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Authors

Mahurkar-Joshi, Swapna

Chang, Lin

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Epigenetic Mechanisms in Irritable Bowel Syndrome

Swapna Mahurkar-Joshi and Lin Chang*

G. Oppenheimer Center for Neurobiology of Stress and Resilience, Division of Digestive Diseases, Department of Medicine at UCLA, Los Angeles, CA, United States

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*Correspondence:

Lin Chang
linchang@mednet.ucla.edu

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Irritable bowel syndrome (IBS) is a brain-gut axis disorder characterized by abdominal pain and altered bowel habits. IBS is a multifactorial, stress-sensitive disorder with evidence for familial clustering attributed to genetic or shared environmental factors. However, there are weak genetic associations reported with IBS and a lack of evidence to suggest that major genetic factor(s) contribute to IBS pathophysiology. Studies on animal models of stress, including early life stress, suggest a role for environmental factors, specifically, stress associated with dysregulation of corticotropin releasing factor and hypothalamus-pituitary-adrenal (HPA) axis pathways in the pathophysiology of IBS. Recent evidence suggests that epigenetic mechanisms, which constitute molecular changes not driven by a change in gene sequence, can mediate environmental effects on central and peripheral function. Epigenetic alterations including DNA methylation changes, histone modifications, and differential expression of non-coding RNAs (microRNA [miRNA] and long non-coding RNA) have been associated with several diseases. The objective of this review is to elucidate the molecular factors in the pathophysiology of IBS with an emphasis on epigenetic mechanisms. Emerging evidence for epigenetic changes in IBS includes changes in DNA methylation in animal models of IBS and patients with IBS, and various miRNAs that have been associated with IBS and endophenotypes, such as increased visceral sensitivity and intestinal permeability. DNA methylation, in particular, is an emerging field in the realm of complex diseases and a promising mechanism which can provide important insights into IBS pathogenesis and identify potential targets for treatment.

Keywords: irritable bowel syndrome, IBS, epigenetics, visceral hypersensitivity, DNA methylation, microRNA, histone modifications, long non-coding RNA

INTRODUCTION

Irritable bowel syndrome (IBS) is a complex condition characterized by alterations of bidirectional brain-gut interactions affecting gastrointestinal (GI) function. It is a widely prevalent disorder affecting about 5% to 11% of general population and occurs in children and adults and in men and women although it is considered a female-predominant condition. Hallmark symptoms include the presence of chronic or recurrent abdominal pain associated with altered bowel habits without underlying structural abnormalities (1–4). IBS has been subdivided on the basis of predominant bowel habits into diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), or a mix of

diarrhea and constipation (IBS-M) subtypes (3). IBS can coexist with other GI disorders including gastroesophageal reflux disease and functional dyspepsia, as well as somatic syndromes including fibromyalgia, interstitial cystitis, migraine headaches, and psychological disorders (5). Due to its high prevalence, recurrent nature of symptoms and a negative impact on health-related quality of life (6), IBS is associated with substantial cost to patients, the health care system, and society (7).

IBS is considered to be a multi-factorial disorder, however, its pathophysiology is not completely understood. IBS and other functional GI disorders have more recently been redefined by experts as “disorders of gut-brain interactions (DGBI) classified by 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” (4). The presence of emotional and psychological factors and food intolerance contribute to the clinical presentation and can exacerbate IBS symptoms (8, 9).

Studies have shown that genetic factors have a modest effect in IBS (10). In addition, there is increasing evidence of a strong influence of environmental factors such as stress in its pathogenesis. A number of studies have found that IBS patients have a higher prevalence of stressful events including early adverse life events (EALs), or traumatic experiences during childhood, as well as current stressful life events in adulthood (11–13). The mechanisms underlying long-term effects of stress and EALs may result from epigenetic programming (14). Epigenetic changes refer to molecular alterations that potentially lead to altered gene expression resulting in a change in phenotype in absence of alteration in the underlying gene sequence.

In this review, we summarize the genetic factors associated with IBS and describe the role of epigenetic factors including DNA methylation and histone modifications as links between genes and environmental factors (e.g., stress) in the etiopathology of IBS. We review the current knowledge of epigenetic modifications associated with IBS in patients as well as in early life stress animal models of IBS, and those associated with IBS endophenotypes (defined as intermediate phenotypes of subclinical traits) including stress and hypothalamic–pituitary–adrenal (HPA) axis function, visceral hypersensitivity and abdominal pain, and GI motility. Further, we briefly outline the role of other epigenetic factors including non-coding RNAs (long non-coding RNAs [lncRNAs] and microRNAs [miRNAs]) in IBS. Finally, we will present a schematic model of our current understanding of factors associated with IBS pathogenesis. A better understanding of the epigenetic mechanisms in IBS can open new avenues for the identification of novel therapeutic targets.

GENETIC CHANGES ASSOCIATED WITH IBS

Familial Aggregation and Twin Studies in IBS

IBS is often associated with familial clustering in which patients report a family history of IBS (15–17). However, the strength of

the genetic association varies between studies. One study reported familial aggregation in IBS but found no evidence of association in spouses, suggesting either a possible genetic etiology or an exposure to a shared household environmental factor early in life as an underlying cause of IBS (18). Additional evidence in favor of both a genetic and environmental etiology of IBS comes from twin studies. Twin studies by Morris-Yates et al. (19) and Svedberg et al. (20) provided evidence for genetic basis of IBS in Australian and Swedish populations. In two large studies on 281 twin pairs in the United States (21) and 3334 twin pairs in Norway (22), Levy et al. and Bengtson et al. showed a higher concordance rate among monozygotic twins than in dizygotic twins for IBS. However, one study by Mohammed et al. (23), failed to replicate the differences in the concordance rates between the monozygotic and dizygotic twin groups. Interestingly, Levy et al. also reported that the presence of IBS in the mother was a strong predictor of having IBS. The proportion of twins who had mothers with IBS was 15.2% which was significantly higher than the 6.7% of twins with IBS who had a co-twin with IBS. Since dizygotic twins share about the same number of genes with each other as each twin shares with their mother, this study suggested that in addition to heredity, social learning, and behavior may contribute to the development of IBS (24).

