Towards a systems view of IBS

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Abstract | Despite an extensive body of reported information about peripheral and central mechanisms involved in the pathophysiology of IBS symptoms, no comprehensive disease model has emerged that would guide the development of novel, effective therapies. In this Review, we will first describe novel insights into some key components of brain–gut interactions, starting with the emerging findings of distinct functional and structural brain signatures of IBS. We will then point out emerging correlations between these brain networks and genomic, gastrointestinal, immune and gut-microbiome-related parameters. We will incorporate this new information, as well as the reported extensive literature on various peripheral mechanisms, into a systems-based disease model of IBS, and discuss the implications of such a model for improved understanding of the disorder, and for the development of more-effective treatment approaches in the future.

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Introduction

IBS is the most common functional gastrointestinal disorder, occurring in up to 15% of the population worldwide.[1] Even though the syndrome is defined by chronically recurring abdominal pain and discomfort associated with altered bowel habits in the absence of organic disease, increased trait anxiety, as well as comorbidity with psychiatric and other chronic pain syndromes are common.[2] Despie an extensive body of reported information about peripheral[3–5] and central[6–9] mechanisms involved in the pathophysiology of IBS symptoms, and the development of animal models with high face and construct validity (that is, the models have many features similar to and is based on a similar pathophysiological concept as the human disease),[10] no comprehensive disease model has emerged that would guide the development of novel and effective therapies. This aspect is surprising in view of the comprehensive data as well as clinical experience demonstrating the strong relationship between psychosocial factors and IBS symptoms,[11] and in view of breakthroughs in the identification of several IBS-related biological abnormalities at several levels: the gut epithelium;[12] immune system;[4,5] neuroendocrine mechanisms;[13] brain structure and function;[7] stress response;[14] affective,[8,15] cognitive[6,16–18] and pain modulation[19,20] abnormalities; gene polymorphisms;[3] and the gut microbiome.[21,22] Despite these insights, a comprehensive understanding of how these various factors interact, and particularly to what degree they are involved, in the generation of symptoms in IBS in general or in IBS subsets (as opposed to representing epiphenomena) has not emerged. The long history of IBS research[23] is full of examples of reported abnormalities (including excessive mucus production, alterations in sigmoid colon motility, slow wave abnormalities in smooth muscle, gut inflammation and others), which were reported in small sample cohorts and often not confirmed in validation sample cohorts. Typically, the majority of reported mean differences are small when compared with healthy individuals, often do not take into account sex-related differences, might only be present in subsets of patients and correlations with clinical symptoms are weak. Thus, only a very small number of findings have translated into highly effective therapies.[24] These therapies include the serotonin (5-HT) 5-HT_3 receptor antagonist, alosetron, the 5-HT_4 agonist, tegaserod, the guanylate cyclase agonist, linaclotide, and the chloride channel blocker lubiprostone (for chronic idiopathic constipation).[25] Even though effective, the first two agents were restricted or withdrawn due to serious adverse effects, whereas the latter two are primarily targeted at treating the symptom of constipation.[25]

The brain and gut show reciprocal interactions in health and disease. For example, altered brain outputs via the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis have been shown to influence intestinal motility and secretion,[26] intestinal epithelial permeability,[3,12,27] immune function[28] and gut microbial composition.[21] In addition to these well-established influences of the brain on peripheral target cells, peripheral environmental gut-directed factors—in particular, dietary factors[29] and intestinal pathogens[30]—might have an equally important role on these same processes. Regardless of primary cause for the observed peripheral findings, several of them (in particular immune and microbiota-related signalling) can feed back to the brain, setting up circular regulatory loops (Figure 1).

Major barriers exist for progress towards a comprehensive understanding of IBS pathophysiology, which incorporates such circular regulatory loops. Despite a wealth of information supporting all mechanisms listed already, controversy regarding the primary role of the brain versus peripheral factors has persisted in the field. The majority

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Key points

- Physiological and molecular alterations have been identified in the brain–gut axis of human and rodent models of IBS, yet a comprehensive disease model to guide effective drug development has not emerged
- Studies have identified distinct brain signatures in patients with IBS, which provide plausible neurobiological substrates of many previously reported behavioural and psychosocial observations
- Emerging evidence demonstrates correlations of these brain signatures with alterations in genetics, immune system and gut microbiota in IBS, even though the causality of these interactions remains unknown
- A systems-biology-based model is proposed to integrate the growing number of central, peripheral and behavioural IBS-related alterations, and to identify targets for more effective therapies

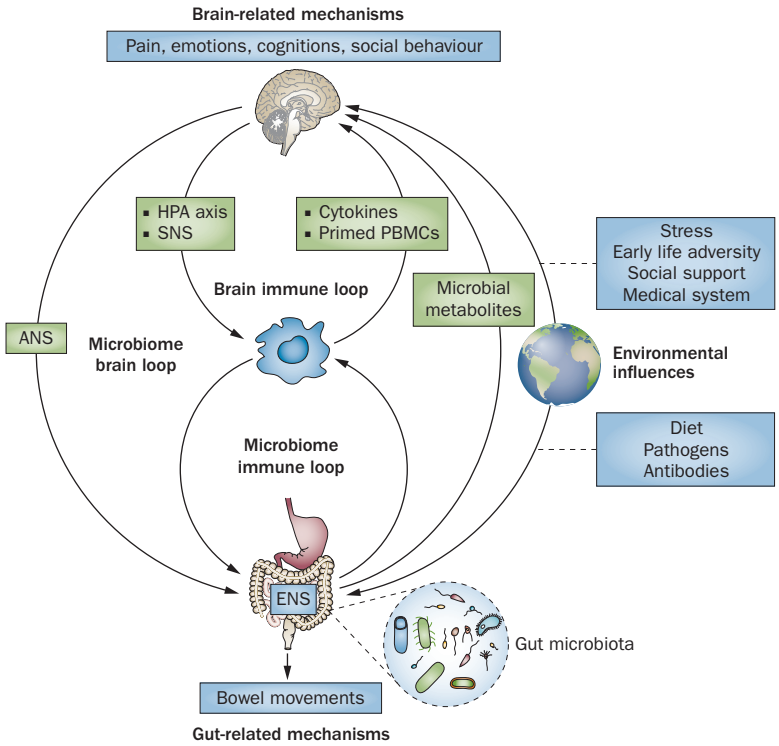

Figure 1 | Brain–gut axis. Schematic of the brain–gut axis, including inputs from the gut microbiota, the ENS, the immune system and the external environment. The model includes both peripheral and central components, which are in bidirectional interactions. Bottom-up influences are shown on the right side, top-down influences on the left side of the graph. Abbreviations: ENS, enteric nervous system; HPA, hypothalamic–pituitary–adrenal; PBMC, peripheral blood mononuclear cell; SNS, sympathetic nervous system. Modified with permission from Nature Publishing Group © Irwin, M.R. & Cole, S.W. *Nat. Rev. Immunol.* **11**, 625–632 (2011).[103]

of research and drug development have focused on single, usually peripheral, targets in preclinical models (such as ion channels or specific receptors on individual neurons). Although such studies have provided major insights into mechanisms of visceral pain,[10] their relevance for IBS symptom generation in humans has not firmly been established. Integration of multiple clinical, psychosocial and biological (genetic, immune, neurobiological) findings into a comprehensive disease model has yet to be achieved. For example, few studies aimed at psychosocial aspects of IBS have taken neurobiological concepts into account. The focus has been on descriptive, symptom-based rather than biology-based disease definitions. For example, different biological mechanisms might be associated with similar clinical presentations. The comprehensive identification of distinct biology-based subgroups of patients (including those based on sex), with different underlying pathophysiological components, who are differentially responsive to specific therapies has also not been achieved. A good example illustrating this point is a report on subsets of patients with IBS based on gut microbial signatures.[31]

