REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Defining Interactions Between the Genome, Epigenome, and the Environment in Inflammatory Bowel Disease: Progress and Prospects

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Recent advances in our understanding of the pathogenesis of inflammatory bowel disease (IBD) have highlighted the complex interplay between the genome, the epigenome, and the environment. Despite the exciting advances in genomics that have enabled the identification of over 200 susceptibility loci, these only account for a small proportion of the disease variance and the estimated heritability in IBD. It is likely that gene-environment (GxE) interactions contribute to "missing heritability" and these may act through epigenetic mechanisms. Several environmental factors, such as the microbiome, nutrition, and tobacco smoking, induce alterations in the epigenome of children and adults, which may impact disease susceptibility. Other mechanisms for GxE interactions are also directly pertinent in early life. We discuss a model in which environmental factors imprint disease risk in a window of susceptibility during infancy that may contribute to later disease onset, whereas other elements of the exposome act later in life and contribute directly to the pathogenesis and course of the disease. Understanding the mechanisms underlying GxE interactions may provide the basis for new therapeutic targets or preventative strategies for IBD.

Keywords: Environment; Epigenome; Genetics; Inflammatory Bowel Disease; Methylome.

The inflammatory bowel diseases (IBD), comprising Crohn's disease (CD), ulcerative colitis (UC), and IBD-unclassified,[1] have a combined prevalence of 0.5%–1% in Western Europe.[2] With incidence rates increasing rapidly in the developing world, these are now considered global diseases.[3] Current therapies are largely aimed at modifying the immune response because the present state of knowledge of the pathogenesis of IBD has not translated into prevention or cure. In reviewing progress in this field, we discuss that genetics only accounts for a limited proportion of the disease variance in IBD, and that gene-environmental (GxE) interactions, including epigenetic changes substantially underly IBD development and progression, and may account for the residual variance and "missing heritability" (Box 1). We explored the concept of the exposome, summarizing key evidence on GxE mechanisms implicated to date. In conclusion, we outline key areas for future research and propose a novel framework for advancing our knowledge in this vital area.

Genetics

Both genetic and environmental factors have been implicated in disease pathogenesis by epidemiologic observations, with early twin studies in Scandinavia,[4,5] providing the first impetus toward an extended phase of genetic discovery, which to date has linked more than 240 loci to disease susceptibility.[6,7] The landscape of polygenic susceptibility in IBD remains to be fully characterized and is likely to involve multiple genetic variants with unknown penetrance. Current estimates of disease variance explained by genetic loci defined in genome-wide association studies (GWAS) are 13.6% for CD and 7.5% for UC.[6] These figures are considerably lower than hereditability estimates derived from twin concordance data and other familial studies; concordance for IBD is higher in monozygotic twins (UC, 15%; CD, 30%) than in dizygotic twins (4% in both diseases).[8] The risk of IBD doubles for each first-degree relative with IBD and is further increased in relatives of patients who developed IBD as children (odds ratio [OR] for UC, 8.4; 95% confidence interval [CI], 6.4–10.9; OR for CD, 10.6; 95% CI, 8.2–13.5).[9] There are a number of possibilities to explain the discrepancy between the estimated heritability and the observed genetic contribution in GWAS,[10] including the presence of yet undefined additional common and rare loci (omigenic model), missing heritability explained by

Abbreviations used in this paper: **CD, Crohn's disease; CI, confidence interval; CpG, cytosine–guanine dinucleotide; GWAS, genome-wide association studies; GxE, gene-environment; HDACs, histone deacetylases; IBD, inflammatory bowel disease; IL, interleukin; LOF, loss of function; miRNA, micro RNA; MR, Mendelian randomization; OR, odds ratio; PUFA, polyunsaturated fatty acid; QTLs, quantitative trait loci; SCFA, short-chain fatty acid; SNPs, single-nucleotide polymorphisms; UC, ulcerative colitis.**

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Box 1. Glossary

DNA methylation: a methyl group is covalently transferred to the C5 position of a cytosine in a CpG.
Epigenetics: the study of mechanisms that record or affect altered gene activity without changing the underlying DNA sequence.
Exposome: the totality of environmental exposures an individual is exposed to from conception throughout life.
Genome-wide environmental interaction studies: studies looking for environmental exposures affecting phenotype dependent on an interaction with genotype and vice versa.
Heritability: the statistic that estimates the degree of variation in a phenotypic trait in a population that is due to genetic variation between individuals in that population.
Histone modifications: post-translational modifications to histone proteins that are responsible for the structure of the nucleosome.
Mendelian randomization: an analytical technique that uses genetic variants to estimate the causal effect of an exposure on phenotype.
miRNAs: short endogenous RNA that regulate gene expression.
Monogenic IBD: high (although typically not complete) penetrance of IBD caused by rare or ultrarare mono- or biallelic genetic variants in a single gene.
Polygenic IBD: classical IBD linked to multiple common and rare genetic variants that collectively contribute to disease risk.

gene-gene interactions that are currently not accounted for in sum score models, and epigenetic or environmental factors in utero, in early life, or later in life.

Monogenic conditions can present as phenocopies of CD and UC, more likely in patients with very early-onset–IBD, in particular when IBD onset is infantile and associated with inborn errors of immunty.[11,12] Monogenic disorders cause IBD susceptibility via inborn errors of immunity, such as regulatory T-cell immune dysregulation defects, defective antimicrobial responses in phagocytes, and epithelial defects,[13] enabling diagnostic pathways to facilitate personalized therapeutic approaches.[14] These conditions are extremely rare beyond infancy-onset disease but have major clinical impacts; most recent data demonstrate a convergence of pathways between monogenic and polygenic IBD.[15] Although tempting to speculate that environmental factors are less relevant in single-gene disorders with IBD, it is likely that environmental factors contribute to the incomplete penetrance. Animal models suggest that even some of the strongest colitogenic gene defects depend on the presence of a proinflammatory microbiome and germ-free mice are protected from intestinal inflammation.

Advanced epidemiologic tools, computational modeling, and laboratory tests allow for the deeper exploration of the genetic basis of disease and identification of new genetic associations. New frameworks have been implemented to understand the impact of genetic variants on IBD biology: single-cell response expression quantitative trait loci (QTLs),[16,17] transcriptome-wide association studies,[18] Mendelian randomization (MR) across multiple diseases,[19] and defining of regulatory noncoding single-nucleotide polymorphisms (SNPs) via interaction networks of proteins, micro RNAs (miRNAs), messenger RNAs, and transcription factors.[20] Other approaches include QTL analysis for features such as accessible chromatin QTLs, methylation QTLs,[21] splicing QTLs,[22] and microbial and metabolic QTLs,[23] and the discovery of GxE interactions via genome-wide environmental interaction studies.[24,25] Single-cell technologies have allowed specific molecular changes to be captured at a cellular basis—these methods are crucial for investigating functional differences between cell populations that would otherwise be missed when using multicellular samples, such as peripheral whole blood or tissue biopsies.[26] Technologies such as these have aided multi-omic approaches and computational strategies for the integration of information across many molecular layers.[27]