Candidate Gene Studies in IBS

IBS has been associated with genetic variants in a number of candidate genes. Genes associated with IBS in various studies are listed in **Table 1**. These include single nucleotide polymorphisms (SNPs) in genes related to signaling systems important in the control of gut motility or sensation in IBS, which includes serotonergic (5-HT) system including tryptophan hydroxylase (TPH), serotonin reuptake transporter (SERT), a, cholecystokinin (CCK), voltage-gated sodium channels (Nav), Catechol-O-methyltransferase (COMT), cannabinoids, and ion channels, such as transient receptor potential (TRP) channels (TRPV1). Immune related SNPs have been of particular interest in IBS based on accumulating evidence showing immune activation in IBS (25). However, findings have been variable across studies and association of genes such as tumor necrosis factor (TNF α) and IL-10 have not been consistent (26). A recent meta-analysis, which included 12 published case-control studies found no significant association with IBS with polymorphisms in genes such as IL-4, IL-6, IL-8, IL-10, TNFA, IL-1R1, and IL-23R. However, SNP rs4263839 which encodes for TNFSF15 was only moderately associated with IBS, in particular with IBS-C (25). Candidate gene association studies in IBS have been comprehensively reviewed by Cheung et al. (27), Camilleri (28), and Gazouli et al. (29).

Despite these genetic associations, it is not entirely unexpected that the effects of an individual polymorphism on the overall phenotype are modest because IBS is a complex, multifactorial condition. Moreover, the development of disease likely involves more than the presence of just a moderately associated common variant. While SNPs of these genes alone may not be sufficient to cause IBS or other complex chronic pain conditions, they may interact with other genes and

TABLE 1 | Genetic changes associated with irritable bowel syndrome (IBS).

Function	Gene	Polymorphism	Endophenotype	PMID
Neurotransmission				
Serotonin biosynthesis	Tryptophan hydroxylase (<i>TPH1</i> and <i>TPH2</i> isoforms)	rs4537731, rs2111105, rs4570625	IBS-D, IBS-C	21073637, 24060757
Serotonin reuptake; Serotonin receptors	Serotonin reuptake transporter (<i>SERT</i> or <i>SLC6A4</i>); 5-HT receptor 3A (<i>HTR3A</i>)	5-HT transporter linked promoter region (5-HTT LPR) deletion; rs25531; rs1062613	IBS-C, IBS; IBS-D, symptom severity and anxiety	12135035, 15361494, 17040410, 17564628, 17074108, 17241856, 18511740, 19426812, 19125330; 19125330, 24069428, 24512255, 21420406.
Adrenergic receptors, Catecholamine metabolism	Adrenergic receptors alpha (<i>ADR2A</i> , <i>ADR2C</i> , <i>ADRA1D</i>), Catechol-o-methyl transferase (<i>COMT</i>)	alpha(2C) Del 322–325; alpha(2A) –1291; rs1556832, val158met	IBS-C, severity, alterations in brain regions, IBS	19833115, 26288143
Neuropeptide receptors	Neuropeptide S receptor1 (<i>NPSR1</i>)	rs2609234, rs6972158, rs1379928, rs1379928	colonic transit, pain and gas	21437260
Cannabinoid mechanisms	Cannabinoid receptor1, (<i>CNR1/CB1</i>), Fatty acid amide hydrolase (<i>FAAH</i>), Corticotropin-releasing hormone binding protein (<i>CRHBP</i>)	AAT repeat frequency, rs806378 C385A, rs10474485	IBS, abdominal pain, IBS-D, colonic motility, transit time, emotional abnormalities	19732772
Barrier function, Immune and Inflammatory Mediators				
Barrier function, adhesion	Toll-like receptor 9 (<i>TLR9</i>), Cadherin 1 (<i>CDH1</i>)	rs5743836	PI-IBS, epithelial cell barrier function	20044998
Cytokines	Interleukin (<i>IL</i>)-6, <i>IL</i> -10, Tumor necrosis factor-alpha (<i>TNFα</i>), <i>IL</i> -8, <i>TNFSF15</i>	rs1800870, rs1800872, rs6478108, rs6478109, rs7848647, rs4263839	PI-IBS, IBS, IBS-D, innate immune response	20044998; 22837345
Ion Channels and Bile acids				
	Voltage-gated sodium channel NaV 1.5 (<i>SCN5A</i>), G protein-coupled bile acid receptor 1 (<i>GPBAR1</i>), Klotho Beta (<i>KLB</i>)	rs11554825, rs17618244	IBS, colonic transit, fecal bile acid	20044998, 21752155, 16279907, 23595519, 12477767, 15765388, 20337945, 22158028, 24409078, 22684480, 21636646, 25824902

Table 1 shows genetic changes associated with IBS and IBS endophenotypes. PMID, PubMed ID; IBS-D, IBS diarrhea subtype; IBS-C, IBS constipation subtype.

environmental factors including EALs and contribute to the disease etiology. Therefore, an alternative approach has been to evaluate an association of gene variants with specific IBS subtypes (IBS-D, IBS-C, and IBS-M) as well as endophenotypes. For example, we found that the SNP rs1556832 in the catecholaminergic gene, adrenoceptor alpha 1D (*ADRA1D*), was associated with IBS symptom severity and morphological changes in brain regions that modulate sensory processing (30). In another study, we demonstrated that the presence of IBS was significantly associated with SNPs in corticotropin releasing hormone receptor 1 (*CRH-R1*) gene. These SNPs were associated with increased GI symptom-related anxiety and acoustic startle response to threat in IBS patients, suggesting that *CRH-R1* is involved in altered stress responsiveness in IBS (30).