In this Review, we will first describe novel insights into some key components of brain–gut–microbiome axis, starting with evolving concepts about alterations in defined structural and functional brain networks. We will discuss emerging evidence on how these brain network alterations are correlated with the immune system and the gut microbiota. We will then incorporate this information, as well as the reported extensive literature on various peripheral mechanisms, into a systems-based disease model of IBS. Rather than simply synthesizing the current knowledge about the disease, we will use this model to discuss the implications for better understanding of the disorder and for the development of more-effective treatment approaches in the future.

The nervous system component

Several clinical observations support a major role of the brain in IBS symptoms: the brain is ultimately responsible for generating the subjective experience of abdominal pain, discomfort and anxiety; stressful life events in early life have a major role in vulnerability to develop IBS, and psychosocial stressors in adulthood play crucial parts in the first onset, and perceived severity of symptoms;[32] centrally targeted pharmacological treatments and cognitive behavioural strategies are some of the most-effective treatment strategies.[33] The role of the enteric nervous system (ENS) in the regulation and coordination of motor and secretory functions of the gastrointestinal tract, in sensory function and its interactions with the brain have been extensively reviewed.[34,35] Consistent with the theme of this Review, the complexity of the interactions between multiple gut-based cell types (intrinsic and extrinsic sensory neurons, enteric glia, immune cells and innervated enteroendocrine cells) has been referred to as the 'gut connectome'.[36] The interactions between the ENS and central nervous system (CNS), two key components of the brain–gut axis, are mediated by the sympathetic and parasympathetic branches of the ANS,[6] and by multiple sensory and endocrine pathways.[34] Although it is difficult to characterize alterations of the ENS or of ANS-mediated brain–gut connections in living humans directly, functional, structural and metabolic brain imaging approaches have become a highly productive avenue to study brain–gut–microbiota interactions in health and disease.[8,9,37,38]

Although it has long been assumed that specific brain functions such as pain processing, emotion or cognition are attributable to the isolated operations of single brain regions, these processes are now viewed as resulting from the dynamic interactions of distributed brain areas operating in large-scale networks (Figure 2). These networks and their properties have been assessed by

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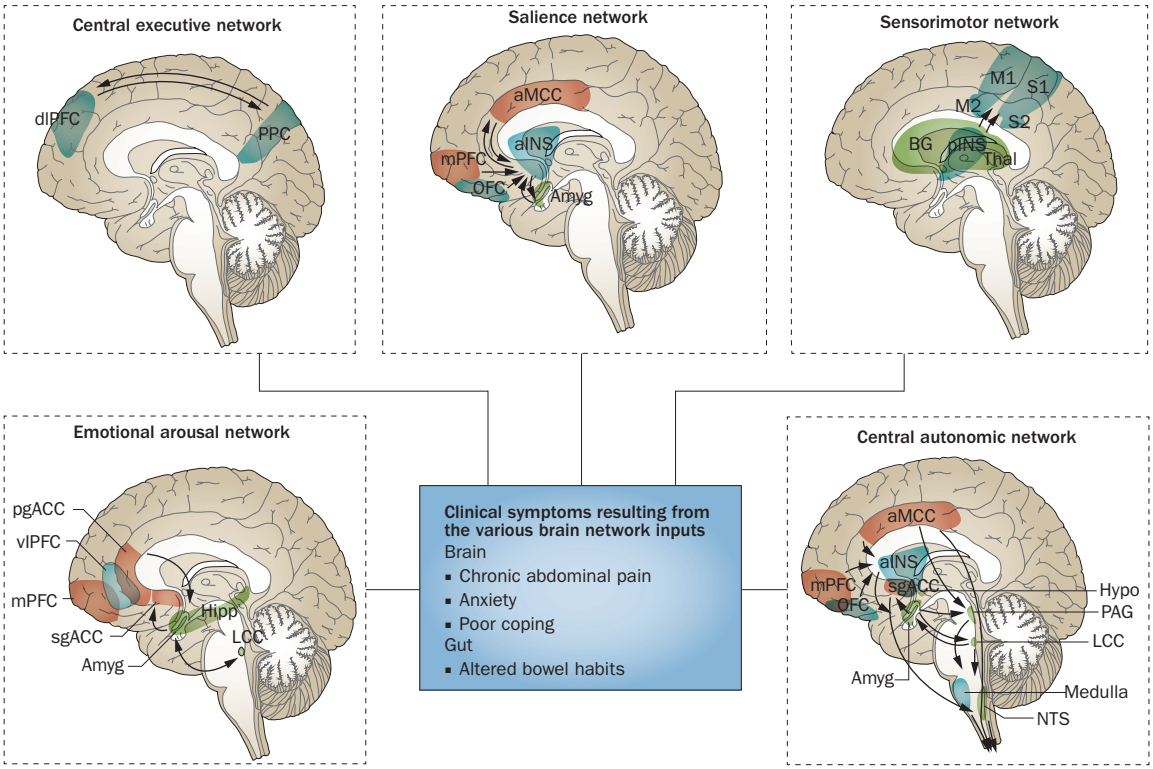

Figure 2 | Brain networks contributing to IBS symptoms. Depicted are task-related brain networks that have been described in the literature and for which structural and functional alterations have been reported in patients wiht IBS. The box in the centre describes the clinical symptoms related to the network inputs. Outputs most relevant for IBS pathophysiology occur in the form of descending pain modulation and autonomic nervous system activity. Abbreviations: Amyg, amygdala; aINS, anterior insula; aMCC, anterior midcingulate cortex; BG, basal ganglia; dlPFC, dorsolateral prefrontal cortex; Hipp, hippocampus; Hypo, hypothalamus; LCC, locus coeruleus complex; M1, primary motor cortex; M2, supplementary motor cortex; mPFC, medial prefrontal cortex; NTS, solitary nucleus; OFC, orbitofrontal cortex; PAG, periaqueductal grey; pgACC, pregenual anterior cingulate cortex; pINS, posteria insula; PPC, posterior parietal cortex; sgACC, subgenual anterior cingulate cortex; Thal, thalamus; vlPFC, ventrolateral prefrontal cortex.

