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REVIEW

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Pharmacoepigenetic Biomarkers in Inflammatory Bowel Diseases: A Narrative Review

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Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, autoimmune disorder characterized by inflammation along the gastrointestinal tract. Global prevalence of the disease is increasing and patients often experience delays in diagnosis accessing effective therapy, highlighting an urgent need to develop a predictive biomarker for therapeutic response to reduce healthcare costs and disease burdens. Despite the advances to identifying genetic biomarkers for prediction of disease remission in IBD, patient responses vary widely, suggesting that inherited genetic variations alone cannot account for these differences. As autoimmune diseases like IBD are largely environmental in etiology, epigenetic modifications like DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) also have the potential to be candidates for predictive biomarkers of patient disease development and treatment response. This review will explore the novel field of pharmacoepigenetics and the development of predictive epigenetic biomarkers for treatment response in IBD, highlighting new research in the field. While research is still in the early stages, the studies reviewed have demonstrated that epigenetic profiling can be utilized to predict treatment response in IBD patients. Additional pharmacoepigenetic cohorts with more diverse participants could help enhance current models, improving predictability of treatment response and clinical outcomes. As research in this field progresses, epigenetic biomarkers should be integrated into the clinical environment to expedite diagnosis, reduce trial-and-error approach to treatment, and lay the foundations for individualized therapeutic strategies for IBD patients.

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Abbreviations: anti-TNF, Anti-tumor necrosis factor; AUC, Area under curve; CD, Crohn's disease; DEGs, Differentially expressed genes; DMPs, Differentially methylated positions; DMRs, Differentially methylated regions; DNAm, DNA methylation; IBD, Inflammatory Bowel Disease; IL, Interleukin; JAK inhibitors, Janus kinase inhibitors; miRNAs, MicroRNAs; ncRNAs, Non-coding RNAs; NR, Nonresponder; PBL, Peripheral blood leukocyte; R, Responder; SCFAs, Short chain fatty acids; SNPs, Single nucleotide polymorphisms; TNF-α, Tumor necrosis factor-alpha; UC, Ulcerative colitis; USTE, ustekinumab; VDZ, vedolizumab.

Keywords: Pharmacoepigenetics, Inflammatory Bowel Diseases, Crohn's Disease, Ulcerative Colitis, Histone Modifications, Therapeutic Outcomes, Treatment Response, Epigenetics, Biomarkers, Systems Biology, DNA Methylation, MicroRNA

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INTRODUCTION

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, autoimmune disorder defined by inflammation along the gastrointestinal tract. Inflammatory bowel disease pathology is characterized by disruptions in immune regulation in several cell types, including T cells, B cells, and macrophages, in addition to increasing the permeability of the gut-epithelial barrier, further exacerbating inflammation and tissue damage [1,2]. Studies have determined that individuals with IBD experience a significant reduction in quality of life compared to healthy individuals [3]. Chronic inflammation in IBD can cause severe complications like fistulas, strictures, and abscesses [4]. Over time, elevated levels of inflammation can increase the risk of malignancies, particularly colorectal cancer [5]. Therefore, early and effective management of IBD is critical to mitigate the risk of comorbidities and improve patient outcomes.

CD and UC affect an estimated 5 million people globally [6] and worldwide prevalence has increased from 1990 to 2021 [7]. Prevalence is elevated in certain regions including Europe, North America, and Oceania [8], however other regions, including East Asia, are seeing increasing disease burdens [9]. Interestingly, while recent trends show that highly industrialized regions currently have stable or even slightly declining incidence rates of IBD, developing and rapidly industrializing areas are showing a significant increase in IBD incidence [7,10].

While it's thought that both genetic and environmental factors are significant contributors in the etiology of IBD, the rapidly increasing prevalence and changing dynamics in epidemiology are evidence that environmental influence is crucial to the onset and progression of IBD. The global trend of westernized diets, characterized by increased consumption of highly processed foods, trans and saturated fats, and refined sugars has been linked to increased IBD risk [11] while daily consumption of fruits and vegetables was demonstrated to improve IBD outcomes [12]. Other common factors of industrialized or industrializing countries appear to be risk factors as well including air pollution [13] and other environmental toxicants including heavy metals, per- and polyfluorinated alkyl substances, and agricultural pesticides [14,15]. Other well-established risk factors include smoking [12,16] and early-life exposure to antibiotics [17].

Given the strong evidence supporting environmental contribution to IBD, epigenetic mechanisms likely mediate the interaction between the environment and gene expression to ultimately influence disease development. Epigenetics refers to molecular modifications such as DNA methylation (DNAm), histone modifications, chromatin structure, and non-coding RNAs (ncRNAs) such as microRNAs (miRNAs) that regulate gene expression without altering the DNA sequence itself [18]. These mechanisms act to alter gene expression levels in rapid response to changing environmental conditions [19]. Critically, epigenetic states are not static and fluctuate over the life course of an individual in response to positive and negative environmental influences such as diet [20], microbial composition [21], inflammatory states [22], and therapeutic interventions [23,24]. This temporal plasticity allows maladaptive epigenetic changes driving disease progression, but also offers opportunities for positive environmental and therapeutic influences to induce beneficial epigenetic modifications. For example, adverse environmental influences can induce epigenetic changes that lead to immune dysregulation, promoting inflammation, and influencing IBD susceptibility and exacerbation of disease [25,26]. While treatment itself, whether through dietary changes [27], corticosteroids [28], or biologic therapies [24] can also directly modify epigenetic states, establishing a bidirectional link between epigenetics and disease management.