Genome Wide Association Studies (GWAS) in IBS

Considering the challenges of identifying individual risk alleles in case-control studies and the difficulty of defining significant gene association with IBS, a GWAS using large samples has been proposed as an alternative approach in an attempt to increase sample size and homogeneity. Ek et al. reported a GWAS study in IBS comprising of 534 IBS patients and 4,932 healthy controls, followed by six independent clinical case-control replication studies from different countries (31) where they identified variants in *KDLER2* and *GRIP2IP* (chromosome 7p22.1) genes to be associated with IBS. *KDLER2* codes for a family of integral membrane protein with seven transmembrane domains involved in intracellular signaling of bacterial toxins

(32), potentially relevant to the role of microbiota in IBS. The *GRID2IP* gene encodes for a protein (delphilin) expressed on fiber-Purkinje cell synapses in the brain involved in glutamatergic neurotransmission, potentially relevant to pain signaling (31, 33). Another GWAS with a smaller sample size (172 IBS cases and 1,398 controls) conducted in an Australian cohort found an association of protocadherin 15 (*PCDH15*) gene, encoding an integral membrane protein that mediates calcium-dependent cell–cell adhesion ($P \sim 9 \times 10^{-9}$).

GWAS studies have also evaluated other SNP associations in IBS. *TNFSF15* was found to be only nominally significant in the GWAS study, contrasting with prior reports as mentioned previously. Similar nominal associations were detected for other genes such as Cell Division Cycle 42 (*CDC42*), Neurexophilin 1 (*NXPH1*) (34), 5-HT Receptor 3E (*HTR3E*) (35), Klotho β (*KLB*) (36) and Sodium Voltage-Gated Channel Alpha Subunit 5 (*SCN5A*). Interestingly, *SCN5A* encodes the α -subunit of the voltage-gated sodium channel NaV1.5. About 2% of patients with IBS were found to carry mutations in *SCN5A*, most of which were loss-of-function mutations that disrupted NaV1.5 channel function (37). Additionally, in a GWAS study on self-reported IBS patients and controls, Bonfiglio et al. identified variants at 9q31.2 locus that were associated with IBS in women suggesting a role for sex hormones in IBS (38). However, most genes associated with IBS thus far represent non-validated findings and therefore their role in IBS needs to be cautiously interpreted. Moreover, such discrepancies are believed to arise from multifactorial nature of the disease, phenotype heterogeneity (including variability in endophenotypes) and/or sample sizes, among others.

Additionally, the mechanisms involved in pain sensitization and altered motility are likely multifactorial as demonstrated in multiple clinical and animal studies in the past decades (39). These functional alterations are mediated through cellular and molecular changes mediated by genetic and epigenetic alterations (40) detailed in the following sections. At the CNS level, proposed mechanisms include plasticity of the endogenous pain modulation system and structural changes in the brain (41, 42). An important step towards understanding the complex pathogenesis of IBS lies in the ability to discover the interface between genetic pathways and epigenetic regulation mediated by gene-environment interaction at peripheral (gut) and central (CNS) levels.

STRESS: AN ENVIRONMENTAL TRIGGER FOR IBS

IBS is associated with various environmental factors including chronic stress in early life and/or adulthood, diet (43–45), and gastrointestinal infections (46, 47). Chronic stress can increase an individual's vulnerability to developing IBS and/or can trigger or exacerbate the symptoms of IBS (48, 49). Stress is the body's reaction to a physical or psychological stimulus that disturbs the homeostasis of an organism. Stress has wide-spread effects on gut physiology, including changes in intestinal motility, mucosal transport, and gut barrier function leading to changes in permeability, and visceral perception. The biological effects of stress are mediated by the sympathetic nervous system and corticotropin releasing factor (CRF)/HPA axis pathways. Glucocorticoids, which are major effector molecules of the HPA axis, bind to their intracellular receptors and regulate the physiological adaptations to stress (50, 51). Glucocorticoids including cortisol/corticosterone initiates negative feedback control *via* binding to glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in brain regions including hippocampus, paraventricular nucleus (PVN), and anterior pituitary gland (52). However, in response to chronic and uncontrollable stressors, maladaptive changes can be elicited resulting in malfunctioning of stress systems affecting the brain structure and function (53, 54).

Stress-Induced Visceral Hypersensitivity and Motility Abnormalities in IBS

Many studies support an important role for stress in the IBS pathophysiology and symptoms (8). The stress-induced activation or augmentation of the CRF and HPA axis systems has been associated with visceral hypersensitivity, an important feature of IBS, in animal models (55–58). IBS patients have a greater reactivity to stress compared to healthy subjects, as manifested by a dysregulated HPA axis response, enhanced visceral perception and gut motility, among other findings (59–61). IBS has been associated with increased prevalence of EALs and a growing body of evidence from both animal and human studies supports the hypothesis that chronic stress, including EALs, represent an important mechanism leading to changes in glucocorticoid receptor (GR) expression, thereby increasing

responsiveness of the HPA axis (62). The HPA axis response is regulated by a negative feedback through binding of cortisol to GRs at multiple levels including the hypothalamus and hippocampus. Impairment of this negative feedback mechanism can lead to a dysregulation of the HPA axis, specifically an enhanced HPA axis response due to reduced negative feedback from reduced expression of GRs. The importance of an early life and adulthood stress on this IBS phenotype was demonstrated in the maternal separation (MS) animal model, where pups that were maternally separated in early life and later subjected to psychological stress as an adult displayed post-stress visceral hypersensitivity, increased corticosterone levels, and reduced expression of GRs in the hippocampus (63, 64). Additionally, stress-induced visceral hyperalgesia has been investigated in repeated water avoidance stress (WAS), a validated rat model of psychological stress that demonstrates many human IBS-like traits. A knockdown of GRs has also been shown to increase visceromotor response to colonic distention in animal models (65). Additionally, a neonatal inflammation rat model suggested a role for inflammatory insult in early life, which upregulates vasoactive intestinal peptide (Vip) in the colon muscularis externa contributing to altered motility and diarrhea-like symptoms as seen in IBS-D patients (66–68).

We found that GR expression was decreased in peripheral blood mononuclear cells (PBMCs) in IBS patients in comparison to healthy controls and that GR expression levels negatively correlated with pituitary responsiveness (ACTH levels) to CRF stimulation (69). That is, reduced GR expression was associated with an enhanced HPA axis response. HPA axis function was assessed in PBMCs because they are accessible and feasible to study. Although GRs regulate HPA axis *via* negative feedback in the CNS, changes in GRs on PBMCs have been reported in psychiatric diseases, including changes in the number and sensitivity of GRs (70, 71) and GR promoter methylation status and mRNA expression (69, 72–74). Furthermore, the transcriptome of peripheral blood has been shown to share >80% homology with genes expressed in the brain, heart, liver, spleen, colon, kidney, prostate, and stomach, and that there is a broad movement of leukocyte subsets to and from the gut at steady state, suggesting that PBMCs can reflect the molecular events at the central and peripheral locations (75).

