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Epigenetics in inflammatory bowel disease

Andreas C. Jenke[a] *and Matthias Zilbauer*[b]

Purpose of review
To briefly summarize some of the principles of epigenetics and assess their potential relevance for the disease pathogenesis of inflammatory bowel diseases (IBDs). To review the results of recent IBD-related epigenetic studies, discuss main challenges as well as highlight the opportunities for future research in this field.

Recent findings
Evidence is accumulating for a major role of epigenetic mechanisms in the disease pathogenesis of several immune-mediated diseases. Recent findings indicate that epigenetics may mediate some of the effects of environment, genetic predisposition and intestinal microbiota on IBD pathogenesis.

Summary
Epigenetics is a rapidly expanding and hugely promising area of research. At best, it may provide a unifying molecular mechanism to explain complex immune-mediated diseases such as IBD. Future research studies must be carefully designed, performed and analysed taking into account the basic principles of epigenetics in order to ensure the true potential of this field is realized in the understanding of IBD.

Keywords
DNA methylation, environmental factors, epigenetics, histone modification, inflammatory bowel diseases

INTRODUCTION

Over the past 50 years, the incidence of inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis has increased, both in children as well as in adults [1–3]. Yet, our understanding of disease pathogenesis remains incomplete. The most widely accepted general hypothesis to explain the development of IBD includes three main factors: genetic predisposition, environmental influences and the homeostasis between the intestinal microbiome and host immunity. The complex interaction of these factors is ultimately believed to cause chronic relapsing inflammation of the intestinal mucosal lining and the well described phenotypes.

Several key questions remain. What mechanisms mediate the effects of environmental factors? How could the microbiome cause and maintain chronic intestinal inflammation? How do the single-nucleotide polymorphisms (SNPs) – identified in recent genome-wide association studies (GWAS) – predispose to the development of IBD?

A fundamental key to all these questions could be provided by the rapidly evolving field of epigenetics.

Epigenetics can be defined as heritable changes to phenotype (e.g. gene expression) that are due to mechanisms other than changes to the underlying DNA sequence. These mechanisms operate at the interface between environmental stimuli and long-lasting molecular, cellular and behavioural phenotypes that are acquired during periods of developmental plasticity [4]. Importantly, such epigenetic marks are not completely erased during gametogenesis in mammals [5] but are transgenerationally heritable and might thus be an important missing link in explaining clearly heritable complex, but non-Mendelian, traits and diseases such as IBD. Additionally, unlike our genetic code, which remains stable throughout life, epigenetic profiles are influenced by exposure to environmental factors (e.g. smoke), diet or even behaviour [6]. When such environmentally induced epigenetic changes are passed on during cell division, they can ultimately

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KEY POINTS

- Epigenetics can be defined as heritable changes to phenotype (e.g. gene expression) that are due to mechanisms other than changes to the underlying DNA sequence and include DNA methylation and posttranslational histone modifications.
- Epigenetic mechanisms are highly influenced by environmental factors (e.g. diet and intestinal microbiota) particularly during periods of developmental plasticity; such induced epigenetic changes are potentially heritable leading to an environmentally acquired, altered phenotype in the offspring.
- Increasing evidence points towards a major role of epigenetic mechanisms in disease pathogenesis of complex traits and immune-mediated diseases. Despite the absence of direct evidence for the role of epigenetics in IBD, the biological plausibility of the concept is raising expectations that this field should provide significant advances for the understanding of IBD in the near future.
- Future studies aiming to investigate the epigenetic mechanisms in IBD have to consider several crucial aspects such as the requirement for isolation and purification of disease relevant cell types obtained from carefully selected patient cohorts.

result in a newly acquired phenotype. Given these potentially major implications, it is not surprising that there is now intense interest in the results emerging from within the field of epigenetics.

In this review, we first provide a brief introduction to the core principles underlying epigenetics. We then outline how epigenetics might help explain some of the remaining uncertainties in IBD pathogenesis, focusing particularly on genetic predisposition, the role of environmental factors and the intestinal microbiota. Finally, we discuss some of the challenges and opportunities that research into the epigenetics of IBD might deliver.