Clinically, both CD and UC are similar in presenting symptoms which include abdominal pain, weight loss, fatigue, and diarrhea with or without blood. However, they can be differentiated through gross appearance from examination by endoscopy and histologic features [29]. Intestinal findings in CD include skip lesions and transmural inflammation [30], whereas findings in UC include partial thickness inflammation and continuous spread beginning at the rectum [31]. Despite these distinctions, patient symptom variability and overlap of clinical features with irritable bowel disease and food intolerances can complicate and significantly delay diagnosis [32,33], increasing the risk of complications and need for invasive surgical treatments [34].

Following diagnosis, most patients require consistent administration of anti-inflammatory treatments to achieve and maintain a state of remission. Most treatment regimens begin with anti-inflammatory medication such as corticosteroids which are used as a "bridge" for maintenance therapy [35]. Given the progressive nature of IBD and lack of a cure, most patients require multiple therapeutic adjustments throughout their life [36]. In some cases, surgery is the best form of treatment when maintenance therapy is not effective [37]. However, treatment response is highly variable between patients, with some individuals experiencing sustained remission for many years on immunosuppressive therapies while others develop resistance or adverse reactions to standard therapy [38]. The inconsistency in patient response necessitates investigation into personalized treatment strategies that consider patient factors beyond clinical presentation, including investigating epigenetic changes that may change

Table 1. Current Treatment Modalities in IBD

Drug Class	Examples	Mechanism of Action	Indications	Advantages	Limitations
Aminosalicylates	Mesalamine	Anti-inflammatory (targets the colonic mucosa)	Mild to moderate UC	Well tolerated, oral and rectal forms available	Ineffective in CD, limited impact on deep inflammation [39]
Corticosteroids	Prednisone, Budesonide	Broad immunosuppression	Acute flares of UC/CD	Rapid symptom relief	Not suitable for long-term use due to systemic side effects [41]
Thiopurines	Azathioprine, 6-MP	Purine analogs that suppress T-cell activation	Maintenance in UC/CD	Steroid-sparing effect, useful for long-term control	Slow onset, risk of hepatotoxicity and myelosuppression [42]
Methotrexate	Methotrexate	Folate antagonist, inhibits T-cell activation	CD maintenance	Alternative to thiopurines, particularly in CD	Teratogenic, risk of hepatotoxicity and bone marrow suppression [43]
Anti-TNF Biologics	Infliximab, Adalimumab	Inhibits TNF-α signaling to reduce inflammation	UC/CD, moderate to severe -Patients with high CRP, severe inflammation	Effective in steroid-refractory disease	Risk of loss of response due to anti-drug antibodies [44]
IL-12/23 Inhibitors	Ustekinumab	Blocks IL-12 & IL-23 pathways to modulate immune response	CD, biologic-refractory cases, IL-23 polymorphism carriers	Alternative to anti-TNF therapy	Expensive, requires weight-based dosing [103]
Integrin Inhibitors	Vedolizumab	Blocks leukocyte trafficking into the gut	UC/CD -Patients with low systemic inflammation	Gut-specific, lower systemic side effects	Slower onset of action compared to anti-TNFs [104]
JAK Inhibitors	Tofacitinib	Inhibits JAK-STAT signaling pathway	UC, moderate to severe	Oral option, rapid onset	Risk of thromboembolism, increased infection risk [43]
Emerging Therapies	Ozanimod	Sphingosine-1-phosphate receptor modulator	UC/CD, refractory cases	Novel mechanisms of action, promising trial data	Long-term safety and efficacy under investigation, potential concerns include cardiovascular risks, including bradycardia and hypertension, as well as the possibility of increased infection rates [49]
Emerging Therapies	Risankizumab	IL-23 inhibitor	UC/CD, refractory cases	Mechanisms of action, promising trial data	Long-term safety and efficacy under investigation, potential concerns risk of opportunistic infections and malignancies [49]

over the course of treatment regimens.

This review will explore the potential for epigenetic biomarkers and the novel field of pharmacoepigenomics to predict treatment response in IBD, highlighting new research in the field. By investigating the interactions between environmental risk factors, epigenetic regulation, and prediction of clinical outcomes, we can develop strategies for personalized treatment of IBD, improving patient outcomes.

CURRENT TREATMENTS IN INFLAMMATORY BOWEL DISEASE

Aminosalicylates, such as mesalamine, are commonly prescribed as the first-line treatment for mild to moderate UC. However, their efficacy in CD is limited, and they are not routinely recommended for its treatment [39,40]. These agents function by reducing inflammation in the gastrointestinal tract and are often well tolerated. In more severe cases, corticosteroids, including prednisone and budesonide, are employed to control inflammation and manage acute flares [41]. However, due to their significant side effect profile, their use is typically limited to short-term induction therapy rather than maintenance treatment.

Immunomodulators, such as azathioprine and methotrexate, are used as maintenance therapy for patients who fail to achieve remission with aminosalicylates or corticosteroids [42]. Thiopurines, including azathioprine and 6-mercaptopurine, are more commonly utilized in UC, whereas methotrexate is predominantly prescribed for CD. Recent studies suggest a shift towards combination therapy of thiopurines with biologics to optimize treatment outcomes and improve long-term remission rates. Table 1 summarizes the current treatment modalities, their mechanisms of action, and key considerations [43,44].

