic guanosine monophosphate.

**Table 2.** Gut Microbiota-derived Molecules Modulating Pathogenesis of Disorders for Gut-Brain Interactions

Metabolites	Targeting cell types	Pathophysiological mechanisms	Key findings
Butyrate and acetate	EC cells	Altered gut motility	These metabolites increase 5-HT biosynthesis from EC cells through stimulatory activities. <sup>75</sup>
SCFAs	EC cells and 5-HTR3 <sup>+</sup> vagal nerves	Altered gut motility	Release of 5-HT from EC cells in response to SCFAs stimulates 5-HTR3 located on the vagal sensory fibers. The sensory information is transferred to the vagal efferent and stimulates the release of acetylcholine from the colonic myenteric plexus, resulting in muscle contraction. <sup>142</sup>
Butyrate	EC cells and TRPV1 <sup>+</sup> cells	Visceral hypersensitivity	Repetitive stimulation of TRPV1 receptor via butyrate-induced 5-HT release desensitize TRPV1 <sup>+</sup> neurons resulting in less pain sensation. <sup>143</sup>
Butyrate	Enterocytes and immune cells	Altered gut immune function and impaired barrier function	Butyrate regulates neutrophil function and migration, inhibits inflammatory cytokine induced expression of vascular cell adhesion molecule-1, increases expression of tight junction proteins in colon epithelia, and exhibits anti-inflammatory effects by reducing cytokine and chemokine release from immune cells. Butyrate or specific species of butyrate producing gut bacteria may be a new target for restoring host immune function and barrier integrity. <sup>144</sup>
Acetate	Enterocytes and EC cells	Abnormal secretion	Gut microbiota alters 5-HT-evoked intestinal secretion in a 5-HTR3-dependent mechanism. Acetate alters 5-HTR3 expression in colonoids. <sup>145</sup>
BAs	EC cells	Altered gut motility	TGR5 receptor on EC cells mediates the effects of BAs on colonic motility. Deficiency of TGR5 causes constipation in mice. <sup>73</sup>
Tryptamine	Enterocytes	Altered gut motility and secretion	Tryptamine accelerates gut transit and increases colonic secretion by activating epithelial 5-HTR4. Genetically engineered bacteria <i>Bacteroides thetaiotaomicron</i> produce tryptamine. <sup>84</sup>
Methane	EC cells and nitroergic neurons	Altered gut motility Visceral hypersensitivity	Methane derived from <i>Methanobrevibacter smithii</i> in the depletes gut 5-HT resulting in slowed gut transit and constipation. <sup>146</sup>
Isovalerate	EC cells	Altered gut motility Visceral hypersensitivity	Isovalerate evoked 5-HT release from EC cells through voltage-gated Ca <sup>2+</sup> channel and modulated 5HTR3 neurons in visceral sensation and gut motility. <sup>12</sup>
Indole	EC cells	Altered gut motility	<i>Edwardsiella tarda</i> metabolized tryptophan to produce indoles that activate TRPA1 on EC cells to produce 5-HT that stimulates enteric neurons and induces gut motility. <sup>51</sup>

EC, enterochromaffin; 5-HT, 5-hydroxytryptamine; SCFA, short chain fatty acid; 5-HTR, 5-hydroxytryptamine receptor; TRPA1, transient receptor potential ankyrin 1 channel; BAs, bile acids; TGR5, Takeda G protein-coupled receptor 5; TRPV1, transient receptor potential vanilloid 1.

**Table 3.** Pharmacological Agents Modulating Serotonin-mediated Mechanism in Disorders of Gut-Brain Interactions

Drug name	Mechanisms of action	Clinical outcome
TCA	TCA has 5-HT and NA reuptake inhibition properties, primarily used for anti-depressant and analgesic. Their mode of action involves mechanisms beyond 5-HT and NA, like blockage of voltage-gated ion channels, opioid receptor activation and also modulates neuroimmune anti-inflammatory effects.	Affect gut motility through anticholinergic and serotonergic mechanisms. Reduces visceral hypersensitivity, intestinal pain sensitivity, mediated either in peripheral nerves or the CNS. <sup>147</sup>
Tetracyclic antidepressant	Boosts both 5-HT and NA neurotransmission, not by blocking their reuptake pumps, but by blocking presynaptic $\alpha$ 2-noradrenergic receptors on NA and 5-HT neurons, resulting in an increased noradrenergic and serotonergic activity	Upregulates the levels of orexigenic hormones and downregulated the levels of anorexigenic hormones. Reduce colonic hypersensitivity and improve gastric emptying. <sup>148</sup>
SSRI	SSRIs are characterized by selective blockade of the presynaptic 5-HT transporter, boosting 5-HT neurotransmission.	Increase colonic contractility and reduce colonic tone during fasting conditions and reduce the colonic tone increase. Decrease IBS scores for abdominal pain and bloating independent of anxiety, depression and colonic sensorimotor function. <sup>149</sup>
SNRI	Primarily block both 5-HT and NA reuptake, boosting 5-HT and NA neurotransmission. Considering the central roles of 5-HT and NA in the descending modulatory nerve pathways, SNRIs are better pharmacological agents to modulate pain sensation.	Increase compliance, relax tone and reduce the postprandial colonic contraction and increase sensory thresholds in response to balloon distensions. <sup>149</sup>
5-HTR4 agonist	5-HT4R agonist target 5-HTR4 to promote peristalsis and secretion through enhanced release of acetylcholine from excitatory motor neurons and interneurons.	Improves GI motility. <sup>57,150</sup>
5-HTR3 antagonist	Abnormal neurotransmission of 5-HT via the 5-HTR3 has been reported in IBS-D patients. Blocking 5-HTR receptors is of clinical relevance in chronic diarrhea as this leads to reduced contractility, slows colonic transit, and increases fluid absorption.	Global improvement in IBS symptoms and relieve abdominal pain and discomfort, improve stool consistency and bowel movements. <sup>150,151</sup>
5-HTR1A agonist	Activation of 5-HTR1A at the level of the CNS increases gastric tone and decrease gastric sensitivity to distension. Peripheral inhibitory effect exerted by the 5-HTR1A agonist improve gastric accommodation.	Enhances fundus relaxation, gastric accommodation and improves postprandial symptoms independently from its anxiolytic effect. <sup>152</sup>

TCA, tricyclic antidepressant; 5-HT, 5-hydroxytryptamine; NA, noradrenaline; CNS, central nervous system; SSRI, serotonin reuptake inhibitor; IBS, irritable bowel syndrome; SNRI, serotonin norepinephrine reuptake inhibitor; 5-HTR, 5-hydroxytryptamine receptor; GI, gastrointestinal; IBS-D, diarrhea-predominant IBS.