using neuroanatomical and neurophysiological studies in animals,[10] as well as different brain imaging techniques and analyses in humans.[39–46] In humans, several types of networks have been reported: functional brain networks based on evoked responses[37] or intrinsic connectivity of the brain during rest;[39–41,44,45] structural networks based on grey matter parameters[47] and white matter properties; and anatomical networks based on white matter connectivity.[48] Both evoked and resting state studies performed in patients with IBS have demonstrated abnormalities in regions and task-related networks linked to emotional arousal,[49–53] central autonomic control,[6,54–56] central executive control,[51,57,58] sensorimotor processing[6,59–61] and salience detection.[57,62] IBS-related alterations in these networks have provided plausible neurobiological substrates for several information processing abnormalities reported in patients with IBS, such as biased threat appraisal and expectancy of outcomes (for example, salience network), autonomic hyperarousal (emotional arousal and central autonomic networks), and symptom-focused attention (central executive network).[63]

In the next section, we will discuss IBS-related changes that have been identified in these task-related networks. The individual networks are depicted in Figure 2, and their basic properties are described in Table 1.

Emotional arousal network

This network acts as an important link between stimulus appraisal (salience network) and ANS output (generated in the central autonomic network) to peripheral targets (gastrointestinal tract, gut microbes, immune system), thereby having an important role in determining the magnitude and duration of autonomic modulation of various gut functions. A reduction in the inhibitory feedback loop within the arousal network has been demonstrated in patients with IBS compared with healthy individuals,[51,64,65] and in healthy individuals as controls after decreasing central serotonin levels by acute tryptophan depletion.[66] Several studies published during the past decade support an increased responsiveness of emotional arousal circuits in relation to both expected and to delivered visceral stimuli, particularly in women.[67–76] A meta-analyses of functional MRI studies published between 2000 and 2010 demonstrated that during controlled rectal distension, patients with IBS show more consistent activation in regions associated with emotional arousal than healthy individuals.[53] Emotional arousal circuit reactivity is associated with 5-HT-related gene polymorphisms.[77] IBS-related functional alterations are accompanied by structural brain alterations in key regions of this network.[78]

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Table 1 | Brain networks with relevance to IBS

Network	Core regions and inputs	Key features
Default mode network	Medial prefrontal cortex, posterior cingulate or retrosplenial cortex, inferior parietal cortex, lateral temporal cortex and hippocampal formation[179,180]	Comprised of brain regions whose activity is greater during rest than goal-directed task performance[181] Associated with self-related processing, including monitoring internal thoughts and future planning[179,182,183]
Emotional arousal network	Amygdala, its positive connections with the locus coeruleus complex and inhibitory feedback projections to the amygdala from prefrontal and anterior cingulate subregions, and from the hippocampus	Activated by perceived or real perturbation of the organism's homeostasis Generates rapid feedback inhibition of amygdala,[184–186] thereby limiting the magnitude and duration of network activity and related activity in the central autonomic network
Central autonomic network	Control centres in the pontine-medulla (including PAG and hypothalamus), the central nucleus of the amygdala, and several cortical regions (including the anterior INS, ACC, prefrontal and motor regions)[187,188]	Regions related to SNS control overlap with the executive-processing and salience-processing networks (including the ventral anterior INS), whereas regions related to parasympathetic control are more associated with the default mode network[154] Provides central control and modulation of the ANS Involved in regulating respiratory, cardiovascular, endocrine and digestive system activity during cognitive, affective and motor tasks and sensation
Sensorimotor network	Core cortical regions are primary somatosensory cortex (S1, post central gyrus), primary motor cortex (M1; precentral gyrus), secondary somatosensory cortex (S2) and supplemental motor area[189] Close connections exist between the posterior INS (primary interoceptive cortex) and S1 Sensorimotor network connectivity to the thalamus, which relays peripheral sensory information to the cortex,[190] is established by 2 years of age[191]	Receives sensory input from the periphery and is important for awareness of body sensations and generation of appropriate motor responses Primary and secondary motor cortex, through their projections to the central autonomic network, might have a modulatory role in the sympathetic control of visceral function[192]
Central executive network	Lateral prefrontal cortices and posterior parietal cortex[193]	Activated during tasks involving executive functions such as attention, working memory, planning and response selection Often coactivated with regions of the salience network,[39] as the brain attempts to focus its limited processing capacity to only salient information via attention, working memory, planning and response selection[193]
Salience network	Dorsal ACC and anterior INS Core regions have strong connections to medial prefrontal and temporal regions, and subcortical regions including the amygdala, PAG and basal ganglia[194–196] Dorsal portions of the anterior INS receives prefrontal input, whilst ventral portions are closely linked with the amygdala and emotional arousal system	Anterior INS can be considered the main hub in the brain, switching from default mode network to activity-related networks, and coordinating and adjusting bodily and behavioural responses to environmental changes Responds to subjective salience of any interoceptive and exteroceptive stimulus reaching the brain, or to the expectation of such stimuli, and coordinates appropriate attentional, behavioural, affective and visceral responses to such stimuli[39,197] Responds with the most appropriate responses to biologically and cognitively relevant stimuli based on maintaining homeostasis[193,194] regardless of whether the subject is awake, engaged in a particular task, or asleep (achieved through close salience network connections with the other networks)

Abbreviations: ACC, anterior cingulate cortex; ANS, autonomic nervous system; INS, insula; PAG, periaqueductal grey; SNS, sympathetic nervous system.

Central autonomic network

An extensive body of knowledge derived from rodent studies[26,79–82] supports an upregulation of brain circuits in response to chronic stress, in particular the input from the locus coeruleus complex to the amygdala and the hypothalamus. Information from human brain imaging studies has been more limited due to the technical difficulties in studying key regions of this network, such as the hypothalamus, locus coeruleus complex and subnuclei of the amygdala, primarily due to limited spatial resolution. However, pharmacological brain imaging studies have implicated alterations in the corticotropin-releasing factor (also known as corticoliberin)–corticotropin-releasing receptor 1[64,83] and norepinephrine–α adrenergic receptor signalling system in this network.[84]

Sensorimotor network

As with other chronic pain disorders,[85–88] evidence indicates that IBS might be associated with alterations in brain networks concerned with the central processing and modulation of viscerosensory and somatosensory information. For example, compared with healthy individuals, patients with IBS showed increased low frequency power of spontaneous brain oscillations (suggesting increased

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neural activity) in regions belonging to the sensorimotor network.[59] These functional changes seem to be accompanied by structural changes in white and grey matter. For example, patients with IBS had widespread microstructural white matter abnormalities in sensory processing and/or modulation regions.[89] Female patients with IBS have cortical thickness increases in sensorimotor areas that correlated with clinical measures of symptom severity compared with healthy individuals.[90] Such grey matter increases in patients with IBS were also seen in the posterior insula (INS), the primary viscerosensory cortex, and these changes were correlated with IBS symptom duration.[91] Volumetric grey matter analyses in a large sample of female patients with IBS revealed increases in the primary somatosensory cortex.[78] Furthermore, examining structural networks in IBS indicated that two key regions of the network (cingulate gyrus and thalamus) were found to be network hubs, indicating that these regions are more critical for information flow in IBS compared with health.[78] When viewed together, current evidence supports the hypothesis that patients with chronically recurring visceral pain and/or discomfort have functional as well as neuroplastic and microstructural alterations within the brain, particularly in regions associated with the processing, integration and modulation of sensory information. The mechanism(s) underlying these alterations include chronically increased viscerosensory information flow from the gut, or from dorsal horn neurons sensitized by descending pain facilitation (see Figure 3).