Despite the availability of these advanced therapies, access remains a significant challenge for many patients. Recent studies indicate that step therapy policies and financial constraints can lead to delays in initiating effective treatment, which is associated with increased rates of hospitalization, disease complications, and lower quality of life [44,45]. Data from recent analyses suggest that patients who face treatment delays due to insurance barriers experience a significantly higher risk of disease progression [45]. Strategies to mitigate these challenges include patient assistance programs, policy advocacy for reduced step therapy requirements, and broader insurance coverage for biosimilars, which have been shown to provide cost-effective alternatives while maintaining therapeutic efficacy. Step therapy policies, often referred to as "fail-first" policies, require patients to try and fail less expensive treatment options before gaining approval for biologics or JAK inhibitors [42]. While these policies are particularly prevalent in the US, they are also observed in socialized healthcare systems, where cost-containment measures restrict the use of high-cost treatments [46]. The financial burden associated with biologics and JAK inhibitors further complicates access, as these therapies can be prohibitively expensive for patients, insurers, and government healthcare programs [44]. The introduction of biosimilars has the potential to mitigate these financial barriers by providing lower-cost alternatives to existing biologic agents, potentially improving affordability and accessibility [47].

Delays in accessing effective therapies due to step therapy policies and financial constraints can have severe consequences for patients. Prolonged inflammation resulting from inadequate treatment can lead to disease progression, increased risk of complications, and a reduced quality of life [41]. In severe cases, delays in receiving appropriate treatment can contribute to increased morbidity and mortality among individuals with IBD [42]. Compounding these challenges, patient responses to biologic therapies vary widely. Some individuals experience primary non-response, where they fail to achieve remission upon initiation of therapy, while others develop secondary loss of response over time [43]. This variability is often attributed to the development of anti-drug antibodies, which neutralize the biologic agent and reduce its efficacy, as well as differences in disease pathophysiology among patients [44]. These factors underscore the need for predictive biomarkers to guide personalized therapy, ensuring that patients receive the most effective treatment while minimizing the risk of inadequate response or treatment failure. Therapeutic drug monitoring optimizes biologic therapy in IBD by measuring serum drug levels and anti-drug antibodies, allowing personalized dosing to enhance efficacy and minimize immunogenicity. Combining therapeutic drug monitoring with predictive biomarkers may help guide biologic switching strategies. For example, high anti-drug antibody levels in a patient on infliximab may warrant a switch to ustekinumab, while primary non-response to anti-tumor necrosis factor (anti-TNF) therapy might indicate a need for Interleukin (IL)-12/23 inhibition [48].

Emerging research has identified several promising biomarkers, including genetic polymorphisms, such as variations in TNF and IL23R genes, which may influence response to anti-TNF and IL-23 inhibitors. Additionally, specific serum proteins, such as C-reactive protein and fecal calprotectin, have been used to assess inflammation and predict treatment efficacy. Microbiome signatures are also being explored as potential predictors of therapeutic response, with studies suggesting that gut microbial composition may correlate with treatment success or failure. These biomarkers hold promise for optimizing biologic

selection and improving patient outcomes, although further validation studies are needed to integrate them into clinical practice. Identifying such biomarkers can assist clinicians in selecting the most appropriate biologic agent for individual patients, thereby enhancing therapeutic efficacy and minimizing adverse effects. Current research has explored various potential biomarkers to predict responses to specific biologic classes. For instance, the gut microbiome has been investigated for its role in influencing treatment outcomes. A study by Schierova et al. [49] examined the predictive value of gut microbiome signatures for therapy intensification in IBD patients, suggesting that certain microbial profiles may be associated with the need for escalated treatment. However, the study concluded that while the gut microbiome holds potential as a biomarker, further research is necessary to validate these findings and enhance their generalizability [49,50].

The role of fecal calprotectin as a non-invasive biomarker has been extensively studied. Calprotectin levels correlate with intestinal inflammation and monitoring its concentration can aid in assessing disease activity and predicting response to therapy. A review by Mosli et al. highlighted the utility of fecal calprotectin in guiding treatment decisions, noting its association with mucosal healing and clinical remission [51].

Recent studies have highlighted the role of epigenetic modifications, such as DNAm, in influencing drug response variability among patients with inflammatory diseases. For instance, methylation of the tumor necrosis factor-alpha (TNF-α) promoter region has been implicated in the regulation of TNF-α expression, a cytokine central to inflammatory processes [52]. Altered methylation patterns in this region can lead to either upregulation or downregulation of TNF-α production, thereby affecting the efficacy of anti-TNF therapies commonly used in conditions like rheumatoid arthritis and inflammatory bowel disease.

Despite these advancements, no single biomarker has yet fulfilled all criteria for reliably predicting response to any specific biologic treatment in IBD. A comprehensive review by Verstockt et al. [53] emphasized the complexity of IBD pathogenesis and the multifactorial nature of treatment response, indicating that a combination of biomarkers may be necessary to achieve accurate predictions [53,54].

Predictive biomarkers have the potential to transform IBD treatment by guiding personalized therapy selection. Incorporating biomarker-driven decision-making could optimize biologic use, reduce treatment failures, and improve long-term disease control, reduce the burden of trial-and-error treatment selection.

THE CASE FOR EPIGENETIC BIOMARKERS IN IBD

Several genetic variants, such as mutations in *NOD2*, *IL23R*, and *ATG16L1*, have been associated with IBD susceptibility, particularly in Northern European populations [55]. However, despite extensive research, genetic factors alone do not fully explain the variability in disease onset, progression, or treatment response, particularly in non-European cohorts [56-59]. This has led to growing interest in epigenetic mechanisms, such as DNAm, histone modifications, chromatin remodeling, and ncRNAs, which mediate gene-environment interactions and contribute to disease heterogeneity [60].

Beyond genetics, epigenetic modifications play a crucial role in the regulation of gene expression, immune function, and intestinal epithelial integrity. DNA methylation, a key epigenetic process, involves the addition of methyl groups to DNA. If the promoter or key regulatory regions of the gene are methylated, it can decrease the expression of the gene or shut down gene expression, effectively "turning off" the gene [61]. This process plays a critical role in modulating gene expression related to immune responses and epithelial function. For instance, hypermethylation of the *CLDN2* gene has been linked to increased intestinal permeability, and barrier dysfunction, a hallmark of UC pathology [60]. The *CLDN2* gene encodes claudin-2, a tight junction protein that regulates paracellular transport, and its hypermethylation results in decreased expression, compromising mucosal barrier function and increasing susceptibility to inflammation-driven epithelial damage [2,62].