Stress, Intestinal Epithelial Barrier Function and Immune System

Various animal models representing different stress paradigms (e.g. restraint stress, WAS, neonatal MS, etc.) as well as studies in human subjects have demonstrated an impairment in mucosal barrier function, the enteric nervous system (ENS), and immune system (76–78). These stress-induced changes result in alterations in GI functions including increased intestinal permeability, altered ion transport and hypersecretion, and mucus secretion and are mediated by neuro-immune mechanisms including the CRF system, which consists of CRF, urocortins 1–3 (Ucn) and their receptors CRF-1R and CRF-2R (79, 80). Barrier dysfunction may also occur early in IBS and is hypothesized to contribute to low-grade intestinal immune activation and increased visceral perception (81), specifically in

IBS-D patients (82, 83) and post-infection IBS (PI-IBS) (84). Additionally, an increase in paracellular permeability has been correlated with the magnitude of visceral pain in IBS-D patients (83). Furthermore, an exaggerated response to CRH infusion in IBS patients was associated with an increase in cytokine levels suggesting a correlation between stress and increased cytokine levels (85). This is hypothesized to be mediated by glucocorticoid-related epigenetic changes leading to inadequate suppression of proinflammatory cytokines (40). It conceivable that this contributes, at least in part, to the higher plasma levels of cytokines reported in some IBS patients (86, 87).

Both local (intestinal) and systemic factors contribute to the altered epithelial barrier function. Recent data indicate that soluble mediators from fecal supernatants and mucosal homogenates of IBS patients affect the epithelial integrity, thereby increasing colonic permeability (88, 89). These studies showed that the impairment of barrier integrity may be mediated *via* the release of various mediators such as serine proteases or histamine. Additionally, molecular alterations such as expression of genes involved in barrier function (90) can mediate permeability changes, which can in turn lead to permeation of bacteria and their products.

EPIGENETIC MODIFICATIONS: A BRIDGE BETWEEN ENVIRONMENT AND GENES IN IBS

Epigenetic mechanisms alter gene expression without alterations of underlying DNA sequence (91) and are key to the normal development, cellular function, and differentiation into specific lineages (92). These mechanisms broadly include DNA methylation, histone modification, and non-coding RNA mediated gene regulation as shown in **Figure 1**. Epigenetic mechanisms play a role in synaptic plasticity, learning, and memory (93), as well as in various neuropsychiatric conditions including depression and pain (94). Long-lasting epigenetic changes have been linked to early stress, childhood trauma or abuse. Epigenetic changes are amenable to exogenous influences and involve complex and dynamic interaction between the DNA sequence, DNA and histone modifications and environmental factors, all of which combine to produce the phenotype, thus providing an important link between environment and phenotype (95).

DNA Methylation in Animal Models of IBS and IBS Patients

In vertebrates, DNA methylation occurs mostly in the context of CpG dinucleotides by a covalent attachment of a methyl group to the C5 position of cytosine (91). CpG islands (CGIs) are short interspersed DNA sequences (usually 1000 base-pairs) with a high concentration of CpG residues, which are normally non-methylated in contrast to the rest of the genome, which is globally methylated. CGIs typically occur at or near the transcription start site of genes (96) and when a CGI in the promoter region of a gene is methylated, expression of the gene is

repressed. The exact mechanism of DNA methylation mediated repression of gene expression has begun to be elucidated in recent years. DNA methylation results in binding of methyl-binding-domain (MBD) proteins, which are associated with large protein complexes that contain histone deacetylases (HDACs) and recruit histone methyl transferases (HMTs) leading to chromatin remodeling (97). Both DNA methylation and the proteins associated with MDBs are being investigated as promising therapeutic targets (98). Additionally, recent studies have demonstrated that methylation of CpG sites in the gene body are positively correlated with gene expression and is a potential therapeutic target in cancer (99). The quantification of DNA methylation in diseased or environmentally impacted cells could provide useful information for detection and treatment of the disease.

DNA methylation changes, in particular, have been studied in various chronic conditions including cancer (100), chronic pain (101), and psychiatric diseases (102). Stress and other environmental factors including EALs, diet and gut microbial metabolites can potentially trigger epigenetic alterations (103, 104). For example, studies have demonstrated that maternal care influences HPA axis function through epigenetic programming of GR (coded by Nuclear Receptor Subfamily 3 Group C Member 1, or *NR3C1*) expression and that environment-induced remodeling of the epigenome, or during chronic stress, can result in long-term changes in gene expression (105–107). **Table 2A** lists the epigenetic modifications reported in association with IBS or animal models of IBS. The role of central epigenetic regulatory mechanisms in stress-induced visceral hypersensitivity has been demonstrated in MS and WAS rat models. While MS animal models mimic the early life stress, WAS simulates both acute and chronic effects of a psychological stressor on colonic sensitivity, which have been extensively reviewed by Greenwood-Van Meerveld et al. (108). Stress-induced visceral hypersensitivity has been associated with an increase in DNA methylation in the GR gene promoter and a decreased expression of the GR gene in the amygdala of WAS rats (109, 110). Additionally, the study identified a decrease in DNA methylation and increased expression of the CRF gene associate with visceral hypersensitivity in the amygdala of the stressed rats. Hong et al. demonstrated that chronic stress increased methylation of genes that regulate visceral pain sensation in the peripheral nervous system of rats. They reported that chronic stress resulted in increased promoter methylation and reduced expression of the *NR3C1* (or GR) gene in L6-S2 dorsal root ganglia (111). In human subjects, DNA methylation in brains of suicide victims with a history of childhood abuse was associated with increased methylation and decreased expression of GR gene compared to suicide victims with no history of childhood maltreatment (106). However, no clear consensus exists regarding DNA methylation of the GR gene in IBS patients.