Central executive network

Evidence indicates that patients with IBS might have functional impairments in cognitive processes associated with the central executive network.[16,17] Preliminary evidence based on administration of the Attentional Network Test[92] suggests that patients with IBS have greater behavioural efficiency during the alerting and orienting function of attention than healthy individuals. In comparison with healthy individuals, this greater efficiency was associated with greater activation of anterior midcingulate and insular cortices, confirming the previously reported close interactions between the central executive network and the salience network.[39] Converging evidence also suggests that increased attention to gastrointestinal symptoms and contexts have an important role in the increased perceptual sensitivity to visceral stimuli characteristic of IBS.[26] Patients with IBS show deficient activation of inhibitory cortical regions involved in downregulation of pain and emotion as well as attention during expectation and experience of aversive gastrointestinal stimuli.[53] Selective recall of negative and gastrointestinal sensation words, as well as selective attention to threat-related stimuli, has been demonstrated in patients with IBS.[93–96] Furthermore, a reduction in the effective connectivity of the central executive network circuitry (including parietal, dorsal lateral prefrontal cortex) during repeated exposure to the anticipation and experience of a threatening gastrointestinal stimulus (repeated exposure to balloon inflations) was associated with a reduction in IBS hypersensitivity.[97] Data from a sample of Japanese patients with IBS, compared with healthy controls, indicated that alterations in error feedback mechanisms were associated with decreased dorsolateral prefrontal cortex activity.[16] A strong negative correlation between the cortical thickness and grey matter density of the dorsolateral prefrontal cortex and pain catastrophizing has been reported.[98,99] Evidence from the University of California, Los Angeles research group indicates prepulse inhibition, a process by which an organism can filter the flow of information from its internal and external environments, is altered in IBS compared with health.[60] Together, these data suggest that patients with IBS have specific abnormalities in attentional processes that have a role in the increased perception of visceral stimuli and in IBS symptom severity.

Salience network

Studies performed during the past decade on brain responses to delivered and expected rectal distension have consistently reported increased engagement of the core regions of the salience network, the anterior INS and anterior midcingulate cortex in patients with IBS,[7,37] which initially but incorrectly were referred to as the "pain matrix". In addition, a close relationship between increased affect, central emotional arousal processes and enhanced visceral stimulus perception has been reported in patients with IBS.[65,100,101] Three recent reports published between 2013 and 2015 in female patients with IBS have identified disease-related alterations in anterior INS activity and connectivity in the resting state[58,59] and during an ambiguous abdominal pain threat,[102] confirming a key role of salience network alterations in IBS. Reported alterations in the response and connectivity within the salience network are consistent with the prediction error characteristic of patients with IBS about the likelihood and severity of future gastrointestinal symptoms (catastrophizing).[15]

Integration of brain networks

An extensive body of literature supports the model of the central role of aberrant salience computation underlying key clinical IBS symptoms (depicted in Figure 3). Well-established biological and behavioural consequences exist with such a biased appraisal: the engagement of altered ANS outputs to targets in the gastrointestinal tract (including ENS activity, gut permeability, gastrointestinal motility and secretion,[3] gut microbial composition and metabolites),[21,22] and to extraintestinal targets (including the immune system);[103] shifting of the balance of endogenous pain modulation systems towards increased descending pain facilitation;[20] increased engagement of the central executive network resulting in selective attention to gastrointestinal symptoms; and development of prediction errors about likelihood and severity of symptoms (so-called catastrophizing).[104,105] The model also provides a plausible biological basis for the effectiveness of different behavioural interventions such as cognitive behavioural therapy (normalizing salience, executive control and emotional arousal networks), self-relaxation techniques (normalizing emotional arousal and central autonomic networks) and mindfulness-based stress reduction (normalizing salience and executive control networks).[63]

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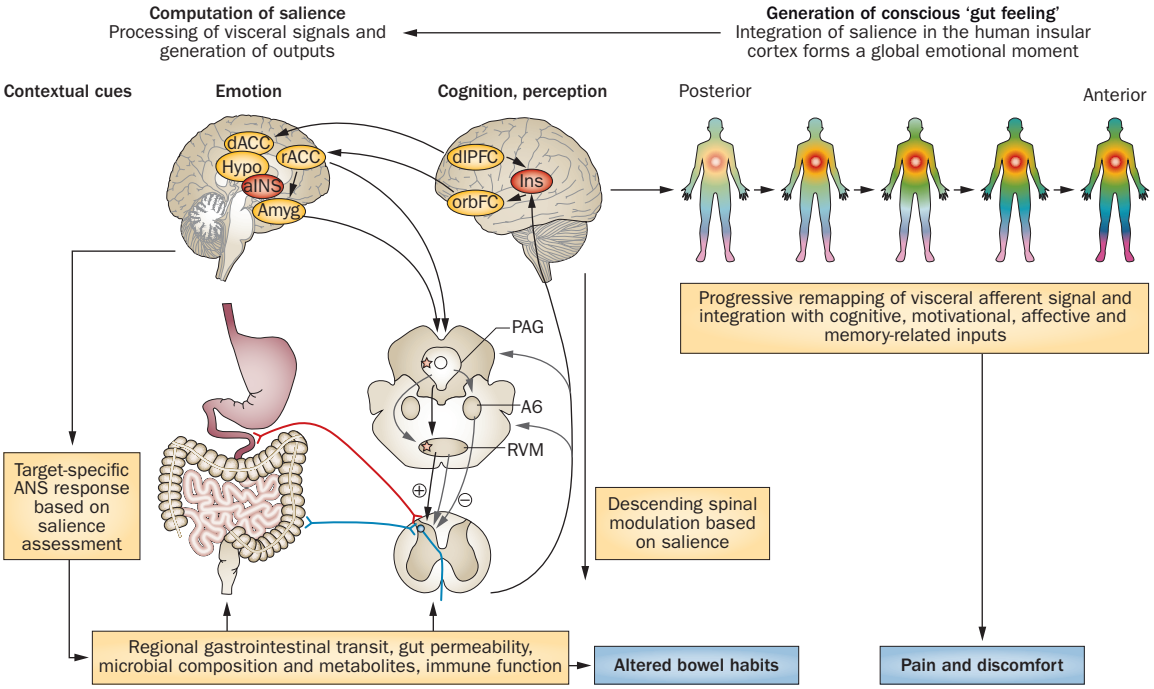