PRINCIPLES OF EPIGENETIC MECHANISMS

Waddington [7] first described the concept of epigenetics in 1942. He coined the word 'epigenetic landscape' as a portmanteau of genetics and epigenetics. Although at the time the role of genes in heredity was unknown, Waddington used his model to explain how genes might interact with their environment to produce a phenotype. To date, epigenetics is defined as heritable changes to phenotype that are caused by mechanisms other than changes in the underlying DNA sequence. The two most studied epigenetic mechanisms in mammals are histone modification (e.g. acetylation and methylation) and DNA methylation (Fig. 1 [8]) [9,10▪▪]. More recently, also small noncoding RNAs such as micro RNAs (miRNAs) have been found to operate as epigenetic factors; however, details are beyond the scope of this article and the authors refer the interested reader to some excellent recent reviews [11,12].

DNA methylation occurs almost exclusively on the 5′ position of the pyrimidine ring of cytosines in the context of CpG dinucleotides (5mC). This process is catalysed by DNA methyltransferases (DNMTs), which are responsible for both maintaining DNA methylation profiles (i.e. DNMT 1) and de-novo methylation (i.e. DNMT 3a/b) [13]. In principle, hypermethylation of regulatory genetic elements (e.g. 5′ promoter regions) is associated with silencing of the associated gene and vice versa. Although exact mechanisms remain ill defined, regulation of gene expression via DNA methylation is thought to occur through its effect on chromatin structure [14]. Chromatin is defined as the combination of DNA and proteins, particularly histones, which organize DNA into structural units called nucleosomes. Densely packed, transcriptionally silenced chromatin is referred to as heterochromatin. In contrast, euchromatin is lightly packed and favours active transcription of associated genes and genomic areas (Fig. 1a [8]). The chromatin state can be altered not only by DNA methylation, but also through posttranscriptional modifications (PTMs) of histones, a process that represents the second major epigenetic mechanism.

PTMs occur mainly in histone tails (N-termini) and include acetylation, methylation, phosphorylation and others [15,16▪]. These PTMs can alter the degree of chromatin compaction and ultimately create chromatin structures favourable either for active transcription (euchromatin) or repression (heterochromatin) of genes. For example, euchromatin is associated with high levels of acetylation and trimethylation of H3K4 (i.e. methylation of lysine 4 of histone 3), H3K36 and H3K79, whilst heterochromatin is characterized by low levels of acetylation and high levels of H3K9 and H3K27 methylation (Fig. 1b) [15,16▪]. It is becoming increasingly clear that epigenetic mechanisms are interconnected, such that DNA methylation can influence histone modifications and vice versa [17,18]. Importantly, DNA methylation and histone modifications have been shown to play a major role in several fundamental biological processes such as X-chromosome inactivation, silencing of repetitive elements, genomic imprinting, cellular differentiation, as well as regulating tissue and cell-type