Even with these technological advancements and revised estimates of heritability from more recent twin studies, it is apparent that the majority of disease variance is associated with nongenetic factors and that the calculated heritability is not accounted for by the loci that GWAS have identified to date. GxE interactions are likely to contribute to the so-called "heritability gap," with epigenetic alterations as potential mediating links.[28]

Emerging Data to Define the Contribution of Epigenetics in the Pathogenesis

Epigenetics—literally "above" or "in addition to" genetics—is the study of mechanisms that influence transcription without modification to the genetic sequence. Epigenetic mechanisms include DNA methylation, histone modifications, and miRNAs, all of which may have evolved to allow plasticity of the biological response and phenotype in an individual, depending on the environment. In this context, the impact of environment-driven epigenetic mechanisms is the most plausible explanation to account for the dramatic increases in the incidence of pediatric IBD, which have been reported in Scotland,[29] Scandinavia,[30–32] Germany,[33,34] and North America,[35,36] as well as more recently in newly industrialized countries.[29] Many of the interindividual differences observed in IBD, including disease location, behavior, progression, age at onset, response to treatment, and extraintestinal manifestations, may partially be explained by the impact of different environmental exposures, either alone or in combination with genetic background. Parallels can be drawn between IBD and other complex diseases, such as type 2 diabetes, multiple sclerosis, and rheumatoid arthritis, which all exhibit both genomic and environmental bases for disease.[37]

DNA Methylation, Histone Modifications, and miRNA Synthesis

All epigenetic modifications, including DNA methylation, miRNA synthesis, and histone modifications, are dynamic regulators of gene expression (Figure 1). DNA methylation is the most widely studied mechanism and occurs when a methyl group is covalently transferred to the C5 position of cytosine in a cytosine–guanine dinucleotide (CpG) by a methyl transferase enzyme, with the resultant modified base termed 5-methylcytosine.[38] CpGs are found less frequently than would be expected by chance, probably due to a propensity for the spontaneous mutation of methylated cytosine to thymine, but density varies across genes, with dense regions ("CpG islands") frequently associated with gene promoters.[39] Classically, DNA methylation (especially at transcription start sites) is associated with the silencing of expression, as seen in genetic imprinting, but the relationship is more complex in IBD and other complex diseases in which smaller shifts in methylation at nontranscriptional start site CpGs, often outside of CpG islands, are seen.

Histones are involved in the regulation and packaging of DNA into chromatin. They are the central complex of a nucleosome subunit, which is formed by an octamer containing 2 copies of 4 histone proteins (H3, H4, H2A, and H2B) wrapped by a 147-bp segment of DNA.[40] The histone tails are then subjected to post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications are correlated with chromatin accessibility, with open chromatin (euchromatin) offering easier access to transcription machinery and increased transcription compared with closed chromatin (heterochromatin).[41]

miRNAs are short single-stranded RNA molecules averaging 22 nucleotides in length that act to regulate gene expression.[42] They can be found throughout the genome in both intergenic and intronic regions of both protein-coding and noncoding genes. MiRNAs are processed from a hairpin structure (pre-miRNA) cleaved from a primary transcript of approximately 3 kb.

Progress Toward Defining the IBD Epigenome

Epigenetic alterations studied in intestinal inflammation and inflammatory bowel disease include alterations in histone proteins, DNA, and RNA (including miRNA). A body of literature describes histone alterations in patients with IBD.[43] Moreover, disease-specific miRNAs have been described in depth in both colonic tissue and whole blood.[44] However, the most consistent progress has been made in relation to understanding DNA methylation changes in IBD. As previously observed in defining the genetics of IBD using genome scanning, the progress has been led by technological innovation and the growing availability of cost-effective microarrays to assess genome-wide methylation has led to extensive progress in the field of epigenetic studies in complex diseases (Figure 2). [21,45,46]

Data from epigenome-wide association studies of peripheral blood from patients with IBD have been subject to a recent systematic review and meta-analysis with consistent findings observed.[47] The data are most compelling in studies of newly diagnosed children and adults in Northern Europe in whom a highly reproducible signature has emerged.[21,45,46] One caveat to note, however, is the need to extend these studies into ethnically diverse populations. Notwithstanding this, these Northern European datasets suggest that the consistent alterations of DNA methylation in the circulation detected in patients with IBD are reflective of 4 overlapping categories. First, there are changes associated with markers of systemic inflammation or mucosal inflammation in active IBD, which have been best demonstrated in inception cohort studies[45] and may resolve with treatment.[48] Second, methylation changes associated with other pathways have been found, notably studies looking for biomarkers to predict disease course, progression, or response to specific biologics.[49] Third, methylation QTLs, wherein the methylation alteration level is associated with a local or distant genetic variant, have been described in IBD. Finally, and of particular interest in addressing pathogenesis and interaction with the exposome, there are methylation loci that have been implicated by causal inference testing. Most notably in formal MR analysis ribosomal protein S6 kinase 2 (*RPS6KA2*) hypomethylation has been implicated as having causal association.[48] This locus has been identified consistently as a differentially methylated site across multiple IBD whole blood studies.[47]

The stability, or conversely the plasticity, of the circulating methylome in patients with IBD over time is an important consideration.[50] Most recently, correlations between genome-wide methylation profiles were assessed in blood samples taken from individuals with IBD, with a median interval period of 7 years between samples. Although 60% of DNA methylation changes showed low stability over time, more than 40,000 markers showed good or excellent correlation, and 5% of markers showed highly stable methylation, with intraclass coefficients >0.90. The stable markers included the HLA region and other genomic regions that may potentially be modulated by environmental exposures.[50]

In parallel with studies in blood, there are initiatives to define the intestinal epigenome in IBD, both at the tissue and cellular levels. Data defining methylation changes in patients' intestinal epithelium[51–53] suggest that these remain stable over time in newly diagnosed pediatric patients[52] and in adults.[54] This is an area in which single-cell technology will have a clear advantage over bulk analysis. Organoids may provide an advantageous tool for assessing disease-relevant tissue, and they have been observed to mimic the phenotype and gene expression of human colonic epithelium.[55,56] Furthermore, these observed epigenetic changes are stable; they maintain epigenetic signals during cell culture and correlate with epigenetic marks found in whole blood, epithelial cells, and immune cells.[52]

Epigenetic Programming in Early Life

The development and homeostasis of the immune system is profoundly influenced by microbial imprinting during early life.[57] Although the relative importance of specific