Figure 3 | Cross-sectional integrated brain–gut model of IBS pathophysiology. Proposed model for involvement of brain–gut axis in the generation of cardinal IBS symptoms (chronic abdominal pain associated with altered bowel habits). Under normal circumstances, visceral and external signals are evaluated by the salience network, which generates brain outputs in terms of targeted ANS responses (regulating gastrointestinal and immune function) and descending pain modulatory activity (regulating pain sensitivity at the dorsal horn level). Target organ alterations (either peripherally or ANS stimulated) are signalled back to the brain via neural, endocrine or immune-related channels. These signals are processed within subregions of the INS, and depending on their subjective salience, are consciously perceived (associated with activation of anterior INS) as normal gut sensations, discomfort or pain. IBS symptoms can arise from several primary peripheral or central mechanisms, but once brain–gut interactions are altered, causality is difficult to determine. Abbreviations: Amyg, amygdala; ANS, autonomic nervous system; dACC, dorsal anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; Hypo, hypothalamus; INS, insula; orbFC, orbitofrontal cortex; PAG, periaqueductal grey; rACC, rostral anterior cingulate cortex; RVM, rostral ventromedial medulla.

Genetics and epigenetics

As with many other chronic diseases that involve the brain, IBS probably has a strong developmental component, which starts with the interactions of genetic and epigenetic factors early in life, including the prenatal period. In addition to the reported associations of gene variations with various peripheral mechanisms,[106] imaging genetics studies performed in large samples of well-phenotyped individuals with and without IBS have identified interactions between early environmental factors,[107] candidate gene polymorphisms and brain networks related to emotional arousal and/or central autonomic control, salience and somatosensory integration. The reported genes were related to the regulation of the HPA axis: corticotropin-releasing hormone receptor 1 (*CRHR1*, single nucleotide polymorphisms [SNPs] rs7209436, rs110402 and rs242924); glucocorticoid receptor gene (*NR3C1*, SNPs rs2963155 and rs33389);[108,109] female sex hormones (progesterone receptor or *PGR*, SNPs rs1042838 and rs10895068);[109] 5-HT signalling system (*HTR3A* c.–42C>T SNP rs1062613),[77] inflammation-related genes (*IL1B*, SNP rs16944);[108] and catecholaminergic signalling (*ADRA1D*, *SNP* rs1556832; *ADRA2B*, SNP rs1042717; *COMT*, SNP rs174697).[110] As discussed in the next section, these interactions are markedly influenced by epigenetic factors[111,112] (such as a history of early adverse life events [EALs]) and by the sex of the study participant (Figure 4).

As with other polygenic disorders, it has become clear that no single gene variation is sufficient to explain the full clinical phenotype in IBS. However, interactions between multiple genes, early life experiences and sex probably make a small contribution to the overall variance of the peripheral and central endophenotypes.[107] Validation studies in larger samples are required to confirm such contributions.

The external environment component

Various influences originating in the external environment (Figure 1) can markedly affect the development, chronicity and severity of IBS. Although some of these influences are mediated by the brain (psychosocial stress, social support, societal responses to symptoms) others are mediated by the gastrointestinal tract, including the gut microbiota, diet, gastrointestinal infections and medications.

Brain-mediated influences

Clinical literature on the role of psychosocial factors in the development, symptom persistence and symptom flares in IBS is extensive.[18] In addition to the well-documented

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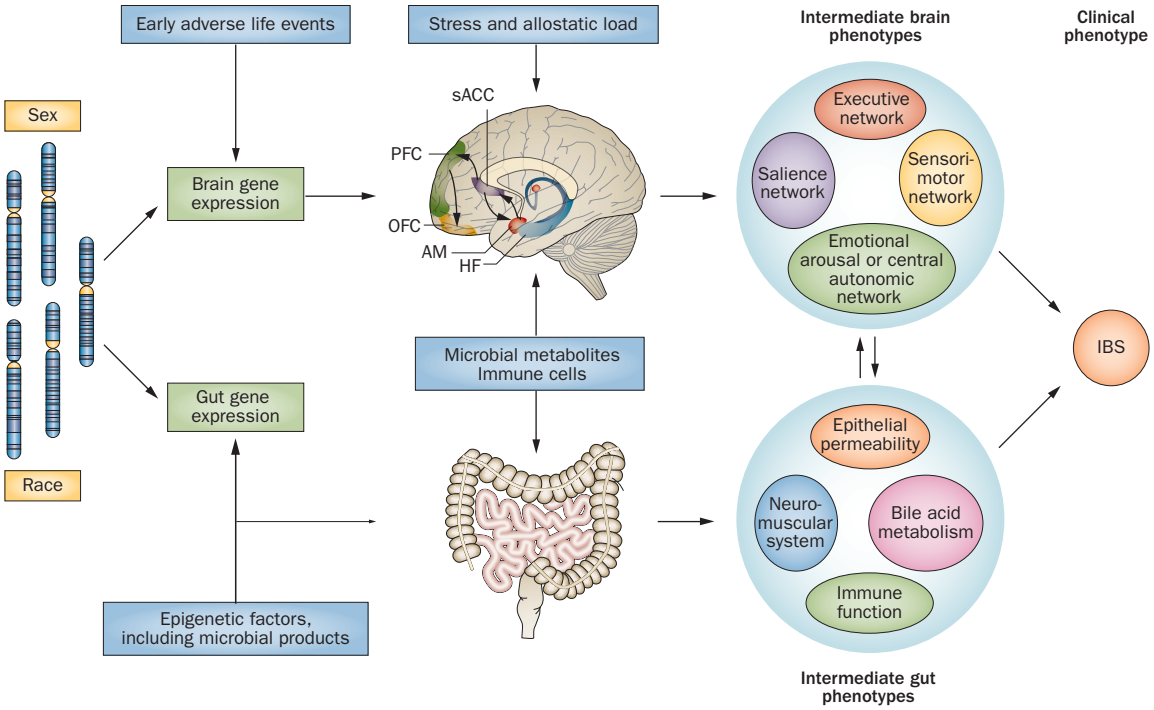

Figure 4 | Longitudinal brain–gut model of IBS pathophysiology. The interaction between genetic and epigenetic influences result in central and peripheral gene expression profiles that underlie the shaping of brain-based and gut-based intermediate phenotypes. Epigenetic factors provide the input from environmental influences on the development of intermediate phenotypes. Brain and gut intermediate phenotypes interact bidirectionally to shape the clinical phenotype of IBS. Abbreviations: AM, amygdala; HF, hippocampal formation; OFC, orbitofrontal cortex; PFC, prefrontal cortex; sACC, subgenual anterior cingulate cortex.

role of stressful life events in adults in preceding or exacerbating IBS symptoms,[32] a history of EALs is associated with an increased vulnerability to IBS,[113] disorders of mood and affect,[114,115] as well as to a wide range of other chronic diseases.[116] In contrast to the earlier emphasis on the role of a sexual abuse history, it is now clear that a variety of factors that disturb the quality of the interactions between the primary care giver and the child during the first 18 years of life (including serious illness of the mother, marital discord, divorce, verbal and emotional abuse) can have equally detrimental effect on adult disease vulnerability.[116]