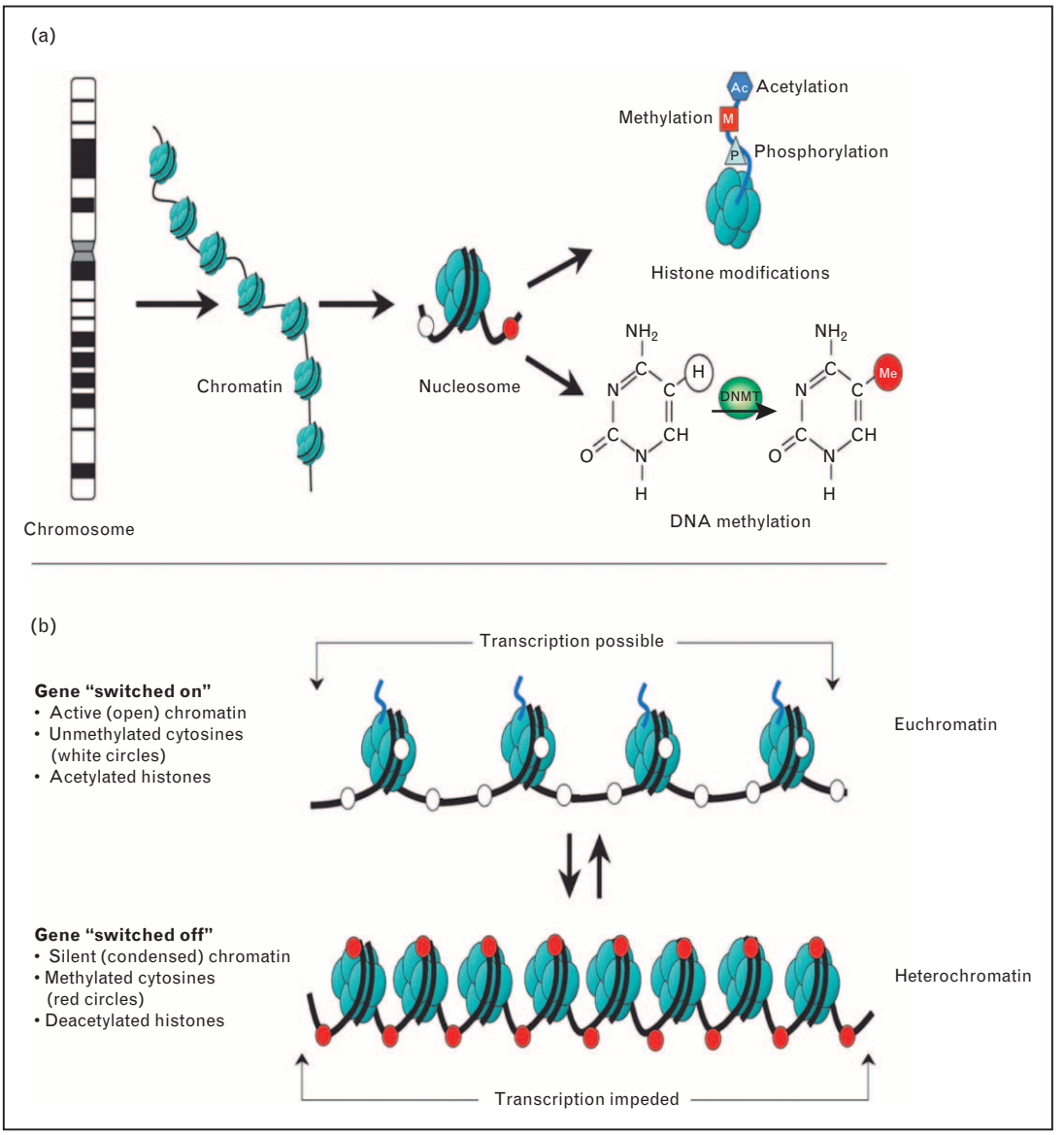

FIGURE 1. Epigenetic modifications, their effect on chromatin state and gene transcription. (a) DNA wrapped around histone octamers forming nucleosomes, which represent the principal component of chromatin. Reversible and site-specific posttranslational histone modifications occur at multiple sites through acetylation, methylation and phosphorylation. DNA methylation occurs at 5′ position of cytosine residues in a reaction catalysed by DNA methyltransferases (DNMTs). (b) DNA methylation and histone modifications regulate gene transcription through their effect on chromatin state. Densely packed, transcriptionally silenced chromatin is referred to as heterochromatin, whilst the lightly packed euchromatin favours active transcription. In principle, unmethylated DNA (white circles) as well as high levels of acetylated histones (e.g. histone 3, lysine 4 – H3K4) lead to euchromatin formation and active transcription of associated genes. Modified with permission from [8].

specific gene expression [10▪▪,19▪▪,20,21]. Particularly, the latter implies that in multicellular organisms epigenetic mechanisms play a key role in defining cellular phenotype. Additionally, unlike our genetic code, which remains stable throughout life (with the exception of some spontaneous somatic mutations), epigenetic profiles are more easily and rapidly influenced by and can respond to environmental factors. Such induced epigenetic changes can then be passed on during cell division, ultimately resulting in the adoption of a new permanent phenotype that can itself be further inherited.