In a genome-wide methylation scan followed by targeted sequencing, we previously demonstrated an association of DNA methylation of several CpG sites in PBMCs in IBS patients compared to healthy controls (112). We reported an

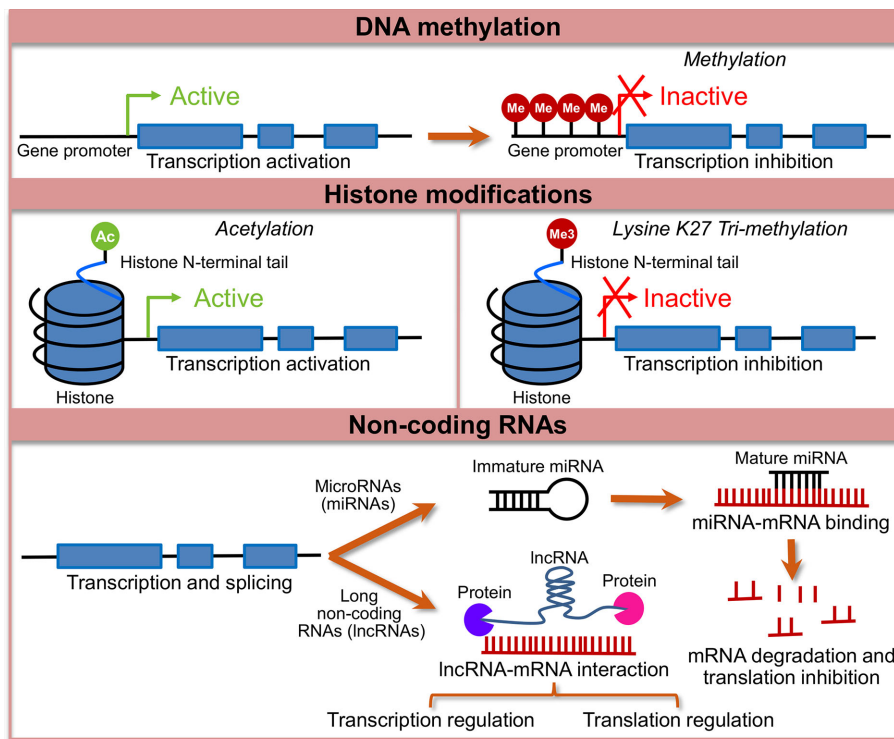


FIGURE 1 | Major epigenetic changes studied in the context of irritable bowel syndrome (IBS). Shows a conceptual model of major epigenetic changes studied in the context of IBS. Lines with blue boxes represent genes with promoter regions. Blue boxes represent exons, lines before exon 1 represents promoter region and the lines between exons represent introns. The top panel shows active transcription in the unmethylated state of the gene, which when methylated (Me) at the promoter region leads to transcription inactivation. Middle panel shows two representative histone modifications, histone acetylation at the N-terminal tail, which is usually associated with activation of transcription and histone methylation, specifically, addition of a tri-methyl group (Me3) at 27th lysine (K) on the N-terminal tail, which is associated with transcription repression. The bottom panel shows mechanism of transcription regulation by non-coding RNAs. MicroRNA genes are transcribed to immature precursor miRNAs that are processed to form mature miRNAs, which bind to miRNAs either leading to mRNA degradation or inhibition of translation. Long non-coding RNAs regulate transcription and translation, and function at the level of chromatin via interaction with RNA binding proteins.

increase in DNA methylation in genes including sub-commissural organ (SCO)-Spondin (*SSPO*), glutathione-S-transferases mu 5 (*GSTM5*) and tubulin polymerization promoting protein (*TPPP*) in IBS patients compared to healthy controls. *SSPO* is associated with neuronal function (113) and has been suggested to play a role in depression and evidence suggests that SCO secretory activity is regulated by the serotonin system, which plays an important role in stress-related pathways and in IBS (114). Additionally, an increased methylation of *GSTM5*, a gene that codes an enzyme that plays an important role in antioxidant defense was associated with decreased gene expression compared to controls. Although a role for oxidative stress and the significance of epigenetic silencing of *GSTM5* in IBS is not known, DNA methylation mediated repression of *GSTM5* gene expression has been shown in other conditions (115). Although larger independent studies may be required to confirm the functional role of the associated genes, these studies highlight the importance of epigenetic changes in IBS. DNA methylation changes in blood cells can provide insights into

systemic changes associated with IBS and can serve as important diagnostic and prognostic biomarkers (116).

Epigenetic changes in the gut mucosa can provide important insights into the peripheral mechanisms of IBS. A recent study investigated the genome-wide methylation predominantly in promoter regions of genes, and gene expression in the colon of rat WAS model and suggested an association of Notch signaling and focal adhesion pathways with psychological stress (117). In a recent study that included a relatively large cohort of IBS subjects and healthy controls (n=102 and 36, respectively), we found several DNA methylation changes in PBMCs as well as colonic mucosa that were associated with IBS. There was increased methylation of stress-related genes such as *NR3C1*, *CRHR1*, brain-derived neurotrophic factor (BDNF) in PBMCs and/or colon (118). In the colonic mucosa of IBS patients, we identified distinct clusters of DNA methylation patterns highlighting the heterogeneity in the epigenetic profiles of colonic mucosa of IBS patients. A hyper-methylated cluster was associated with higher symptom severity and abdominal pain compared to clusters with

lower methylation levels and included genes such as protocadherins (PCDHs), cadherins (CDHs), VIP, TRPV4, and Guanylate Cyclase 1, Soluble, Beta 3 (GUCY1B3) which were significant after correcting for multiple comparisons. Thus, these studies suggest that DNA methylation changes are important pathophysiologic mechanisms in IBS and should be further evaluated.

Histone Modifications in Animal Models of IBS

In eukaryotic cells, genes complex with histone and other chromosomal proteins to form a chromatin scaffold. Histone modifications play an important role in regulation of gene expression. The histone tails undergo a variety of covalent modifications, that include lysine acetylation, methylation, ubiquitination, and sumoylation, among others (119) (**Figure 1**). Acetylation and methylation are some of the most studied histone modifications so far. In general, acetylation of core histone tails leads to open chromatin structure to allow transcription and the histone deacetylases (HDACs) oppose the effects of histone acetylases and are predominantly transcriptional repressors (120). Histone methylation is more complex and can occur on a specific lysine or arginine residue. Depending on the residues being methylated and the number of methylation molecules added (each methylated lysine residue can exist in a mono-, di-, or tri-methylated state), histone methylation may be associated with either an active or a silent state of chromatin. For example, H3K27me3 is associated with transcription repression whereas, H3K4me3 is generally associated with active transcription.