Evidence from brain imaging studies have identified structural and functional brain alterations associated with self-reports of EALs. Structural alterations in regions of the emotional arousal circuitry[78] and in regions associated with modulating somatosensory and viscerosensory processes[90] were correlated with such reports. Alterations in the activity in the brainstem and amygdala in response to noradrenergic stimulation has been associated with increasing levels of EALs, consistent with an upregulation of central autonomic circuits in IBS.[117] Self-reports of EALs were also found to be correlated with brain activity in networks involved in determining the salience of somatic, visceral or environmental stimuli in IBS.[57] These results suggest that the experience of adversity early in life can lead to altered resting state activity in the salience and in the central executive network of adults with IBS, possibly leading to permanent alterations in salience computation of viscerosensory signals by the brain. The observed alterations in the functional connectivity of the emotional arousal[51] and salience networks[57] found in IBS might be driven by altered central noradrenergic modulation.[50,64] Similar changes have also been observed in animals exposed to early life stressors,[118] and have been linked to increased sympathetic nervous system responses.[119] EALs are also associated with altered signalling within the HPA axis.[120,121] A study published in 2009 demonstrated that self-report of EALs was associated with exaggerated HPA axis responses to an aversive visceral stimulus, an effect that was more pronounced in male participants.[113]

Considerable preclinical and clinical evidence supports the concept that gene expression can be influenced by EALs through epigenetic mechanisms, including DNA methylation, and that these effects can persist throughout adult life.[115,122,123] As depicted in Figure 4, interactions of EALs, sex and vulnerability gene polymorphisms might increase the risk of developing IBS by shaping the connectivity of relevant brain networks (see previous section). Even though the prevalence, clinical importance and underlying molecular mechanisms of EALs have been studied in great detail, there are other factors through which the external environment can influence brain function. These include, but are not limited to, the beneficial role of a strong social support system, which can mitigate the negative effects of EALs, and societal responses to patients' symptom reporting, which can drive a vicious cycle of symptom amplification.[124]

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Gastrointestinal-tract-mediated influences

Factors arising from the external environment have been implicated in the pathophysiology of IBS and in the modulation of IBS symptoms. These factors have been reviewed elsewhere and so will not be discussed in detail here: dietary factors;[125–128] pathogenic microorganisms;[129,130] and antibiotic treatment.[131,132] It remains to be determined if patients with IBS have abnormal mucosal responses to any of these factors, if symptoms reported in relation to these factors are mediated by alterations in the gut microbiota (see section on gut microbiota), or if it is simply the sensitivity of visceral perception that determines if somebody develops symptoms or can tolerate the same factors without any symptoms.

The immune system component

IBS is not an inflammatory disease, but a growing body of research suggests that dysregulation in immune function might nevertheless contribute to its aetiology or symptoms.[4,5,133] Mixed data exist on whether plasma or intestinal mucosal cytokine levels are associated with IBS.[5] However, other research has linked IBS to an increased reactivity of blood monocytes and increased numbers of mucosal mast cells.[134–139] Some studies also indicate that peripheral blood mononuclear cells (PBMCs) from patients with IBS show abnormal release of proinflammatory cytokines such as IL-6, IL-1b and TNF.[5]

These observations in IBS are consistent with the broader role of the nervous system in regulating immune cell development and gene expression via neuroendocrine signals from the brain (for example, cortisol from the HPA) and activation of sympathetic nerve fibres in the one marrow and other lymphoid organs (for example, via the sympathetic neuroeffector molecule norepinephrine).[103,140–142] These regulatory interactions enable the CNS to integrate information regarding the general internal state of the body with information regarding real and perceived environmental threats (as detected by the salience network) and historical or developmental influences (for example, a history of EALs[143]). As one example of these regulatory effects, studies have found that stressful life circumstances are associated with the activation of a so-called conserved transcriptional response to adversity (CTRA) in PBMCs that is characterized by increased expression of proinflammatory genes and decreased expression of genes involved in innate antiviral responses (for example, type I interferons) and IgG antibody production.[103,140,144,145] *CTRA* gene expression can also be experimentally invoked by social stress in animal models[145,146] and is mediated in part by sympathetic nervous system (SNS)-induced increases in bone marrow haematopoietic production of immature and immunologically primed monocytes ($CD16^-$ in humans, Ly-$6c^{hi}$ in mice).[146] These primed monocytes can also be reciprocally recruited into the CNS by exposure of mice to social threat and aggression.[147] The integration of these observations suggests a new hypothesis regarding the immune system's role in the pathogenesis of IBS: high levels of SNS activity during early developmental periods (stemming from either genetic or environmental triggers such as EALs) might lead to increased production of immature primed monocytes that both traffic into the gut to alter local function and ENS plasticity, and traffic into the brain to affect CNS plasticity (including structures involved in salience processing and autonomic regulation). The result can be viewed as a physiological Hebbian association between gut biology and brain function, resulting in a self-perpetuating feedback system in which a sensitized gut generates ongoing adverse sensory experiences (symptom flares), to which a neurally sensitized brain responds with both greater aversion and increased sympathetic outflow, resulting in upregulated monocyte production that further promotes neural alterations in both the gut and the brain.

Several areas of empirical evidence are consistent with this systems-level brain–immune–gut hypothesis, including: brain regulation of primed monocyte production via the SNS;[148] stress-induced migration of primed monocytes to the brain;[147] inflammation-induced neuroplastic changes in the brain[149] (for example, altering affective behaviour[147]); reinstatement of previous stress effect on brain and behaviour by subsequent exposure to mild stress weeks after the initial sensitization;[148] and the observation that patients with IBS (and animal models of EAL) show increased stress responsiveness (for example, SNS activity[150]) and increased responsiveness of brain circuits related to salience detection, emotional arousal and autonomic response.[151,152]

Additional evidence was examined from a pilot study in which the PBMC gene expression profiles from 20 patients with IBS (12 female) and 20 healthy individuals (nine female).[153] Analyses identified 280 gene transcripts showing >10% differential expression across groups (134 genes upregulated in PBMC from IBS, and 146 downregulated). Promoter-based bioinformatics analysis implicated several transcription control pathways in structuring the observed transcriptome differences, including increased activity of CREB transcription factors (which mediate β-adrenergic signalling from the SNS), growth control pathways (for example, the MAPK-responsive transcription factor ELK1), oxidative stress response pathways (NRF2), and pathways involved in growth factor and cytokine signalling (STAT). However, PBMCs derived from patients with IBS did not differ from those from healthy individuals in the activity of proinflammatory transcription factors such as NFκB or AP-1. Transcript origin analyses[154] indicated that IBS upregulated genes derived predominately from monocytes and dendritic cells. Additional transcriptome representation analyses suggest that these effects stemmed at least in part from upregulation of immature ($CD16^-$) monocytes within the PBMC pool of patients with IBS. These results are all consistent with a pattern of increased myeloid lineage cell development in patients with IBS, which might stem from tonically increased SNS signalling to bone marrow myelopoietic processes.[146]

Consistent with the hypothesis that CNS processes might mediate relationships between IBS and peripheral myelopoiesis, differential signalling by the myeloid lineage transcription factor MZF-1 was positively

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associated with the morphometry of brain regions of the emotional arousal network[155] (previously reported to be altered in IBS[78,90,156]). These associations included bilateral amygdala, hippocampal and anterior INS volumes, and bilateral anterior INS cortical thickness (all $P<0.05$). Moreover, these associations were reasonably specific in that other brain regions showed either no association, or negative associations (for example, bilateral cerebellar volumes).