INTERPLAY BETWEEN EPIGENETICS AND THE ENVIRONMENT

It is becoming increasingly accepted that the environment can have lasting effects on phenotype. Particularly in multifactorial or complex diseases, environmental triggers are thought to play a major

role in pathogenesis. Yet, except for very few examples (e.g. smoking and lung cancer), even large-scale epidemiological studies have struggled to identify individual environmental factors, or provide potential mechanisms, by which these factors cause disease. The same holds true for IBD. By providing mechanistic insights into how these factors induce disease, the study of epigenetics may eventually allow us to identify the causative environmental triggers themselves [22▪].

One of the most striking and best-studied examples of how environmental factors can influence the phenotype via epigenetic modification is seen in the agouti mouse model [23,24▪]. A specific gene locus (agouti viable yellow A^{vy} allele) in these genetically identical mice has been shown to be differentially methylated. Specifically, if unmethylated, the agouti gene is aberrantly expressed, leading to a yellow coat colour, obesity and diabetes. In response to a maternal methyl donor rich diet, the agouti gene becomes hypermethylated in the offspring and hence is not expressed [23,24▪]. This results in a normal phenotype, that is, brown coat colour, normal body weight and no diabetes. This example illustrates how early life environmental factors, such as maternal diet (e.g. adequate intake of folate, a crucial co-factor for DNA methylation, during pregnancy) can have a major impact on phenotype and disease susceptibility in subsequent generations, a concept also very relevant for the gastrointestinal tract. In fact, substantial evidence is already available indicating that epigenetic mechanisms are likely to mediate the impact of dietary components on the development of gastrointestinal malignancy [25,26], but little is currently known with regard to gastrointestinal inflammatory disease. However, a recent study by Schaible *et al.* [27] provides some early evidence for these mechanisms being relevant in the gut. The authors demonstrated in mice that a maternal methyl-donor rich diet left offspring more susceptible to dextran sulphate sodium (DSS)-induced colitis – an effect shown to be, at least in part, because of aberrant DNA methylation of several anti-inflammatory genes, including Cpn2.

Given these findings, one may hypothesize that significant changes to the human environment over the last decades (such as fast food diet), leading to an altered epigenome, might at least partly explain the rising incidence of IBD in our society.

EPIGENETICS ORCHESTRATING INTESTINAL HOMEOSTASIS

In addition to dietary antigens, the gastrointestinal mucosa is exposed to both commensal microflora and pathogenic organisms. The intestinal immune system is therefore constantly required to modulate its response to maintain tolerance or respond to pathogens. It increasingly appears that differential epigenetic signatures seem to play an important role in the orchestration of this homeostasis. For example, it has been shown that expression of the pattern recognition receptors Toll-like receptor 2 (TLR2) as well as TLR4 and its co-receptor MD2 in intestinal epithelial cells (IECs) is regulated by DNA methylation and histone deacetylation [28–30]. Interestingly, epigenetic signatures at these loci seem to be influenced by commensal flora. Takahashi *et al.* [31] found that in the large intestine of germ-free mice, TLR4 methylation levels were much lower than in their fully colonized wild-type counterparts. In a similar way, intestinal epithelial expression of the antimicrobial peptide human β-defensin 2 (hBD2) has been shown to be regulated by histone modification [32].

Taken together, these studies indicate that epigenetic marks seem to respond directly to the presence of the local microflora [32]. This molecular crosstalk and the resulting epigenetic modulation therefore provide a biologically plausible explanation as to why exposure to microbes during early infancy and childhood can protect from immune-mediated diseases such as IBD, a concept described in 'the hygiene hypothesis' [33]. In fact, recent studies performed by Richard Blumberg's group provide further compelling evidence supporting this hypothesis using an elegant mouse model. Specifically, the authors were able to show that accumulation of invariant natural killer T (iNKT) cells in the colonic lamina propria, along with the associated susceptibility to IBD, was markedly influenced by exposure to commensal bacteria early in life. The authors were able to demonstrate that expression of CXCL16 (chemokine receptor ligand) was regulated by DNA hydroxymethylation (a second form of DNA methylation also shown to regulate gene transcription), with epigenetic marks at this locus being dependent on early life exposure to bacteria [34▪]. These data not only further highlight the importance of commensal bacteria in regulating intestinal tolerance via epigenetic mechanisms, but also indicate that there are crucial time periods during which priming must occur.