Histone modifications, through acetylation and methylation of histone tails, alter chromatin structure and regulate the accessibility of transcription factor binding sites in DNA, thereby impacting gene expression [61]. They play a crucial role in regulating gene expression in IBD, with specific marks associated with either activation or repression of inflammatory pathways. H3K4me3 and H3K27ac, known as activating marks, are enriched at promoters and enhancers of pro-inflammatory genes, while H3K27me3 and H3K9me3 are repressive marks linked to silencing anti-inflammatory pathways [60,63]. Dysregulation of these marks has been observed in inflamed intestinal tissues. Although the exact contributions of histone modifications to IBD pathogenesis remain under active investigation, they represent a significant area of ongoing research [60].

ncRNAs, particularly miRNAs, are increasingly recognized for their regulatory role in post-transcriptional gene expression. In most cases, miRNAs bind to target mRNAs, preventing their translation into proteins [64]. Some miRNAs contribute to inflammatory responses and tissue repair mechanisms, highlighting their relevance in

Table 2. Genetic vs Epigenetic Contributions to IBD

Category	Specific Marker	Function	Clinical Relevance	Reference
Genetic Markers	NOD2	Regulates bacterial sensing, activates NF-κB	Associated with CD, predicts early disease onset but not treatment response	Jostins et al., 2012 [55]
	IL23R	Regulates Th17 cell differentiation	Linked to CD susceptibility, potential marker for ustekinumab response	Liu et al., 2015 [59]
	ATG16L1	Autophagy-related gene	Increased risk of CD, but no direct link to therapy response	Cleynen et al., 2016 [66]
Epigenetic Markers	TNF-α promoter methylation	Regulates TNF-α expression	Associated with anti-TNF response; hypomethylation correlates with therapy resistance	Somineni et al., 2019 [52]
	FOXP3 methylation	Modulates regulatory T cell function	High methylation linked to loss of immune tolerance, severe UC	Howell et al., 2018 [63]
	CLDN2 hypermethylation	Affects tight junction integrity	Increased methylation associated with UC, barrier dysfunction	Ventham et al., 2016 [60]
	MIR21 overexpression	Post-transcriptional regulator of immune genes	Upregulated in active UC, associated with corticosteroid resistance	Venkateswaran et al., 2023 [62]
	Histone H3K27 acetylation	Enhances inflammatory gene expression	Increased in IBD patients, marks active disease sites	Somineni et al., 2019 [52]

the complex network of IBD pathogenesis, and its upregulation in active UC has been strongly associated with corticosteroid resistance [62]. This overexpression leads to persistent immune activation and inflammation, preventing effective resolution of disease flares despite corticosteroid therapy.

For instance, miR-21 has been implicated in the regulation of immune responses and intestinal barrier function, with its overexpression associated with increased intestinal permeability and inflammation in IBD patients [62]. Additionally, miR-7-5p has been shown to target trefoil factor 3 (TFF3), a protein involved in mucosal healing, with elevated miR-7-5p levels leading to decreased *TFF3* expression and impaired mucosal repair in IBD [2].

FOXP3 methylation plays a crucial role in regulating regulatory T cell function, which is essential for maintaining immune homeostasis. Increased methylation of the *FOXP3* promoter leads to reduced expression of regulatory T cells, contributing to a loss of immune tolerance and the exacerbation of inflammation in UC [63]. This epigenetic alteration is particularly significant as regulatory T cells play a central role in suppressing excessive immune responses, and their dysfunction is associated with more severe disease phenotypes in IBD. Additionally, histone H3K27 acetylation, a marker of active chromatin and inflammatory gene expression, is significantly increased in IBD patients, further contributing to the chronic activation of pro-inflammatory pathways [52].

GENETIC VS EPIGENETIC CONTRIBUTIONS TO IBD

While genetic markers such as *NOD2* and *IL23R* polymorphisms indicate inherited susceptibility, they do not change over time and have limited diagnostic utility for guiding treatment, necessitating a deeper examination of epigenetic regulatory mechanisms. Epigenetic modifications are dynamic and reversible, offering a potential mechanism for precision medicine approaches in IBD. Epigenetic alterations, including DNA methylation, histone modifications, and miRNA expression changes, have been implicated in disease severity, immune cell function, and response to biologic therapy [63,65]. Table 2 contrasts genetic and epigenetic influences in IBD and highlights epigenetic biomarkers currently under investigation for disease stratification and treatment optimization.

The dynamic nature of epigenetic modifications suggests that epigenetic profiling could be leveraged for personalized IBD management, improving diagnostic accuracy and therapeutic decision-making. Pharmacogenetic research has demonstrated that genetic markers, such as *NOD2* variants, may predict non-response to anti-TNF therapy, but their predictive power remains limited [66].