Recent studies have highlighted antinociceptive effects of histone acetylation and lysine tri-methylation in inflammatory and neuropathic pain models (121, 122). In the partial sciatic nerve ligation model of neuropathic pain, an increase in the expression levels of monocyte chemoattractant protein-3 (MCP3), a pro-inflammatory cytokine was associated with reduced levels of repressive histone methylation, H3K27me3 (123). A role for histone acetylation has been suggested in the pathophysiology of visceral hypersensitivity induced by early-life stress in the MS animal model of IBS (124). Moloney et al. showed that HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), reversed visceral hypersensitivity, and the effects of stress on fecal pellet output in animal models of early life stress highlighting the importance of histone acetylation in stress-related conditions (125). Hong et al. demonstrated an increased expression of histone acetyltransferase EP300, which induced acetylation of histone H3 of promoter of nociceptive endovanilloid TRPV1 gene in the chronic WAS model of IBS. Moreover, they demonstrated that siRNA mediated knockdown of EP300 prevented visceral hyperalgesia (126).

Animal models suggest that neonatal inflammation may contribute to altered gut motility *via* histone modification. In rats subjected to neonatal inflammation, Vip levels increased, which reduced the interaction of histone deacetylase 3 (HDAC3) with α 1C-subunit of Cav1.2b channel (Cacna1c or α 1C1b). This

resulted in increased acetylation of histone H3 lysine 9 (H3K9) in the promoter region inducing the transcription of α 1C1b which may result in gut dysmotility and diarrhea (67). Similarly, neonatal immune challenge led to an upregulation of tyrosine hydroxylase in the locus coeruleus, mediated by epigenetic programming (127). The study showed a cascade of events involving upregulation of norepinephrine, activation of adrenergic receptors, and involvement of enhanced pCREB binding to the cAMP response element, which resulted in recruitment of histone acetyltransferase (HAT) to the brain derived neurotrophic factor (BDNF) gene. This led to an enhanced expression of the BDNF and aggravated visceromotor response to colorectal distension.

MicroRNA in Animal Models of IBS and IBS Patients

MiRNAs are endogenous noncoding RNAs of small size (18–25 nucleotides) that have been characterized as important gene expression regulators *via* binding through complementary sequence homology to the 3'-untranslated region (UTR) of target mRNAs thereby causing repression of translation or mRNA degradation (128) (**Figure 1**). Involvement of miRNA in cancer is well established and emerging research indicates a role of miRNA in the regulation of genes that play a role in nociceptive circuits (129). It has been suggested that miRNAs interacting with nervous and immune systems may act as “master switches” regulating a network of genes orchestrating the pain response and may be targeted for therapeutic purposes contrasting with the current strategy focusing on single targets (129). This approach is highly relevant to the GI tract where neuroimmune interactions are key contributors to the control of GI functions.

Recent translational studies in IBS have identified several miRNAs (**Table 2B**) that appear to be important in regulating the expression of genes involved in visceral pain response or intestinal permeability. In a study conducted in two independent cohorts of IBS-D women in the UK and Germany, there was an association between the c.*76G>A variant in the 3'UTR of the serotonin receptor 3 subunit gene (*HTR3E*), leading to increased expression of the 5HT3E subunit, and (131). Using luciferase assays, this variation was located in the binding element sequence of miR-510 suggesting a functional implication of the *HTR3E* variation in the ability of miR-510 to regulate its gene expression.

Fourie et al. investigated whether circulating miRNAs are differentially expressed in a small number of IBS patients compared to healthy controls (132). This study found an upregulation of miR-150 and miR-342-3p, which are involved in inflammatory (133) and pain pathways, in IBS patients compared to healthy controls (134). Subsequent studies from Zhou et al, using a miRNA microarray approach, revealed increased expression of miR-29a in blood microvesicles, small bowel and colonic biopsies from IBS-D patients compared to healthy controls, and it was associated with increased intestinal permeability (135). Glutamine synthetase was confirmed as a

TABLE 2A | Epigenetic changes associated with irritable bowel syndrome (IBS).

Functional category	Gene	Sample	IBS vs controls	Phenotype	PMID
DNA methylation					
Oxidative stress	Glutathione-S-transferases mu 5 (<i>GSTM5</i>)	PBMCs	Hyper-methylated	IBS-D	26670691
Neuronal genes	SCO-Spondin (<i>SSPO</i>)	PBMCs	Hyper-methylated	IBS; HAD [#] depression	26670691
	Tubulin polymerization promoting protein (<i>TPPP</i>)	PBMCs	Hyper-methylated	IBS-C	26670691
	SSX family member 2 interacting protein (<i>Ssx2ip</i>)	Colon of WAS [§]	Hyper-methylated	Visceral hypersensitivity	30106160
	Par-3 family cell polarity regulator (<i>Pard3</i>)	Colon of WAS [§]	Hyper-methylated	Visceral hypersensitivity	30106160
	Vinculin (<i>Vcl</i>)	Colon of WAS [§]	Hyper-methylated	Visceral hypersensitivity	30106160
	Glucocorticoid receptor (<i>Nr3c1</i>)	MS Amygdala/DRG [¶] neurons in WAS [§]	Hyper-methylated	Visceral hypersensitivity	25263804; 23084728
	Corticotropin-releasing factor (<i>Crf</i>)	Amygdala/DRG neurons in WAS [§]	Hypo-methylated	Visceral hypersensitivity	23084728
	Cannabinoid receptor 1 (<i>Cnr1</i>)	DRG [¶] neurons in WAS [§]	Hyper-methylated	Visceral hypersensitivity	25263804
Histone modifications					
Neuronal genes	Transient receptor potential cation channel subfamily V member 1 (<i>Trpv1</i>)	DRG [¶] neurons in WAS [§]	Increased histone (H3) acetylation	Visceral hypersensitivity	25263804
	Brain derived neurotrophic factor (<i>Bdnf</i>)	Neonatal inflammation	histone acetylase transferase (HAT)	Visceral sensitivity	28439935
Calcium channels	<i>Cacna1c</i>	Neonatal inflammation	Reduced interaction with histone deacetylase 3 (HDAC3)	Altered motility and diarrhea	23886858