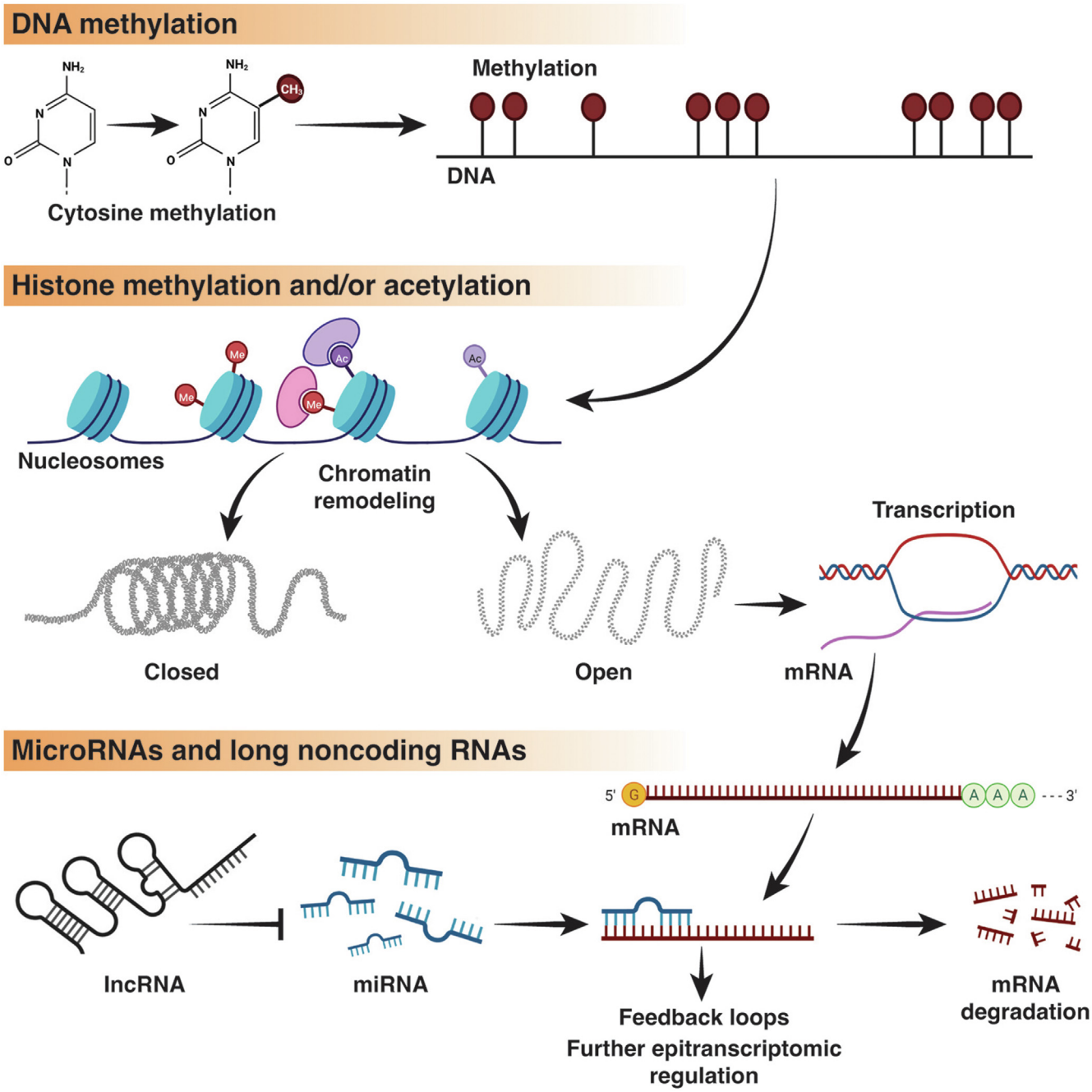

Figure 1. Epigenetic regulator mechanisms consist of DNA methylation, histone modifications (methylation/acetylation), and miRNAs and long-coding RNAs. They are heritable changes that do not alter DNA sequences. Each of these epigenetic regulators work in concert with one another and are crucial for development and differentiation of cell lineages. Created with BioRender.com.

genetic and environmental influences may differ between IBD occurring in the very young and adults, environmental exposures may influence disease risk immediately and with consequences later in life. This phenomenon known as "developmental origins of health and disease"[58] suggests that environmental exposures may have both immediate- and long-term consequences on disease risk. The most critical window for human early conditioning of disease is considered to be the first 1000 days of life (conception until 2 years of age).[59,60]

Epigenetic programming begins shortly after conception, and DNA methylation and histone modifications are reset during germ cell maturation and early embryonic development.[61,62] Some known exceptions include methylation persisting in some active retrotransposons[63] and imprinted loci. It is unclear whether epigenetic marks associated with IBD can be passed onto the next generation, or if these changes are only acquired through direct environmental exposures. Inheritance of epigenetic marks in humans is controversial and beyond the scope of this review, and

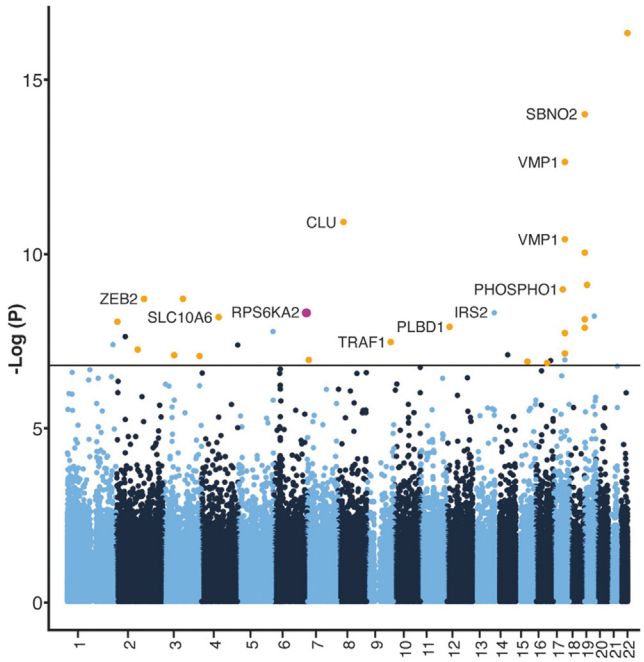

Figure 2. Manhattan plot of differentially methylated positions (DMPs) in patients with IBD vs controls. *Yellow dots* represent hits that replicate in the Scandinavian as well as the UK cohort and the *purple dot* represents hits that replicate in Northern Europe and Spain. Figure is reproduced from Kalla et al 2022.[55] Top hits include *SBNO2* (strawberry notch homolog 2), an *IL6*-regulated gene, *VMP1* (vacuole membrane protein 1), a key regulator of autophagy, and *RPS6KA2* (ribosomal protein S6 kinase 2), which has a diverse set of cellular processes including cell growth and proliferation. Created with BioRender.com.

evidence in both human and nonhuman models is inconclusive. Data from epidemiologic studies supports the hypothesis that certain environmental exposures early in life are more strongly associated with IBD risk.[64] For instance, early life shaping of the microbiome may modulate genetic effects on the immune system, leading to IBD pathogenesis.

Epigenetics and Aging

The concept of biological aging and the discrepancy between "biological" and "chronological" age are of great interest in health and disease. Global levels of DNA methylation gradually decrease with age,[65] but specific loci have been found throughout the methylome that are strongly correlated with age. Biological age has been found to exceed the actual age of patients with many inflammatory conditions, leading to a phenomenon called "age acceleration."

The Horvath epigenetic clock is one of several clocks available to estimate biological age in a mixture of human tissue and cell types at any age (prenatal to supercentenarians).[66] In a study of treatment-naïve IBD patients (IBD character: 678 participants, 63 aged 3–18), age acceleration (an increase in biological age relative to chronological age) was detected from whole blood samples in both CD (1.19 years) and UC (0.74 years) patients compared with non-IBD controls.[21] Further research is required to validate these findings and to determine if they are reversible or if there are any associations with disease course or treatment response.