Table 3. Analysis of Pharmacoepigenetic Studies in Inflammatory Bowel Disease

Study	Mishra et al., 2022 [69]	Lin et al., 2024 [24]	Joustra et al., 2025 [77]
Objectives	Identifying biomarkers for early prediction of therapeutic outcomes	Identifying biomarkers associated with anti-TNF drug concentrations	Identifying biomarkers of clinical response to VDZ and USTE
Treatments	Infliximab, adalimumab, or VDZ	Adalimumab and Infliximab	VDZ and USTE
Study population and sample size	Discovery cohort: n=14 Replication cohort: n=23	Adalimumab: n= 187 Infliximab: n=198	Amsterdam Discovery Cohort: VDZ n= 64, USTE n=62 Oxford validation cohort: VDZ n= 25, USTE n=33
CD or UC	Discovery cohort: 4 CD and 10 UC Replication cohort: 14 CD and 9 UC	All participants had CD	All participants had CD
Sex distribution %female	Discovery cohort: 50% Replication cohort: 47.8%	Adalimumab: 47.1% Infliximab: 56.1%	Amsterdam Discovery Cohort: VDZ: 50%, USTE: 68% Oxford validation cohort: VDZ: 36%, USTE: 55%
Average age	Discovery cohort: 38.6 Replication cohort: 37.1	Adalimumab: 37.2 Infliximab: 35.3	Amsterdam Discovery Cohort: VDZ - R: 36, NR: 28 USTE - R: 38, NR: 35 Oxford validation cohort: VDZ - R: 41, NR: 42 USTE - R: 43, NR: 30
Ethnic background of participants	Not explicitly stated. Discovery cohort was likely German and Replication cohort was likely Hungarian based on where the studies were conducted	Adalimumab: 94.1% White, 2.1% South Asian, 3.7% other Infliximab: 95% White, 2% South Asian, 3% Other	Amsterdam Discovery Cohort: VDZ - 75% White USTE - 76% White Oxford validation cohort: VDZ - 88% White USTE - 85% White
Sample type	Whole blood DNAm and RNA	Whole blood DNAm	PBL DNAm
Sampling period	RNA: Baseline, 4h, 24h, 72h, week 2, week 6, week 14 DNAm: Baseline, week 2, week 6	Baseline, week 14 week 30, week 54	Baseline and 6-9 months post treatment

Results	- 85,728 DMPs, 357 DMRs, and 3043 DEGs in remitters - 58,347 DMPs, 1163 DMRs and 389 DEGs in non-remitters - Methylation changes appeared more stable in remitting patients - No clear predictive signature for treatment failure at baseline, however week 2 DNAm and RNA response to treatment appeared to predict remission or treatment failure	- 4999 DMPs were identified between baseline and 14 weeks - 323 DMPs were associated with elevated drug concentrations (sign of positive response) - 125 DMPs could be correlated to patient clinical biomarkers - Very few (13) DMPs were found when comparing infliximab and adalimumab	- Machine learning could predict response with an area under the curve (AUC) of 0.87 for VDZ and 0.89 for USTE. - VDZ had a sensitivity of 0.769 and a specificity of 0.67 - USTE had a sensitivity of 0.73 and a specificity of 0.73 - The models developed in Amsterdam correctly predicted non-response in 88.9 % of VDZ NR and 92.3% of USTE NR
Limitations	- Small cohort and sample size - Lack of discussion of patient demographics like ethnic background - Combined CD and UC groups may prevent accurate DNAm patterns - Whole blood DNAm may confound results	- Outcome data may be improved with endoscopic outcomes - Whole blood DNAm may confound results - Patient background was grand majority European	-Lower statistical power in the USTE group - Approximately 70% of participants had less stringent response assessments as they could not receive endoscopies - PBLs are a heterogeneous cell population and subject to confounding

In contrast, epigenetic signatures may offer a more precise tool for therapy selection, particularly in the context of biologic response variability.

Multi-marker panels combining therapeutic drug monitoring and pharmacoepigenetics may further optimize biologic selection and dosing in IBD. For example, TNF promoter methylation levels correlate with anti-TNF efficacy, suggesting that epigenetic screening could help identify patients at risk of primary or secondary loss of response [48]. Similarly, studies have indicated that histone modifications and miRNA expression patterns may influence response to JAK inhibitors and integrin inhibitors, potential stratifying patients for optimal therapy [63,65]. With further validation, it could revolutionize IBD management by reducing the need for trial-and-error biologic selection.

PHARMACOEPIGENETICS FOR IBD

IBDs arise from a complex interplay of genetic predisposition and environmental factors. While genetic mutations contribute to disease susceptibility, they cannot fully explain why some individuals develop IBD while others remain unaffected. Moreover, treatment responses vary widely between patients, suggesting that inherited genetic variations alone cannot account for these differences [67]. These challenges highlight the limitations of pharmacogenetic studies, which primarily examine single nucleotide polymorphisms (SNPs) and other inherited factors to predict drug metabolism, efficacy, and toxicity [68]. Although this approach has been beneficial in conditions with strong genetic determinants, such as cancer and monogenic disorders, its predictive power in IBD is limited due to the significant role of environmental exposures, immune system dynamics, and gut microbiome interactions in shaping disease progression and treatment outcomes [45].

Recognizing these challenges, researchers have recently turned to pharmacoepigenetics as a promising alternative for understanding treatment response in IBD. Unlike genetic variations, which are static, epigenetic modifications are dynamic and reversible, meaning they can be influenced by external factors such as diet, infections, and inflammation [69]. By identifying epigenetic markers associated with drug response, pharmacoepigenetics offers a targeted approach to personalizing therapy in IBD patients [24]. Due to their ability to change in response to disease activity, epigenetic modifications may provide a more adaptable predictive model for treatment response in IBD compared to static SNPs [69].

The impact of commonly prescribed medications on the epigenetic profiles in IBD is a growing area of research. Disease modifying antirheumatic drugs like methotrexate and sulfasalazine, both commonly prescribed

as treatment for IBD, have been shown to interact with genetic and epigenetic factors, potentially affecting treatment outcomes and disease progression [70,71]. While such effects have been studied in rheumatic diseases like axial spondyloarthritis and rheumatoid arthritis, studies specific to IBD are lacking. Interestingly, metformin, a drug more commonly prescribed for type 2 diabetes, can also alter epigenetic states [72], which may explain the mechanism of how metformin can ameliorate IBD symptoms [73]. Metformin has been demonstrated to influence the activity of several epigenetic enzymes, including histone acetyltransferases, histone deacetylates, and DNA methyltransferases [72], resulting in a reversal of adverse epigenetic states and preservation of the epigenome [74,75]. These findings highlight the bidirectional relationship between pharmaceutical therapies and the epigenome: epigenetic states can influence drug efficacy, while some drugs can, in turn, alter epigenetic states.