In conjunction with the published data reviewed here, these preliminary results suggest that key regions of the emotional arousal and/or central autonomic networks, which differ structurally between IBS and healthy individuals are also related to differential gene regulation in the peripheral immune system. We hypothesize that these chronic influences of the brain on peripheral monocytes ('top down') might have a role in the reported PBMC abnormalities in IBS, whereas primed monocytes migrating to the brain might have a role in the ('bottom up') generation of visceral hypersensitivity, anxiety and neuroplasticity during recurrent stressors.

The gut microbiota component

Evidence from rodent studies supporting bidirectional interactions between the gut microbiota and the nervous system (both CNS and ENS) has been summarized in numerous review articles.[157–160] Even though various signalling mechanisms underlying such interactions have been proposed, detailed mechanistic studies regarding the relative contributions of neural, hormonal, metabolite or immune-mediated factors are required to draw definitive conclusions. Evidence from human studies using different endpoints (symptoms, brain imaging studies) confirming the rodent findings, or identifying a definitive pattern of dysbiosis in patients with IBS are limited.[158]

Conflicting evidence exists regarding alterations to the organization and function of the gut microbiota in patients with chronic abdominal pain and in adult and paediatric patients with IBS.[21,22] Several studies examining faecal samples from patients with IBS reported decreased proportions of the genera *Bifidobacterium* and *Lactobacillus*, and increased ratios of Firmicutes:Bacteroidetes at the phylum level, even though a causal role of these microbial changes in clinical symptoms has not been established. On the other hand, one might speculate that some of these changes might be related to alterations in regional gut transit and secretion secondary to altered ANS output. A study in a cohort of well-phenotyped patients with IBS highlights the complexities of brain–gut–microbiota alterations in IBS.[31] IBS subgroups were identified based on hierarchical clustering of operational taxonomic unit information from 16S ribosomal RNA analyses.[31] Two IBS clusters were clearly separated from each other, from a 'normal-like' IBS sample and from the healthy control sample. Although the normal-like IBS sample showed normal diversity and normal Firmicutes:Bacteroidetes ratio (as in, similar to the pattern in the healthy control sample), another cluster showed diminished, and a third cluster showed increased diversity. Both of these clusters showed increased Firmicutes:Bacteroidetes ratios. Only the normal-like IBS group showed a markedly elevated rate of depression symptoms (40%). In addition, prolonged colonic transit times, common in patients with depression correlated with the prevalence of 17 taxa.[31] In a randomized, placebo-controlled study of healthy men and women, psychological distress and anxiety improved after taking a *Lactobacillus*-containing and *Bifidobacterium*-containing probiotic compared to those taking a matched control product, although another study using a different *Lactobacillius* probiotic failed to confirm these findings.[161,162]

Findings from brain imaging studies in healthy individuals and patients with IBS has provided some evidence supporting reported rodent gut microbiota–brain interactions. One study has shown that chronic ingestion of a probiotic consortium (*Bifididobacterium animalis* ssp. *lactis*, *Lactococcus lactis* spp. *lactis*, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus*) for 4 weeks altered functional brain responses to an emotional face recognition task in healthy women.[52] Compared with two control groups (one group received a nonfermented milk product and the other no treatment), the women who had ingested the probiotic had a reduced response to the task across a wide network of brain regions that included sensory and emotional regions. Although no treatment-related changes in self-report of symptoms of anxiety or depression were seen, the findings suggest a basic change in responsiveness to negative emotional stimuli in the environment. No organizational changes in the gut microbiota were observed in this and a previous nonimaging study using the same intervention.[163] The effects of the probiotic intervention on brain responsiveness were probably mediated by a change in microbial-derived metabolites; this hypothesis will have to be confirmed in future studies. Preliminary evidence investigating correlations between gut microbial metabolites and brain structure in healthy individuals and patients with IBS demonstrated statistically significant correlations between several metabolites and structural aspects of several brain regions.[153]

An integrated model

Hypothesis

On the evidence reviewed here, the following model is proposed that lends itself to experimental evaluation. The hypersensitive brain, and presumably ENS, shows increased responses to a variety of viscerosensory and exterosensory stimuli, which by themselves might not be consciously perceived in healthy individuals or in patients with inflammatory bowel disorders in whom intact descending inhibitory bulbospinal influences reduce dorsal horn excitability.[20,164] This hyper-responsiveness might be a primary genetic or epigenetic alteration in certain brain networks (autonomic, emotional arousal or salience), or might be secondary to chronic experience of increased sensory input from the gut. Such increased viscerosensory signalling could originate from any of the elements of the gut connectome. Altered brain networks generate altered signals, which are transmitted to the

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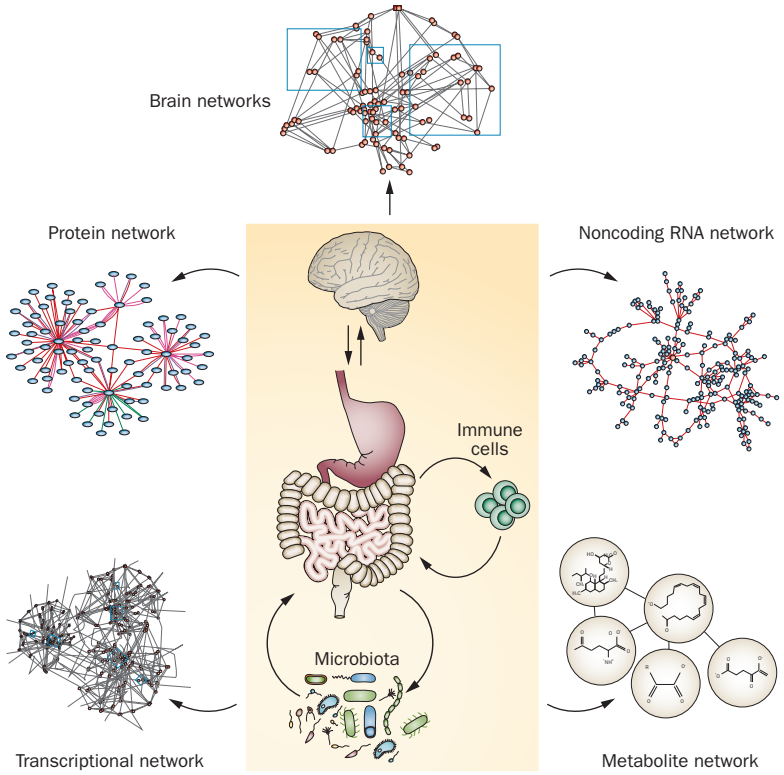