Table 2A shows epigenetic changes, including DNA methylation and histone modifications associated with IBS or IBS models. [#]HAD, hospital anxiety depression scale; PMID, PubMed ID; [§]WAS, water avoidance stress; [¶]DRG, dorsal root ganglia; IBS-D, IBS diarrhea subtype; IBS-C, IBS constipation subtype; Hyper-methylation, increased methylation; Hypo-methylation, decreased methylation.

target of miR-29A and was significantly reduced in the small bowel mucosa in IBS patients suggesting a relationship between miR-29a, glutamine dependent signaling pathways and intestinal permeability in IBS patients. In a randomized placebo-controlled trial, glutamate was shown to safely and effectively reduce IBS symptoms in post-infection IBS-D patients with increased intestinal permeability (136). Subsequently, Zhou et al. showed increased levels of miR-29A/B and reduced expression of NFKB Repressing Factor (*NKRF*) and Claudin 1 (*CLDN1*) genes in intestinal tissue from IBS-D patients as well as TNBS colitis and WAS rat models of IBS (137). Additionally, they showed that miR-199a was significantly decreased in IBS-D patients compared to controls and an upregulation in animal models decreased visceral pain *via* inhibition of TRPV1 signaling.

Subsequently, the role of other miRNAs has been identified in IBS. CGN and *CLDN2*, associated with barrier function were shown to be the targets of hsa-miR-125b-5p and hsa-miR-16, which were downregulated in jejunal mucosal samples of IBS-D (138). Similarly, occludin (*OCLN*) and zonula occludens 1 (*ZO1/TJP1*), which are associated with intestinal permeability, were identified as direct targets of miR-144 in the colon of IBS-D rat models (139). In addition, the role of miRNAs in visceral hyperalgesia has been suggested by altered levels of miRNAs, including miR-200a which targets cannabinoid receptor 1 (*CNR1*) and serotonin transporter (*SERT*) (140), miR-214 which targets *SERT* (141), and miR-16 and miR-103 which target *HTR4* (142) in a rat model of IBS-D and human IBS-D colonic epithelial cells.

These studies have led to an increased understanding of the molecular mechanisms underlying some of the endophenotypes of IBS. Thus, they may be explored as diagnostic tools and have potential to form a basis for the therapeutic interventions being proposed in IBS (135). However, further studies examining their exact mechanisms in IBS and that can reproduce previous findings in a larger population are needed. These translational discoveries have prompted growing interest in miRNA-based therapy for IBS, although delivering drugs targeting miRNA to the intestinal tissue currently stands as a major obstacle and is being actively investigated (143).

Long Non-Coding RNAs in IBS

lncRNAs are transcripts that measure more than 200 nucleotides in length and are processed similar to protein-coding mRNAs (144). Although the functional mechanisms of most lncRNAs are not fully understood, they are known to exhibit diverse functional roles, including the gene regulation by chromatin remodeling, modulation of gene expression, localization, and stability (145) (**Figure 1**). Recently, Videlock et al. investigated the entire colonic mucosal transcriptome and found that a lncRNA, GREHLOS, which regulates the expression of motilin involved in smooth muscle contraction, was downregulated in IBS patients compared to healthy controls (130). Recently, increased expression of a lncRNA, X inactivate-specific transcript (*XIST*) was associated with decrease *SERT* transcription and increased visceral hypersensitivity in mouse

TABLE 2B | Non-coding RNAs associated with irritable bowel syndrome (IBS).

	Targets	Endophenotype	Sample	miRNA regulation in IBS/model	PMID
MicroRNAs					
miR-510	5-hydroxytryptamine receptor 3E(HTR3E), PRDX1	IBS-D	Colonic mucosa, and cells	Downregulated	18614545 26787495 31934286
miR-150 and miR-342-3p	Exploratory	Inflammatory and pain pathways	Whole blood	Upregulated	24768587
miR-199a	Transient receptor potential cation channel subfamily V member 1(TRPV1)	IBS-D, visceral pain	Colonic biopsies	Downregulated	25681400
miR-29a	Glutamate-ammonia ligase (GLUL), Aquaporin (AQP) 1, AQP3 and AQP8	Intestinal permeability	Colon and duodenum of IBS; colonic epithelial cells of IBS-D rat models	Upregulated	19951903 29156760
miR-16	HTR4 CLDN2	Intestinal sensitivity and motility; permeability	Colon of IBS-D; Jejunum of IBS-D	Downregulated	29089619 28082316
miR-103	5-hydroxytryptamine receptor 4 (HTR4)	Intestinal sensitivity and motility	Colon of IBS-D	Downregulated	29089619
miR-125b	Cingulin (CGN)	Permeability	Jejunum of IBS-D	Downregulated	28082316
miR-144	Occluding (OCLN), Zona Occludens1 (ZO1)	Intestinal permeability	Colon of BS-D rat model	Upregulated	29258088
miR-200a	Cannabinoid receptor 1(CNR1), Serotonin transporter (SERT)	Visceral hypersensitivity	Colon of IBS-D rat model	Upregulated	30347941
miR-24	SERT	Pain and nociception	Epithelial cells of colon and mouse model of IBS	Upregulated	26631964
LncRNAs					
GHRLOS	Motilin	Smooth muscle contraction	Colonic mucosa of IBS	Downregulated	Vidlock et al. (130)
XIST	SERT	Visceral hypersensitivity	Colon of mouse model of IBS	Upregulated	32446903

Table 2B shows epigenetic changes including miRNA and long non-coding RNA expression changes associated with IBS or IBS models. IBS-D, IBS diarrhea subtype.

model of IBS-D. The study suggested a role for XIST in recruiting DNA methyl transferases, DNMT1, DNMT3A, and DNMT3B to reduce SERT transcription *via* promoter methylation.