Current Understanding of GxE Interactions: Defining the Exposome

A range of environmental exposures has been linked to IBD pathogenesis through epidemiologic and molecular studies, in particular the microbiome, diet, and tobacco smoking, with the totality of environmental exposures often referred to as the exposome.[67] The exposome is a complex construct that encompasses many exposures that fall into two potentially inter-related categories: internal exposures, including inflammation and the microbiome, and external exposures, including carcinogens (eg, environmental pollution), lifestyle factors (eg, diet and exercise), and sociodemographic factors (eg, education).[68] In comparison with the genome, the exposome is highly variable and dynamic over a lifetime; hence, new approaches are needed to understand its involvement in disease.[67]

Further research is required to link epidemiologic observations with plausible mechanistic effects to understand their role in the development of IBD. An increasing number of exposome factors have epidemiologic evidence to support their involvement in the development of IBD.[64] Vitamin D, physical activity, the hygiene hypothesis, and social determinants of health (eg, education, housing, and early childhood development) have also been found to have epigenetic effects.[69–73] Antibiotic use, breastfeeding, mode of delivery, and exposure to bacterial/viral infections may have indirect effects via impacts on the microbiome. An important issue is how epigenetic mechanisms facilitate effects on the pathogenesis of IBD. Vieujean et al[74] have performed a comprehensive review of the evidence for epigenetic modifications induced by the extended exposome in environmental models. We summarize recent data that provide insights into the GxE mechanisms by dissecting epigenetic and nonepigenetic findings with a focus on early life exposures: diet and smoking. Although highlighting areas in which epigenetic interactions may be critical in GxE effects, we also identify the need for deeper mechanistic insights.

Early Life Exposures and the Risk of IBD

The establishment of a stable microbiome occurs early in life and then undergoes major adaptation during human infancy.[75] Two factors have been identified to be critical during microbial colonization: mode of birth and breastfeeding vs formula nutrition.[76] Furthermore, the timing of weaning is crucial for healthy imprinting, and antibiotic use during this infantile age window can increase susceptibility to inflammation and changes to the mature ecosystem.[77]

The interplay between host genetics and gut microbiome is complex. The underlying genetics can predispose an

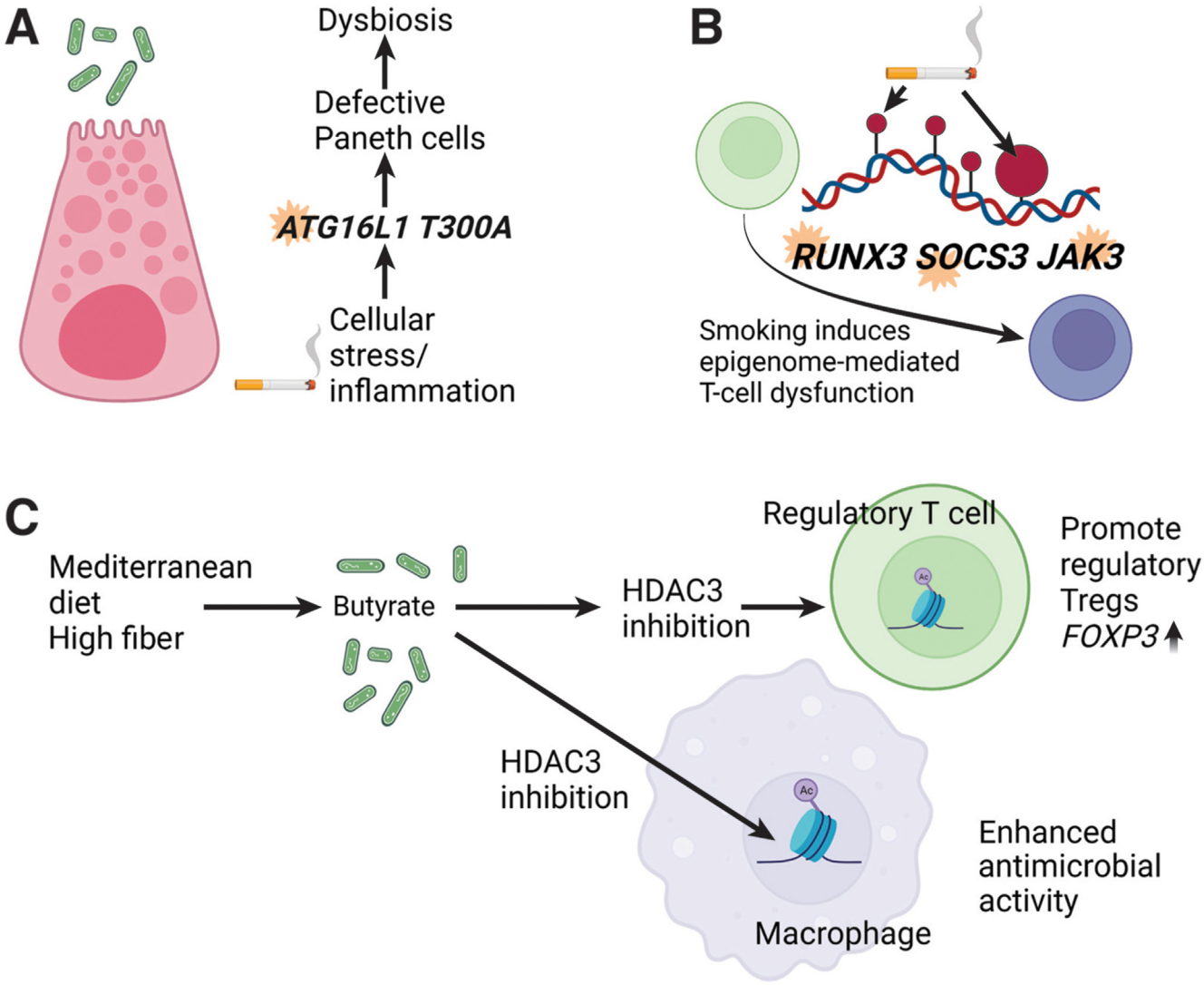

Figure 3. Selected epigenetic examples. (*A*) Genetic variance at the IBD susceptibility locus *ATG16L1* is associated with impaired autophagy in Paneth cells leading to the ineffective clearance of *Yersinia enterocolitica* when exposed to tobacco smoke. (*B*) In patients with IBD, tobacco smoke induces differential DNA methylation at key IBD susceptibility locus genes, which have functional implication on T-cell development. (*C*) Butyrate acts as an HDAC3 inhibitor impacting on regulatory T cells in human and mouse models and has antimicrobial activity via HDAC3 on metabolism as well as antimicrobial peptides. Created with BioRender.com.