Figure 5 | Systems biological model of IBS. Schematic illustrating a systems biological view of components involved in the development of IBS at the cellular and molecular level. Systems-based interactions between central and peripheral components of the brain–gut axis can be studied at the level of the genome, epigenome, transcriptome, proteome, metabolome and brain connectome.

periphery through the ANS, HPA axis and descending modulation to the dorsal horn. The chronically increased ANS output, results in an extensive remodelling of various peripheral cells in the immune system, ENS, gut epithelium (permeability), and in the composition and function of the gut microbiota, all contributing to sensitization of visceral afferent pathways, and increased viscerosensory feedback to the brain, reinforcing the circular regulatory loops. Memory formations at the level of the immune system, the nervous system and the gut microbiota early in life are likely to contribute to the chronicity of symptoms.

Systems biology view

The evidence reviewed above strongly suggests that IBS is a systems disease (Figure 5), involving not only complex individual systems—nervous, immune, digestive, microbiota and the environment—but also their complex, nonlinear, reciprocal interactions. Thus, we propose a systems biological view of IBS pathophysiology, an approach taken in other areas of biology and disease[165,166] involving a large number of functionally diverse, interacting components, each of which contributes only a small fraction to the variance of the symptoms. These components interact highly selectively at multiple scales and typically in a nonlinear fashion to produce coherent behaviours and outcomes.[167] Applied on a macroscopic level, we propose this model to characterize the interactions of the nervous system, immune system, the gut environment (motility and secretion), the gut microbiota and the external environment (Figures 1 and 5). The nonlinearity of the model is reflected by the complexity of these macroscopic components and their macroscopic interactions, which form circular regulatory loops. Applied to the microscopic level, the model proposes the interactions between highly diverse cells types making up the various macroscopic components (Figure 5).

Given the complexity of the expanded gut–microbiota–brain–environment axis, and with the rapid advance of analytical tools, in particular of '-omics' technologies—in the case of IBS studies, primarily genomics, transcriptomics, proteomics, metabolomics, brain connectomics and their integration into, for instance, gene and protein networks—we believe that systems biological approaches are essential to fully understand symptom generation and to identify novel treatment approaches in the future.

The multiorgan, systemic view of IBS leads to the prediction that disease models focusing on individual cellular components at the macroscopic level (brain, immune system, gut, microbiota, environment) will have limited validity. Furthermore, this view predicts that therapies targeted at single organs (for example, brain, immune system, gut), single mechanisms (for example, secretion, motility, gut microbiota, pain, diet), or single molecular targets (such as ion channels or receptors) are not likely to be successful in treating the entire syndrome, as evidenced by the limited success in drug development for IBS to date, reflected in rather small effect sizes on overall changes in IBS symptoms. This problem is compounded by the fact that different subsets of patients can be characterized by different patterns of interactions between the components of the overall system, resulting in differential responses to a particular therapy.

Indeed, each isolated mechanism proposed over the past 30 years only explains a small fraction of the variance of the clinical phenotype[26] and no isolated therapies have had better effectiveness than ~10% over placebo.[168] In addition, the systems view predicts that a single agent targeting multiple components of the system (tricyclic antidepressants, 5-HT_3 antagonists), or combined therapies, aimed at simultaneously targeting multiple organs and mechanisms (combination of laxatives, antidiarrhoeal agents, probiotics and centrally acting drugs) are likely to be more effective in the clinic, than individual treatments by themselves. Although no controlled studies have been conducted yet and are needed, in our opinion, this suggested integrated approach is in part supported by current clinical practices and the consensus of most physicians.

Although still in their infancy, integrated multiomic approaches could be essential in the future to better understand the disease spectrum for IBS at the system level and the underlying mechanisms. Ideally, one would like to combine high-throughput microscopy and brain imaging to obtain brain connectome variables, and sequencing and mass spectrometry methods to obtain complete measurements of genomic, epigenomic, transcriptomic, proteomic and metabolomic variables simultaneously in multiple organs and tissues (in particular in gut and brain), including the microbiota. Data derived from these

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–omics approaches can be integratively analysed in combination with other sources of information ranging from public databases to the literature to identify, for instance, targetable hubs. This integrated –omics approach faces major obstacles stemming from the obvious difficulties of performing some of these analyses in human patients (for example, nervous system tissue). However, important steps can be taken in this direction both using rodent models[10,81,169,170] and by performing partial measurements in humans.

Some of these analyses should be performed at multiple time points along the circadian cycle. Circadian rhythms are found at the molecular level in all tissues, both centrally and peripherally, and have a fundamental role in coordinating the physiology and homeostasis of the organism. Indeed integrative system biology analyses[171] have revealed that about 10% of all transcripts and metabolites oscillate in a circadian manner in any tissue,[172] with little overlap across different tissues beyond the core molecular clock comprising a transcriptional–translational negative feedback loop coordinated by a dozen genes.[173] Furthermore, the list of oscillating molecular species in a given tissue is altered by genetic, epigenetic and environmental perturbations and thus provides a characteristic physiological signature of a tissue and its health state.[174–176] Furthermore, complex reciprocal interactions exist between the molecular rhythms found in different brain regions and in other organs. Thus, in short, it is reasonable to predict that IBS should result in perturbed lists of transcripts and metabolites that oscillate in a circadian manner both centrally in specific brain areas as well as peripherally, for instance in the gut.

Conclusions

Clearly, none of the growing list of individual abnormalities identified in patients with IBS by itself can account for the variance of the clinical phenotype. For the same reason, such individual abnormalities are unlikely to represent reliable biomarkers and are not likely to represent suitable targets for the development of highly effective treatments. Rather, as depicted schematically in Figures 1 and 5, the clinical phenotype emerges from the interactions of multiple systems in the periphery (gut connectome, microbiome, genome and epigenome) and in the brain (connectome) interacting with each other in bidirectional ways. Most consistent with a systems view is the concept that central and peripheral abnormalities form circular loops that reinforce each other. In the absence of comprehensive phenotyping studies in patients with IBS performed longitudinally with or without therapeutic interventions, and without targeted mechanistic animal studies, it remains unclear which of these reported abnormalities are primary and which are secondary. On the basis of this assessment, we suggest that in the future, high-throughput –omics measurement across both tissues and time, combined with comprehensive characterization of clinical, behavioural and brain endophenotypes, should enable more accurate differential analyses, uncover complex system-level interactions and, ultimately, help develop more efficient, multipronged therapies.[177] Such a system biological approach might not only hold promise when applied to IBS and related functional gastrointestinal disorders, but also for inflammatory diseases of the gut, such as IBD and coeliac disease.[178]

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Author contributions

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