Microbiome and Diet as Environmental Factors Mediating Epigenetic Changes in IBS

Recent studies are starting to investigate an interaction of microbiome, diet, and epigenetics defined as “microbiota-nutrient metabolism-epigenetics axis” in complex diseases (146). Evidence suggests that epigenetic events are dynamic and responsive to changing nutrient availability and microbiome (104, 146, 147). Although the role of microbes and their metabolites on epigenetic machinery in the manifestation of IBS symptoms has not been investigated, there is indirect evidence for the role of microbial products involved in epigenetic modifications in eliciting visceral hypersensitivity (148). These interactions may be mediated by metabolites synthesized by commensal bacteria including neurotransmitters or short-chain fatty acids (SCFAs) (149). SCFAs, including butyrate, propionate, and acetate produced by the fermentation of host dietary polysaccharides, have neuroactive properties (150) and may play an important role in the brain-gut microbiome axis in IBS (151). SCFAs have been shown to regulate post-translational modifications of histones by inhibiting histone deacetylases, promoting active chromatin state and thereby promoting transcription (152, 153).

Nutrigenomics, the study of interaction of diet and genomic factors is an emerging topic in the context of IBS (154, 155). The majority of patients with IBS report meal-related symptoms and dietary modifications is an increasing treatment intervention used in IBS. For example, a low FODMAP (fermentable oligo-, di-, and mono-saccharides and polyols) diet has been associated with alleviation of IBS symptoms (156–157). Additionally, nutrition or diet can affect the epigenomic state. The role of diet in regulating epigenetic pathways is highlighted by a recent study, which showed that calorie restriction changes gene expression and DNA methylation profile of subcutaneous adipose tissue (147). It is suggested that diet and microbial metabolites influence the epigenome by impacting the pool of compounds or enzymes involved in epigenetic pathways (102). In particular, dietary components, co-factors, and vitamins including, S-adenosyl methionine (SAM), folate, vitamin B12, vitamin B6, acetyl-CoA have been shown to play a role in regulating histone modifications or DNA methylation levels (146). Therefore, investigating interactions between diet, microbiome and epigenetic factors may be important in understanding the etiology of IBS and developing personalized therapy for IBS.

MODEL FOR ETIOPATHOGENESIS OF IBS

Genetic, epigenetic, and other factors associated with IBS have been summarized in a schematic figure (**Figure 2**). IBS is a

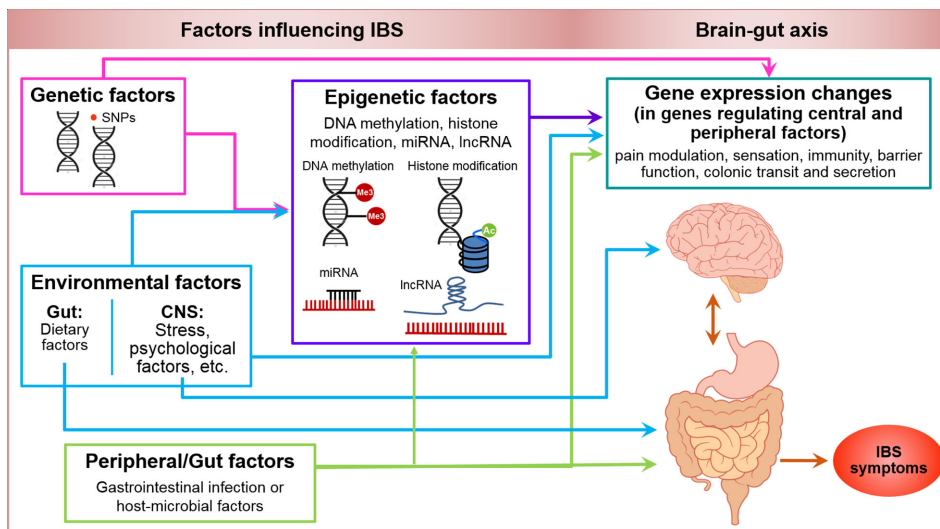


FIGURE 2 | Genetic, epigenetic, environmental and peripheral factors in irritable bowel syndrome. Shows a schematic model of genetic and epigenetic factors influencing IBS. Pink arrows illustrate that genetic factors including SNPs can influence the gene expression either directly or mediated by epigenetic factors including DNA methylation, histone modifications, miRNA and lncRNA expression (purple arrow). Environmental factors including stress and psychological factors at CNS level and dietary factors at gastrointestinal level can induce changes in gene expression mediated by epigenetic or non-genetic/epigenetic factors, and can have a direct influence on CNS and gut function (blue arrows). Peripheral or gut factors including GI infection or other host or microbial factors, can potentially modify the function of genes mediated by epigenetic or non-epigenetic factors, and influence the CNS and gut function (green arrows) such as, pain modulation, sensation, immunity, barrier function, colonic transit and secretion to manifest the symptoms of IBS (orange-red arrow).

multifactorial disorder of gut-brain interactions. In addition to stress, diet, and other environmental factors, changes at molecular level including genetic and epigenetic factors may contribute to pain modulation at the CNS level and/or periphery, and affect immune function, oxidative stress, mucosal barrier function, and GI motor and secretory function at the peripheral level in IBS. Moreover, gut microbiota and their metabolites likely contribute to this integrated system and play a major role in the pathogenesis of IBS. Given that IBS is a complex, multifactorial disorder, we propose that epigenomic mechanisms imprint dynamic environmental effects on the fixed genome resulting in alterations in phenotype leading to a disease state. These changes are potentially reversible and can be powerful diagnostic and prognostic markers and therapeutic targets (158).

CONCLUSION

Understanding the role of neuroimmune, genetic, epigenetic, and microbial underpinnings in IBS is crucial to understanding the pathophysiology of IBS. The mechanisms of visceral pain and neuro-motor dysfunction, resulting in the symptoms of IBS are influenced by several factors including stress, genetic, epigenetic as well as microbiota. An in-depth investigation of these factors independently, as well as integratively, in a sufficiently large,

well-characterized patient and control populations is crucial in understanding the etio-pathology of IBS and in identifying reliable and validated diagnostic biomarkers and therapeutic targets in IBS.

AUTHOR CONTRIBUTIONS

SM-J reviewed the literature and wrote the manuscript. LC reviewed the literature, wrote and edited the manuscript and provided the resources.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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