individual with IBD to microbial dysbiosis,[78] in which there is reduced intestinal microbial diversity.[79] Many genetic variants associated with IBD have functional implications for the interaction between the immune system and the microbiome[80] (Supplementary Table 1). For example, nucleotide oligomerization domain 2 (*NOD2*) is a pathogen-sensing gene that can recognize bacterial peptidoglycans and trigger the induction of autophagy via autophagy-related 16-like 1 (*ATG16L1*).[81] *NOD2* and *ATG16L1* variants are strongly linked with CD.[82,83] Another pathogen recognition gene, caspase recruitment domain-containing protein 9 (*CARD9*), has several susceptibility loci for both CD and UC and is found to cause impaired immunoglobulin G responses in individuals with risk variants.[84] Although loss-of-function (LOF) variants confer protection from IBD, biallelic LOF variants cause susceptibility to infections caused by *Candida albicans*.[84] LOF variants in the interleukin (IL)-23 receptor (*IL23R*) protect against CD but predispose to microbial infections.[85,86] Consequently, blocking IL12/23p40 using ustekinumab and IL23p19 using Risankizumab has therapeutic efficacy in IBD.[87]

Furthermore, alterations to the microbiota have even been observed in healthy controls who have an increased genetic susceptibility to IBD (due to variants in *NOD2*, *CARD9*, and *ATG16L1*), specifically a reduction in the protective *Roseburia* species that converts acetate to butyrate.[88] Understanding the tipping point between susceptible individuals and individuals with the disease is valuable for future research into IBD pathogenesis. Many of these genes implicated in host-microbe interactions are also linked to

the response to other environmental exposures such as smoking (eg, *NOD2*, *IL10*, *IL23R*, and *ATG16L1*; Supplementary Table 1 and Figure 3*A*). This highlights the complex nature of GxE and the difficulty of understanding when investigated in isolation.

The microbiome can have both direct and indirect effects on the epigenetics of the host. The altered abundance of certain bacteria may elicit a response that causes alterations in the epigenome, which can then impact the immune homeostasis in the host. Intracellular pathogens, such as *Listeria monocytogenes*, have been shown to target a chromatin repressor gene, bromo adjacent homology domain containing 1 (*BAHD1)*, and activate interferon-stimulated genes.[89] A lower abundance ratio of *Bacteroides:Firmicutes* has been observed in patients with IBD. *Bacteroides vulgatus* can induce nuclear factor kappa light chain enhancer of activated B cells (NF-kB) and thus trigger proinflammatory gene expression in intestinal epithelial cells through the induction of histone deacetylases (HDACs).[90]

Epigenetic effects can also be caused by the actions of microbe-derived metabolites, such as short-chain fatty acids (SCFAs), gram-negative bacterial lipopolysaccharides, and products of amino acid metabolism (most notably tryptophan). SCFAs, such as butyrate, acetate, and propionate are produced by anaerobic fermentation of dietary fiber in the intestine.[91] Butyrate protects the intestinal barrier by nourishing colonocytes and has significant epigenetic effects. It inhibits HDACs impacting on gene expression and has anti-inflammatory effects via the nuclear factor kappa light chain enhancer of activated B cells pathway. *Faecalibacterium prausnitzii*, a prolific butyrate producer, is one of the strongest environmental protective factors against IBD,[92] which further supports the integral protective role butyrate plays in intestinal homeostasis.[93,94] Moreover, increased intestinal butyrate induces differentiation of macrophages with antimicrobial effects in the host via inhibition of *HDAC3*[95] (Figure 3*C*). Butyrate also promotes regulatory T cells in the colon by inhibiting *HDAC3* activity and increasing forkhead box P3 activity[69] (Figure 3*C*). A further study assessed whether supplementation with SCFAs is sufficient to mimic bacterial fermentation in mice. Although the magnitude of the effects was smaller in germ-free mice supplemented with SCFA, there was similarity in histone post-translational modifications (acetylated and methylated peptides) and transcriptional clustering profiles. There was also more than 50% overlap of differentially expressed genes compared with those of conventionally raised mice.[96]

Mode of Delivery at Birth

There is mixed evidence regarding the mode of delivery as a risk factor in both CD and UC.[97–99] Direct exposure to vaginal microbiota is crucial for the development of the microbiome, whereas the microbiota of infants delivered using Cesarean section resembles more closely their mothers' skin microbiome, with delayed establishment of diversity[100] and reduced abundance of bacteria from the *Bacteroidaceae* family and *Bacteroides* genus.[75,101] DNA methylation differences have been shown in cord blood samples from Cesarean and vaginal deliveries in key immune regulatory genes including CXXC finger protein 5 (*CXXC5)*, spi-1 proto oncogene (*SPI1)*, and serpin family B member 9 (*SERPINB9)*,[102] although these methylation changes may be a result of differences in cell type proportions in cord blood between vaginal and Cesarean deliveries.

The Role of Breastfeeding and IBD

Breastfeeding plays a protective role against the development of IBD. A meta-analysis including 2 cohort studies and 40 case-control studies confirmed a protective effect of breastfeeding,[64] with the greatest impact seen in Asia, Australia, and New Zealand.[64] A dose-dependent protective effect of breastfeeding has been observed in IBD;[103] infants who were breastfed for 12 months were protected against CD, whereas 3–6 months of breastfeeding was associated with protection against UC.[104] Breastfeeding is key for microbial colonization of the gut. Immunomodulatory factors in breast milk interact with the infant microbiome and can thereby mediate many of the health benefits associated with breastfeeding.[105] In animal models, the suckling period is a key time for epigenetic development of intestinal stem cells, with methylation changes in CpG islands correlated with transcriptional activity of glycosylation genes.[106] Bioactive compounds in breastmilk include miRNAs,[107,108] immunoglobulins, and antimicrobial peptides, which aid in the selection of commensals in the gut.[109] Therefore, breastfeeding is a highly complex environmental exposure that primes the organism to maintain intestinal immune homeostasis.

Early Life Exposure to Antibiotics

Exposure to antibiotics is correlated with an increased risk of IBD, both in early life and in later life.[110] In Swedish children the use of antibiotics was associated with an increased risk of IBD.[111] Further work assessing IBD subtypes has also found an increased risk in both CD and UC, with the most pronounced risk evident when antibiotics were received at younger than 18 years of age.[112] Antibiotic use in the first year of life was associated with an adjusted hazards ratio for IBD of 5.51.[113–115] There is also evidence to link prenatal exposure to antibiotics with IBD.[64,116] Furthermore, a study assessing very early–onset IBD found an increased risk due to exposure to antibiotics during pregnancy; however, it did not find the same association when exposure occurred in infancy.[117] Whether direct epigenetic effects of early life exposure to antibiotics exist in addition to effects on the evolving gut microflora requires further investigation.[74] Animal models have provided some evidence of miRNA dysfunction in T cells exposed to doxycycline, metronidazole, and isotretinoin.[118] Moreover, SCFAs are depleted after antibiotic treatment, which may also play a role in sustained T-cell–mediated dysfunction, resulting in susceptibility to infections.[119] Intriguingly, when butyrate is administered in parallel with antibiotic treatment, prevention of T-cell dysfunction is

observed, with enrichment for pathways of genes involved in histone and chromatin modifications, including DNA-methyltransferase 1 (*Dnmt1)*, bromodomain containing 3 *(Brd3),* chromatin assembly factor 1 subunit A *(Chaf1a)*, SIN3 transcription regulator family member A (*Sin3a)*, and Dpy-30 histone methyltransferase *(Dpy30)*.[119] Further work has shown that antibiotics are able to suppress tumorigenesis in an inflammation induced model (azoxymethane/dextran sodium sulfate-treated mice) via DNA methylation in the CpG islands of cerebellin 4 precursor (*Cbln4)*, FosB proto oncogene (*Fosb)*, and Msh homeobox 1 (*Msx1).*[120]