Building on these principles, recent research suggests that DNAm patterns correlate with responsiveness to biologic therapies, particularly anti-TNF agents such as infliximab, adalimumab, and certolizumab pegol [24,69]. The ability to monitor epigenetic changes over time could enable more precise patient stratification for therapy selection [24], ensuring that patients receive the most effective treatment while minimizing unnecessary exposure to ineffective medications.

REVIEW OF RECENT PHARMACOEPIGENETIC STUDIES IN INFLAMMATORY BOWEL DISEASES

Several studies provide compelling evidence that epigenetics plays a role in predicting biologic therapy response and are summarized in Table 3. In one of the first studies of its kind, a longitudinal multi-omics study by Mishra et al. [69] demonstrated that early shifts in gene expression and DNAm correlated with clinical outcomes in patients undergoing infliximab, adalimumab, or vedolizumab therapy. A discovery cohort of IBD patients (n=14) undergoing first time treatment with TNF antagonists was used to identify potential biomarkers and epigenetic signatures associated with therapeutic response. Participants were tracked for a total of 14 weeks and therapy was given at baseline (just prior to starting therapy), week 2, week 6, and at week 14. A replication cohort of 23 IBD patients currently treated with vedolizumab (anti-α4β7 integrin antibody) was used to validate discovery cohort findings. Patient blood samples were collected at baseline at various time points during treatment for gene expression and DNAm analysis. The discovery and replication cohorts were used together to build a model using a machine learning with a random forest approach. Area under curve (AUC) and receiver-operating characteristic curve (ROC) curves were used to evaluate model accuracy. The study did not appear to identify a clear signature present at the baseline treatment that could predict success or failure of infliximab treatment. However, longitudinal analysis revealed that transcriptomic and epigenetic changes that occurred in week 2 after the drug was administered could predict therapeutic outcomes of clinical remission or non-responsiveness at week 14. Their findings suggest that epigenetic profiling could help identify responders and non-responders before treatment initiation, potentially reducing the trial-and-error approach in therapy selection [69].

The sample size of the Mishra et al. [69] study was small (n=14), and the primary results combined CD and UC patients within the same treatment groups. However, this study found distinct molecular signatures when comparing CD and UC patients, suggesting that biological mechanisms to TNF antagonist therapy may vary between the two conditions. And indeed, the authors state that when they separated CD and UC samples, models were better able to predict treatment response at baseline than when all IBD samples were combined. While a recent meta-analysis of newly diagnosed IBD patients did find that there is a significant overlap in methylation patterns between CD and UC, there were still some distinct epigenetic features that could differentiate the two conditions [76]. When possible, stratifying UC and CD into separate groups in pharmacoepigenomic studies may yield more accurate results. This approach can help identify disease-specific molecular signatures and improve the precision of therapeutic predictions for future studies.

Similarly, Lin et al. [24] investigated the role of DNAm as a biomarker to predict anti-TNF concentrations in patients with CD with the purpose of identifying which patients may benefit from dose optimization at the start of biologic therapy. The study utilized data from the Personalized Anti-TNF Therapy in Crohn's Disease (PANTS) study, a multi-center prospective cohort located in the UK that investigates the treatment failure rates of anti-TNF drugs including infliximab (Remicade or infliximab biosimilar CTP13) and adalimumab (Humira). Whole blood was collected from 385 participants for DNA methylation analysis from participants at baseline (prior to first dose), and weeks 14, 30, and 54 after the start of treatment. Only 87 participants provided samples at all four study visits. DNA methylation changes were analyzed using beta values, representing the proportion of methylation at individual CpG sites. Analysis considered confounding factors such as aging, smoking, and cell composition, which can influence DNAm. Epigenome-wide association study analyses were conducted using linear mixed effect models which included time on anti-TNF treatment as a fixed effect and random effect was used to assess individual-level effects. The models

adjusted for anti-TNF treatment type and cell composition within the sample.

Between baseline and week 14 of anti-TNF treatment, 4999 differentially methylated positions (DMPs) were identified and associated with 2376 genes. Many of the DMPs became progressively hypomethylated over time, indicating dynamic changes as treatment continued. From baseline to week 14, epigenome-wide association identified 323 DMPs associated with higher anti-TNF drug concentrations, an indication that the patient is receiving adequate drug exposure. Of the 323 DMPs associated with anti-TNF concentrations, 125 DMPs were also correlated with traits like body mass index and C-reactive protein levels.

Although these DMPs show potential as predictive biomarkers for treatment response, the effect sizes were relatively small, suggesting limited utility [24]. However, authors stated that the classification of remission vs non-response was mainly determined on clinical symptoms and CRP levels and that it is possible that utilizing better indicators of remission like endoscopic findings could better refine the data. Analysis between infliximab and adalimumab treatment groups was performed and no significant difference in DMPs was identified at baseline, however 13 DMPs were identified when investigating differences between the two groups post-treatment. Considering there were 4999 common changes between baseline and 14 weeks and only 13 DMPs identified that were different comparing infliximab and adalimumab, this could indicate class-specific effects of anti-TNF drugs. These results reinforce the potential of epigenetic modifications as indicators of treatment efficacy.