EPIGENETICS, GENETIC RISK AND HERITABILITY

There is clear evidence for genetic involvement in the development of IBD and over the last decade, large-scale GWAS have successfully identified a

significant number of DNA polymorphisms and haplotypes that predispose to Crohn's disease, ulcerative colitis or both [35,36]. However, the effect of individual variants, as well as the cumulative effect of several variants, remain relatively small and are thought to account for only around 20–25% of all IBD cases. Additionally, in monozygotic twins, reported disease concordance rates are only 50% for Crohn's disease and 20% in ulcerative colitis [37–39]. Hence, it seems unlikely that DNA variants are able to fully explain the 'missing heritability' in IBD. Also, given the stability of the human and mammalian genome over the last century, genetic changes alone cannot explain the significant increases in the incidence of IBD. Importantly, the vast majority of identified disease-predisposing SNPs were located in noncoding regions of the genome.

Taken together, some of the key questions that remain unanswered include what are the mechanism(s) through which these genetic changes mediate the increased risk of disease? How can we explain the occurrence of sporadic disease, that is, missing heritability? What causes the accumulation of IBD in families or ethnic subgroups? Epigenetics may provide crucial clues to the answers.

Epigenetic model to explain sporadic and familial occurrence of inflammatory bowel disease

In principle, epigenetic signatures are completely erased during gametogenesis. However, if this process of epigenetic reprogramming is incomplete, environmentally induced phenotypes could potentially be passed on to future generations, a concept referred to as 'transgenerational epigenetic inheritance' [40▪]. Incomplete erasure of epigenetic marks in the germline could therefore provide an explanation to both sporadic as well as familial occurrence of IBD. Specifically, in sporadic disease an epigenetic mutation occurs in the germline causing disease in the offspring (Fig. 2a [41]). If erasure during gametogenesis is complete, such epigenetic mutations are eliminated and the disease will not be passed on to the next generation. However, if reprogramming is incomplete, repair fails and future generations continue to be affected leading to familial pattern of disease (Fig. 2b [41]). Exact mechanisms responsible for failed epigenetic reprogramming currently remain unknown, although aberrant chromatin configurations are likely to be involved. Additionally, if germline 'epimutations' continue to fail correction they may become more severe leading to what can be defined as 'epigenetic anticipation' with aggravated disease phenotypes occurring earlier in younger generations [42]. In summary, both the sporadic and familial occurrence of IBD could be explained by generic epigenetic molecular mechanisms – the outcome only differing in the efficacy of epigenetic reprogramming during gametogenesis and following fertilization.

Epigenetic mechanisms mediate the effect of disease predisposing genetic variants

Using a mouse model, Borgel *et al.* [43] demonstrated a direct link between genotype and epigenotype. Specifically, they found that certain DNA sequences were able to determine DNA methylation profiles of larger regions, which in turn were crucial in regulating cellular differentiation. Hence, theoretically, any SNP located in such a crucial, regulatory region may ultimately lead to changes in the gene expression via changes in DNA methylation. In fact, support for this is found in a study investigating the effect of GWAS-identified SNPs on methylation patterns in a cohort of patients with type 1 diabetes [44]. In addition, Ernst *et al.* [19▪▪] mapped chromatin state dynamics in nine different human cell types and identified tissue and cell-type specific enhancer regions or elements. Using these regulatory regions, the authors went on to demonstrate that top-scoring disease-associated SNPs were frequently positioned (i.e. enriched) within the enhancer elements of disease relevant cell types (e.g. SNPs associated with systemic lupus erythematosus were found in lymphoblastoid cells).

These findings highlight the close interaction between epigenetics and genetics in regulating cell-type-specific gene expression and hence their potential implication in mediating the effects of previously identified disease-predisposing SNPs.