Tobacco Smoking and IBD: A Model for Understanding GxE Effects

Tobacco smoking has an impact on disease susceptibility and course in adult-onset IBD, but the effect is markedly different between patients with CD and patients with UC.[121] Smoking is a strong risk factor for CD that is associated with age at diagnosis, disease location,[122] increased disease severity, increased need for surgery, and occurrence of postoperative complications.[123] In contrast, tobacco smoke confers a protective effect on UC susceptibility and is associated with a milder disease course.[124] The prevalence of never-smokers among patients with CD is increasing in the West (27% in the UK and 11% in Sweden over the past 2 decades) as smoking rates decrease; however, this pattern is not observed globally (China has seen a 19% decrease in never-smokers), and smoking remains a risk factor for CD in newly industrialized and developing nations.[125] The risk of UC development is higher in former smokers.[126–128] In a recent South Korean–based population study, a dose-dependent relationship between the amount of smoking and duration before cessation was associated with a risk of UC development.[126]

Tobacco smoke contains thousands of chemicals, many of which are classified as carcinogenic or mutagenic compounds, resulting in several mechanisms of action, including dysfunction of the immune system in the gastrointestinal mucosa.[129] Carcinogenic components include polycyclic aromatic hydrocarbons, tobacco-specific N-nitrosamines, aldehydes, and aromatic amines. Polycyclic aromatic hydrocarbons, in particular, occur after the combustion process but can also be acquired in one's diet (foods cooked over charcoal).[130] Additionally, tobacco smoke impairs nitric oxide–mediated endothelial function through an increase in reactive oxygen species.[131] Reactive metabolites can cause DNA mutations due to DNA adducts, which then cause alterations in gene regulation.[130] Notably, one constituent of cigarettes is nicotine; although it is not carcinogenic itself, it is highly addictive and has been found to activate nicotinic acetylcholine receptors on cancer cells to promote tumor growth.[129]

Smoking provides an important area to dissect GxE interactions in IBD.[132] Direct interactions between IBD susceptibility genes, such as *NOD2* and *ATG16L1*, and smoking have been found (Figure 3*A*).[133] A proportion of SNPs, which are associated with IBD, have also been found to be modified by smoking behavior; 20 of these loci are located within the HLA region.[134] Six SNPs were associated with smoking quantity and behavior in CD. These variants were found to be correlated with a 3.5-fold increased risk of need for surgical procedures in smokers.[135] Furthermore, a study assessing global xenobiotic detoxification genes, which play a protective role against toxic agents, found 65 genes dysregulated in patients with UC compared with both CD and healthy controls.[136] This suggests that smoking modulates the expression of xenobiotic metabolizing enzymes in the colonic mucosa, which aids in normalizing gene dysregulation in UC and is essential to detoxification by xenobiotics.[136]

Epigenetic biomarkers are induced by tobacco smoke and have been well characterized since the introduction of genome-wide methylation analysis. Key inflammatory genes with aberrant DNA methylation in response to tobacco smoke also coincide with IBD susceptibility genes, notably runt-related transcription factor 3 (*RUNX3)*, suppressor of cytokine signaling 3 (*SOCS3)*, and Janus kinase 3 (*JAK3)*[137,138] (Figure 3*B*). The most pronounced methylation change and a biomarker for tobacco smoking is aryl hydrocarbon receptor repressor (*AHRR*). Methylation at this locus (Chr5: 373,378) is decreased in response to smoking,[139] with methylation levels in ex-smokers returning to the pattern seen in nonsmokers after 10 years in non-IBD populations.[139,140] *AHRR* is a negative feedback regulator of aryl hydrocarbon receptor (AhR) activity that competes with AhR for heterodimerization with aryl hydrocarbon receptor nuclear translocator *(ARNT)* and binding to the highly conserved enhancer sequences, termed xenobiotic response elements, which determine cytochrome P450 family 1 subfamily A polypeptide 1 *(CYP1A1)* expression.[141] *AHRR* also contributes to the maintenance of colonic intraepithelial lymphocytes, differentiation of Th17/Tc17, prevention of excessive IL1B production, and enhancement of interferon-gamma production within the inflamed gut.[142] A study assessing the causal effect of smoking on DNA methylation in patients with CD identified seven CpG sites. Two were within the genes B-cell Lymphoma 3 Protein *(BCL3)* and FKPB prolyl isomerase 5 *(FKBP5)* and were found to have an average causal mediation effect ($P < 0.05$).[143]

Maternal smoking during pregnancy is a known cause of adverse effects in offspring, with outcomes including cardiovascular disease,[144] preterm birth,[145] and low birth weight.[146] A recent meta-analysis of 9 studies found that the odds of developing IBD were higher among infants exposed to smoking during pregnancy compared with those who were not exposed (pooled OR, 1.49; 95% CI, 1.17–1.90).[64] Further work specifically examining DNA methylation combined several large birth cohorts and observed differentially methylated probes (P false discovery rate < 0.05) at IBD-associated risk genes in offspring exposed to tobacco, including miRNA 548f-3 (*miR-548F3*) and growth factor independent 1 (*GFI1*).[147]

The relationships between epigenetics, tobacco smoke, and IBD require further resolution; however, the effect of inflammation seems to be an integral aspect of this relationship. With current health policy and legislation changes,

the global effect of the decrease in tobacco smoking is likely to become an intriguing natural experiment, with impacts on IBD incidence, the balance between CD and UC, and disease course and treatment. It will be important to rigorously monitor how changing global patterns of tobacco use correlate with changes in IBD incidence and phenotype of intestinal inflammation.

Interaction Between Dietary Intake, Nutrition, and Genetic Susceptibility

Diet is a key modulator of the microbiome; it also directly impacts intestinal barrier function and the immune system, with likely downstream consequences for IBD development. Westernization of the diet is thought to be a major driver of the increasing prevalence of IBD in both developing and developed nations.[148] A variety of dietary compounds have been associated with IBD risk through human studies and animal models, most notably food additives, such as emulsifiers[149] and artificial sweeteners,[150] or as protective factors, such as the Mediterranean diet[151] or high fiber consumption.[152] As a consequence, the concept of precision nutrition has emerged,[153–155] and nutrigenomics has developed to understand the interplay between diet and underlying genetics, which is crucial for gut health.