In an upcoming paper Joustra et al. identified peripheral blood DNA methylation signatures predictive of response to biologic treatments in patients with CD [77]. This study was a two-center, prospective cohort study that involved 184 adult male and female CD patients that were treated with either vedolizumab (Entyvio—α4β7 integrin antagonist) or ustekinumab (Stelara—IL-12 and IL-23 antagonist). Peripheral blood leukocyte (PBL) samples were collected from patients both before and during treatment. DNA methylation profiling was conducted on the samples to identify differential CpGs among responders and nonresponders utilizing a supervised machine learning approach. The authors used a gradient boosting model on datasets to identify CpG sites that consistently predict treatment response. Each CpG was further validated using permutation tests to avoid overfitting and reduce bias. Internal and external validation was performed and response-predicting models identified specific DNAm changes linked to drug response to these biologics with high accuracy, with AUC scores of 0.87 for vedolizumab and 0.89 for ustekinumab. There was lower statistical power in the ustekinumab group (64.3%) when compared to the vedolizumab group (98.4%), however the model still performed well in predicting patient response vs non-response status. The predictive model had a calculated sensitivity of 0.769 and a specificity of 0.67 for vedolizumab, alongside both a sensitivity and specificity of 0.73 for ustekinumab. When compared to pretest probability of response, the model increases response likelihood for classified patients as 20% and 24% higher for vedolizumab and ustekinumab respectively. The authors noted that due to the COVID-19 pandemic, access to non-essential endoscopies to check treatment progress was limited in 70% of participants. Investigators needed to use less stringent response assessments like inflammatory markers and clinical signs and symptoms to determine treatment response for most patients, similar to the Lin et al. [24] study. If endoscopic data is more available for future studies, it may better improve the accuracy of pharmacoepigenetic predictive biomarkers.

Furthermore, the findings by Joustra et al. indicate that the methylation patterns remained stable over time, but also signify that the markers identified are indicators of treatment response, but not amelioration of inflammation [77]. This is potentially beneficial as it suggests that epigenetic markers might provide a more reliable prediction of treatment response, unaffected by changes in inflammatory parameters. Overall, the study suggests that stable epigenetic markers could pave the way for personalized medicine in CD, potentially offering a more consistent predictor of treatment outcomes compared to SNP-based pharmacogenomics, which may be influenced by genetic variability and and are not predictive of environmental impacts across the lifespan.

Together, these studies highlight the growing role of pharmacoepigenetics in optimizing IBD treatment strategies. While pharmacogenetics has contributed valuable insights into drug response, its limitations in complex immune-mediated diseases like IBD underscore the need for more dynamic approaches such as pharmacoepigenetics. Epigenetic biomarkers provide an evolving framework for improving biologic therapy monitoring and refining personalized medicine in IBD. As research progresses, incorporating epigenetic analysis into clinical decision-making may offer a transformative step toward precision medicine, ultimately improving patient outcomes and reducing reliance on trial-and-error prescribing of biologic therapies.

FUTURE DIRECTIONS

While these studies provide a strong proof of concept that pharmacoepigenomics can be used to develop predictive models of treatment outcomes in IBD, further studies with independent cohorts are needed to validate the findings. Some important questions remain:

Are the DNA methylation patterns identified in these studies dynamic and influenced by aging, inflammatory levels, and severity of disease state or are they truly able to be utilized as predictors of treatment response? These points are not mutually exclusive and indeed, it is very possible that DNAm markers can be both responsive to biological impacts and also useful for prediction. Mishra et al. [69] were unable to identify a specific DNAm pattern to predict treatment response at baseline but were able to find distinct patterns associated with treatment response predictive of remission at two weeks post-treatment. This indicates that DNA patterns are changing in response to treatment, a finding replicated by Lin et al. [24] which found significant differences between methylation levels at baseline and 14 weeks. Joustra et al. [77] were able to determine that several DNA methylated regions were stable in their participants regardless of inflammatory levels and this aligned with a previous study from the same group [78], where certain DNAm markers were present and stable in IBD patients over a period of 7 years.

An investigation into long-term response is also required. Do these epigenetic markers correlate with sustained remission, or just short-term response levels? The Mishra et al. study [69] only investigated remission response into the 14th week after treatment and DNAm samples were only collected until the 6th week. However, Lin et al. [24] and Joustra et al. [77] did follow up until week 54 and 6-to-9 months post-treatment respectively. Further longitudinal investigation is required to determine whether these biomarkers can predict long-term treatment response, especially as many patients treated with biologic therapy can lose response over time [44].

All IBD pharmacoepigenetic studies assessed in depth were conducted in West and Central Europe on majority ethnically White European candidates [24,69,77]. It is important to determine if these results can be replicated in other populations outside of Europe, including non-Western populations. Combining these results in diverse cohorts can enhance the clinical utility of epigenetic biomarkers, leading to more personalized and effective treatment strategies for patients worldwide. This is an especially urgent need with the dramatic increase in IBD incidence in industrializing nations worldwide [7,10], where predictive models of treatment efficacy could have a significant impact on patient care.

An emerging area of interest in IBD pharmacoepigenetics research is the integration of microbiome-epigenome interactions. The gut microbiome has emerged as a critical environmental factor that can alter the epigenetic profile of an individual. Short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are produced by the microbiome and as a byproduct of nondigestible fiber fermentation [79]. Recent studies have demonstrated that SCFAs have the capacity to act as histone deacetylase inhibitors and alter DNA methylation, influencing gene expression in intestinal epithelial and immune cells [80,81]. The interplay of the gut microbiome and inflammatory levels within the gut is complex. Inflammatory states, like those seen in IBD patients, have been associated with gut dysbiosis, involving a decrease in beneficial SCFA-producing bacterial species [21,82]. On the other hand, gut dysbiosis is highly implicated in the development and exacerbation of inflammatory states [83], contributing to aberrant epigenetic states and potentially aggravating disease activity [21,84]. Of high relevance, the gut microbiome and SCFA metabolites have also been utilized as a predictor of biologic therapeutic response and prognosis [85]. Therefore, integrating epigenetic and microbiome data could explain some of the heterogeneity observed in treatment response and aid in developing personalized therapeutic strategies. Future pharmacoepigenetic studies should consider including microbiome profiling to assist in patient stratification and creating an epigenetic biomarker.

All studies assessed were conducted on whole blood [24,69] or peripheral blood leukocytes [77] which contain heterogeneous cell populations. As each cell type has its own epigenome, utilizing whole blood for epigenetic studies is not ideal as cell populations can differ from patient to patient based on inflammatory status, confounding epigenetic data [86]. Approaching pharmacoepigenetic studies by using cell sorting like Fluorescence-Activated Cell sorting (FACS) or cell separation with magnetic beads to isolate specific immune populations from whole blood may be preferable [87]. Additionally, newer technologies allow for single-cell epigenetic analysis like scBS-seq or scRRBS [88]. Unfortunately, both sorted cell populations and single cell epigenetic analysis are significantly more expensive protocols in comparison to whole blood bulk epigenetic analysis. If budget is not a major constraint, multi-omic techniques like scTrio or scNMT can be used to capture epigenomic and transcriptomic changes within a single cell [88]. A compromised approach for clinical utility could be to find potential biomarkers in large patient populations using whole blood or PBL bulk analysis and validate the top candidates using more expensive, but precise multi-omic techniques like for specific immune cell populations. A major weakness in the three studies assessed is that only DNA methylation data was analyzed, with only Mishra et al. [69] also incorporating transcriptomic data. While DNAm is one of the well characterized and therefore more easily interpreted epigenetic marker, DNA methylation, histone modifications, and ncRNAs all have been demonstrated to be involved in the etiology of IBD [89]. Accordingly, both miRNAs and histone modifications have been demonstrated to also show potential for predicting treatment response in other

conditions like rheumatoid arthritis [90], psoriasis [91], major depressive disorder [92], and cancer [93,94]. Furthermore, DNAm, histone modifications, and ncRNAs can work dynamically to regulate each other in many different biological processes, including inflammation [95]. Additionally, while this review previously mentioned the limitations of pharmacogenetics for assessing therapeutic response, it is important to note the influence of the genome on epigenetic states. For example, genetic variations like single nucleotide polymorphisms have been demonstrated to alter DNA methylation patterns through methylation quantitative trait loci (meQTLs), which have recently been evidenced to shape the methylome of pediatric IBD patients [96,97]. Future pharmacoepigenetic studies in IBD would benefit from investigating a broad range of epigenetic markers and should consider taking a multi-omic approach that integrates genomic, epigenomic, and transcriptomic processes within the study.

Finally, a critical aspect in the development of epigenetic biomarkers is the selection of appropriate modeling strategies. In contrast, the Lin et al. [24] study focused primarily on identifying differentially methylated positions associated with anti-TNF drug concentrations and treatment response through a linear mixed effect model approach. While the study did identify a large number of DMPs, many only had modest individual effects. Despite the relatively low individual impact, it is important to note that the identified DMPs showed enrichment in biological processes related to immune function, suggesting that the combined influence of the DMPs is likely significant and impactful. Future studies may benefit by concentrating on considering the overall pattern of epigenetic changes by identifying compound biomarkers or poly-epigenetic risk scores. When possible, multi-omic data should be gathered, including genomic, transcriptomic, proteomic, metabolomic, and microbiome profiles from IBD patients [98,99]. While multi-omic analysis is extremely complex, techniques such as elastic net regularization could be used to identify predictive features within the datasets. For example, elastic net-based techniques have previously been used to predict disease outcomes in multi-omic data [100] and prediction of interferon-beta treatment outcomes in multiple sclerosis patients [101]. Machine learning algorithms, such as random forests and support vector machines, could also be used to construct predictive models [99]. The Joustra et al. [77] study demonstrated this by utilizing supervised machine learning techniques to predict treatment response, enabling the integration of a highly complex DNAm dataset. Once validated, biomarkers could be translated into clinical tools, such as poly-epigenetic or poly-omic risk scores in order to guide treatment decisions [102].

CONCLUSIONS

Despite the advances to understanding the genetic basis of IBD, genetic susceptibility alone cannot explain patient variability in disease development, onset, and therapeutic response. As autoimmune diseases like IBD are largely environmental in etiology, epigenetic modifications like DNA methylation, histone modifications, and ncRNAs have the potential to be better candidates for predictive biomarkers of patient disease development and treatment response.

The dynamic and reversible nature of epigenetic modifications provides a potential avenue for personalized medicine in IBD. While research is still in the early stages, the studies reviewed have demonstrated that epigenetic profiling can utilized to predict treatment response in IBD patients. Additional pharmacoepigenetic cohorts with more diverse participants could help enhance current models, improving predictability and patient outcomes. As research in this field progresses, epigenetic biomarkers should be integrated into the clinical environment to expedite diagnosis, reduce trial-and-error approach to treatment, and lay the foundations for individualized therapeutic strategies for IBD patients. For epigenetic profiling to become a standard part of patient care, regulatory approval, updated clinical guidelines, and the development of cost-effective diagnostic and prognostic tools will be essential for implementation. As research into the pharmacoepigenetics of inflammatory bowel diseases continues, collaboration between researchers, clinicians, and policymakers will be essential to translating these epigenetic discoveries into real-world applications and improving outcomes for patients with inflammatory bowel diseases.

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