However, diet provides a complex GxE interaction to untangle. Factors such as strong behavior effects and dietary changes over the course of one's life are important considerations for GxE human studies.[156] In addition the real practical difficulties in the rigorous and scientific assessment of dietary intake provide potential confounders in interpreting clinical trial data. Significant progress is being made with a number of trials reporting in recent years and the emergence of validated food indices.[157]

One major component of the Western diet is enrichment with polyunsaturated fatty acids (PUFAs). PUFAs can be divided into 2 families, omega-3 (ω-3) and omega-6 (ω-6); ω-3 has been found to be protective for IBD, whereas ω-6 is associated with an increased risk of IBD.[158] A study of newly diagnosed pediatric CD patients searched for SNPs within 3 key metabolic genes: fatty acid desaturase 1 *(FADS1)*, fatty acid desaturase 2 (*FADS2)*, and cytochrome P450 family 4 subfamily F member 2 (*CYP4F3)*. Children who consumed a higher dietary ratio of ω6:ω3 were susceptible to CD if they were also carriers of variants found within *CYP4F3* and *FADS2*.[159] A recent MR study of 24,000 patients with IBD found a protective role for higher levels of ω-3 fatty acids in IBD.[160]

Linking the role of epigenetic mechanisms and dietary intake in IBD currently lacks human data;[161] thus, we rely on animal/experimental models to gain further insight into this field.[74] Germ-free mice provide insight into interactions between diet and the microbiome. In one study, the microbiota mediated both the protective effect of psyllium fiber against colitis and the colitogenic effects of increased protein supply.[162] High protein might induce proteolytic activity of the microbiota, which is high in the feces of patients before UC onset.[163] Direct effects of nutrients on immunometabolism are also at play.[164] In addition, azo dye food colorants (Red 40 and Yellow 6) induced colitis in mice overexpressing IL23 via commensal bacteria capable of metabolizing these compounds.[165]

Maternal PUFA intake during both pregnancy and lactation can reduce fads1 expression and stably differentiate methylation at the same locus in rat offspring. In comparison, adult nonpregnant rats exhibited transient responses to PUFAs, with an increase in methylation at these same sites; however, methylation changes did not persist after a decrease in high-fat diet,[166] highlighting the role of early priming and reversibility changes later in life.

Looking Forward: Defining Strategies for Assessing GxE Interactions

Advancing our understanding of the true effect of environmental exposures in the development of IBD is now a priority area for research. This will require investment into strategic investigation in related diverse fields: basic science and mechanistic laboratory investigations of pathophysiology as well as detailed epidemiologic studies. In population-based studies, well-powered GxE studies in new-

Box 2. Overview: Key Research Objectives

Epidemiologic studies: target large-scale association studies that incorporate patients with IBD within diverse population groups globally and discover rare and polygenic variants that have impacts

- Unmet need—universal consensus on key data that should be collected by biobanks or electronic medical records to better define environmental risks

Basic science: define the mechanisms of GxE interactions

- Unmet need—application of new technological advancements, particularly in single-cell capabilities, to uncover the epigenetic landscape of specific cell populations and allow for investigation into cell-specific exposomal effects

Translational science: understand how the exposome impacts response to IBD treatment

- Unmet need—integration of exposome analysis into clinical trials of new therapeutics

Interventional studies: modify exposome factors (eg, diet and smoking) for genetically susceptible patients with IBD

- Unmet need—stratifying patient groups by genotype when examining exposomal studies

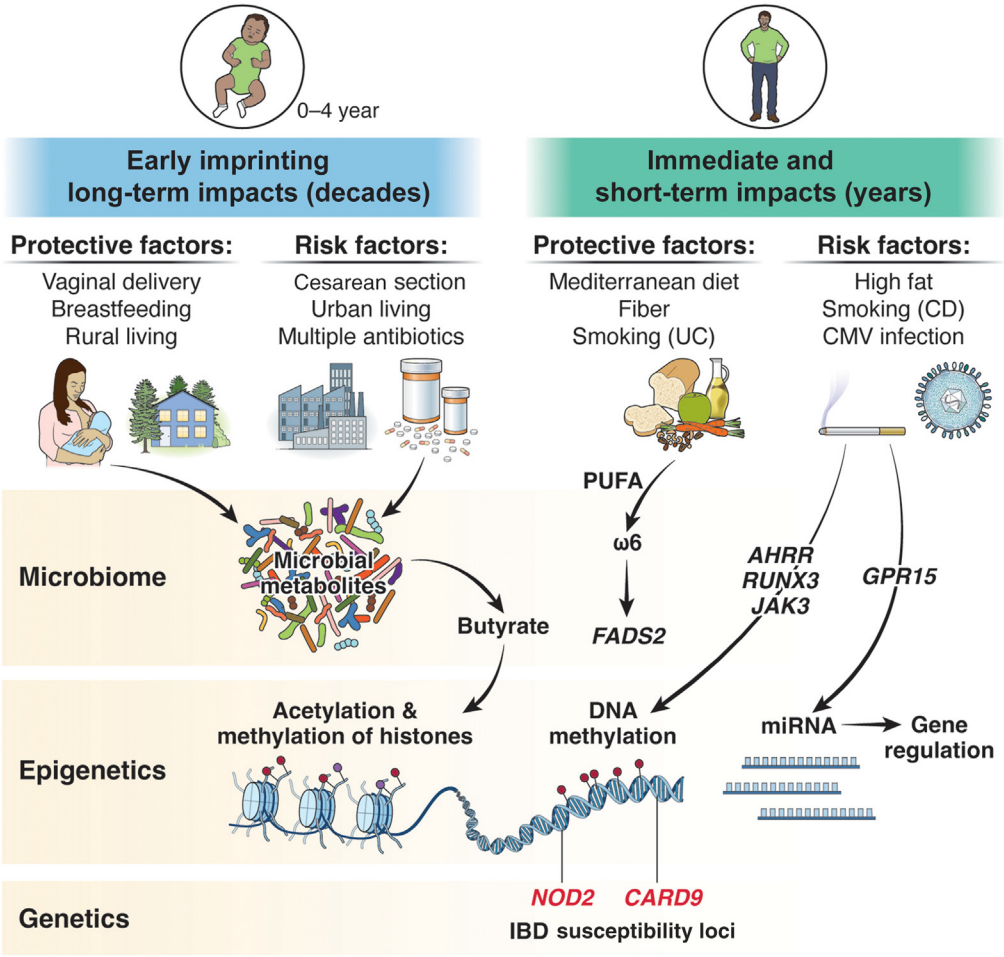

Figure 4. A summary of the layers of complexity observed in the pathogenesis of IBD and the influence of the environment of disease course. Early life exposures, including Cesarean section, urban living, and multiple antibiotics in the first 4 years of life, have been associated with an increased susceptibility to IBD. For adult-onset, acute exposures, high-fat diet, smoking (CD), and cytomegalovirus infection have been reported as potential risk factors for IBD development. Exposomal elements work both directly and indirectly on both the microbiome and epigenetic modifications (histones, DNA methylation, and miRNAs) to regulate gene expression. Underlying genetics underpin each layer of the interaction of the exposome and epigenetics. The overall management of IBD through lifestyle factors, including microbiome, diet, and smoking, can be moderated for better outcomes with a more personalized therapy targeted to both the underlying genetic susceptibility risk loci and epigenetic marks, which have been influenced by an individual's environment. Created with BioRender.com.

onset and established disease offer the potential to further our understanding. Because IBD has been thoroughly explored in GWAS, and large biobanks are available, structures are potentially available that would be capable of undertaking well-powered GxE interaction studies. The sample sizes required to detect GxE interactions are larger than those required for GWAS, although this can be offset to some extent by focusing on GWAS loci or variance QTLs, which have been shown to colocalize with GxE interactions, or using polygenic risk scores or lifestyle risk scores.[167,168] The UK Biobank (with more than half a million participants) and similar cohorts in Europe and North America are new tools for large-cohort studies that offer a pipeline to investigate GxE interactions and their relevance to chronic disease.[67]

Access to inception cohorts and cohorts of patients followed prior to diagnosis are particularly valuable in studies of the exposome and in defining diagnostic or prognostic biomarkers.[169] Two recent studies that included UK Biobank participants demonstrated the feasibility of this approach by examining the potential interactions between genetic risk and lifestyle factors in IBD. [25] Individual genetic susceptibility was estimated using known GWAS variants as a polygenic risk score, and lifestyle was categorized into either favorable, intermediate, or unfavorable categories based on 6 variables (smoking, body mass index, sleep duration, dietary intake, alcohol consumption, and exercise). Genetic and lifestyle factors were independently associated with CD and UC susceptibility and provided intriguing evidence that those individuals at higher genetic risk would be able to reduce their risk dramatically by adhering to a favorable lifestyle.[25] This analysis complements another study that used UK Biobank participants and aimed to identify environmental associations that could predict IBD

independently of genetics. Two GxE interactions were found between previously exposed high- and low-polygenic risk score individuals: smoking for UC and oral contraceptives for both IBD as a whole and UC independently.[24] Most recently, in the same cohort of patients, ultraprocessed food consumption was associated with an increased risk of CD, as well as an influence on disease course.[170] The access to genetic as well as lifestyle data in the UK Biobank and similar cohorts worldwide is of high importance because it allows for analysis into causality to be undertaken, using the technique of MR to link environmental exposure to disease susceptibility through genetics as an intermediate step.[171,172]

The increasing global incidence of IBD highlights the need for these large-scale studies to expand from Europe and North America to the developing world. Data assessing genetic risk in populations of European, East Asian, Indian, and Iranian descent identified 38 significant susceptibility loci.[7] This study highlighted the differential effect size of genetic loci in different populations, leading to the hypothesis that different environmental factors shape genetic susceptibility in different populations and, indeed, that environmental conditions may have population-specific effects. There is a real need for IBD exposome-related studies and assessment of GxE analyses to be carried out in a diverse range of ethnicities and geographic locations to understand disease development in distinct populations.

To truly achieve better power and precision in finding these associations in all populations, further recruitment of large and diverse patient groups into biobanks is needed. Larger study participant numbers are required (in particular in under-represented ethnic and age groups) to provide robust results in complex longitudinal analyses investigating multiple exposures and outcomes. Furthermore, standardization in the collection of clinical measurements and data access from biobanks is a major unmet need. Advancements in technology that can quantify environmental exposures may offer improvements in reporting exposures. Tools such as geographic information systems and mobile health solutions will offer alternatives to self-reporting exposures and electronic health records. Wearable devices that are able to sense surrounding exposures, such as heat, noise, and particle number count, in addition to exploiting the Global Positioning Satellites, are now being discussed to accurately measure personal exposures in urban environments.[173]

Summary

In conclusion, we propose a research strategy (Box 2) that can be implemented for future investigations to incorporate underlying genetic susceptibility, the exposome, and epigenetics (Figure 4). This agenda involves a multilayer investigation of complementary approaches, using basic, clinical, epidemiologic, translational, and interventional studies to understand in more detail the exposome and the potential for modification and translation (Box 2).

Research into GxE interactions and the disease-related exposome is now at an exciting point in time. There is great hope and grounds for optimism that the addition of new computational approaches and cutting-edge technology will allow the identification of underlying biological mechanisms to define causative factors in the exposome and lifestyle. In turn, this will provide a vital opportunity to reduce the disease burden both to individuals and populations. Many of these aspirations are consistent with the high-level objectives for progress defined by the James Lind Alliance and the patient support groups in Europe and North America, notably the understanding of the pathogenetic involvement of microbiome, diet and nutrition, and tobacco are within reach in the next decade.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2023.03.238.

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Supplementary Table 1. Genetic Susceptibility Loci Associated With IBD, Functional Inflammatory Roles, and Exposomal Interactions Including the Microbiome, Nutrition and Dietary Intake, and Tobacco Smoking

		Microbiome	References
Genetic			
CARD9	Pathogen recognition	Deficiency is associated with elevated risk of candidiasis and dermatophytosis infections	1
IL-2RA	IL receptor	Overactive *IL2* promotes microbiota-induced colitis	2
NCF4	Removal of pathogens in oxidative burst	Deficiency in *NCF4* weakens neutrophil defences against pathogens	3,4
PTPN2	Modulator of immune receptor proteins	*PTPN2* maintained intestinal barrier integrity, promoting microbial homeostasis	5
RORC	Th17 immunity	IBD-related *Eggerthella lenta* induces Th17 immunity in the intestine via RORC	6
GPR35	Chemokine signalling	Microbiota converts dietary tryptophan to kynurenine, which activates GPR35	7
Epigenetic			
HDAC3	Chromatin remodelling	Increased intestinal butyrate induces differentiation of macrophages with antimicrobial effects in the host via inhibition of *HDAC3*	8
BAHD1	Heterochromatin formation	Presence of *Listeria monocytogenes* have been found to target *BAHD1*	9
HDAC4	Chromatin remodelling	Induced expression after LPS exposure in macrophages	9
Nutrition and diet			
Genetic			
IL12B	Th1- and Th17-promoting cytokines	Polymorphism and interaction with alcohol intake and risk of UC in a cohort of individuals from Japan	10
CYP4F3	Fatty acid metabolism	Children who consumed a higher dietary ratio of $\omega 6/\omega 3$ were susceptible to CD if they were also carriers of variants found within *CYP4F3*	11
Epigenetic			
HDAC3	Intestinal homeostasis	Butyrate produced from microbes metabolizing dietary fibers suppress *HDAC3*	12
IL23	Maintenance of T helper cells	Overexpressing IL23 was found to induce colitis via commensal bacteria in response to food additives Red 40 and Yellow 6	13
Smoking			
Genetic			
ATG16L1	Autophagy	T300A variant found in CD patients developed Paneth cell defects triggered by tobacco smoke	14
CCL2	Chemokine	Cigarette smoke extract increases *CCL2* secretion from LPS-stimulated macrophages	15
IL23R	Antimicrobial defence	An interaction between variants and smoking behavior in CD	16
NOD2	Pathogen recognition	LOF variant interacts with smoking and shared phenotypes found in CD and smokers	17,18
Epigenetic			
RPS6KA2	Cell growth	Differential DNA methylation found within gene loci between IBD and controls, which is also associated with smoking	19,20
GPR15	T-cell trafficking	Increased expression levels found in patients with IBD who are smokers and in smokers in general	21
BCL3	NF-κB regulation	Mediation effects found between DNA methylation and smoking with CD	22
FKBP5	Immunoregulation	Mediation effects found between DNA methylation and smoking with CD	22
GFI1	Transcriptional repressor	*GFI1* was found to have a causal role after MR analysis of maternal smoking	23

LPS, lipopolysaccharide; NF-κB, nuclear factor kappa light chain enhancer of activated B cells.

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