EPIGENETICS IN INFLAMMATORY BOWEL DISEASE: PRESENT AND FUTURE

Over the past 20 years, research into the role of epigenetics in human disease has been largely restricted to mechanisms of malignancy. One of the main reasons for this lies in the fact that individual genes were implicated in oncogenesis (e.g. tumour suppressor genes) and hence their epigenetic regulation could be more easily investigated. In contrast, for complex, multifactorial diseases, a whole-genome approach is required, tools for which have only recently started to become available [45▪▪]. Promising results have already started to emerge from studies on several immune-mediated complex diseases, such as lupus erythematosus and rheumatoid arthritis [46–49]. Only a small number of recent

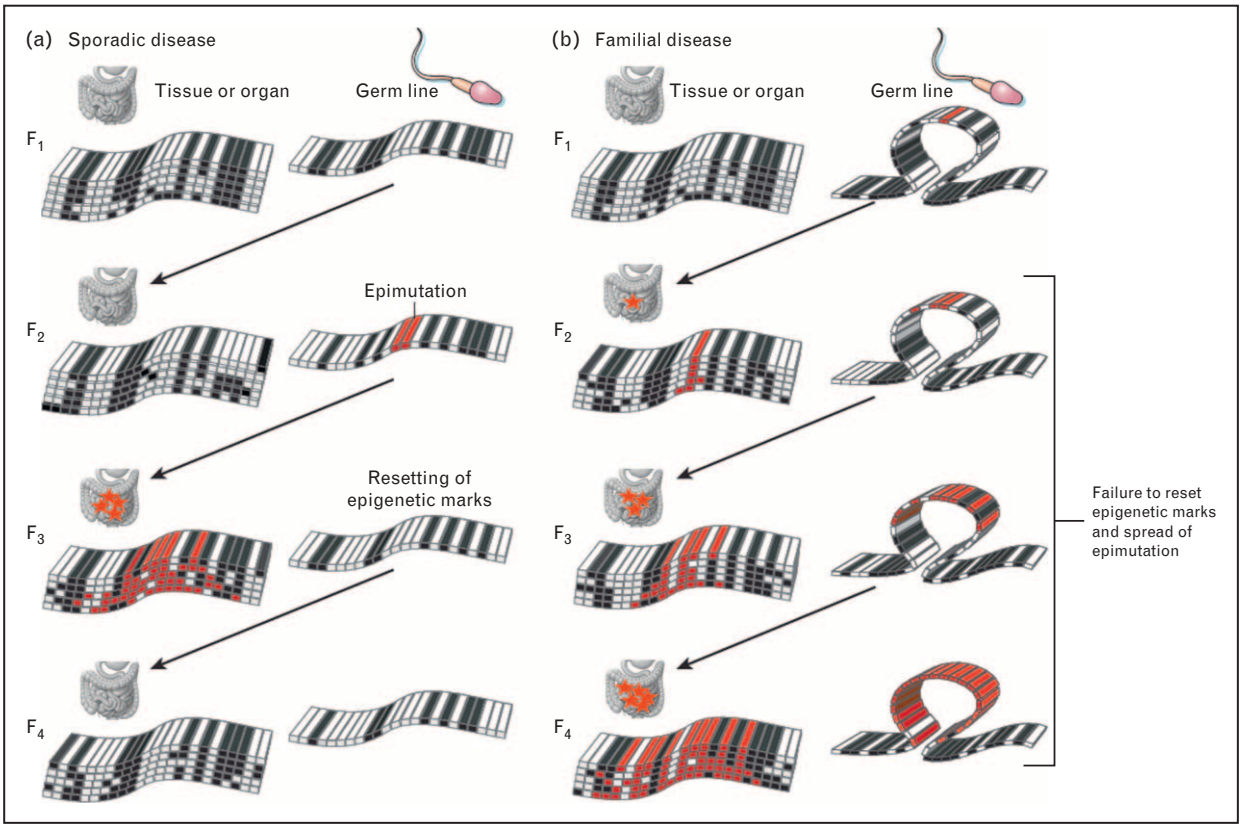

FIGURE 2. Epigenetic model to explain sporadic and familial occurrence of inflammatory bowel disease. DNA methylation profiles are represented by black and white keys with a single layer referring to germline or zygote and multiple layers referring to somatic cells. (a) In sporadic disease, an 'epimutation' (red keys) occurs in the germline (possibly in response to certain environmental triggers) of the second generation (F2). Transmission to the offspring leads to disease in F3 affecting specific tissue and cell types (red stars). Resetting of epigenetic marks because of complete erasure of DNA methylation in the germline leaves F4 generation unaffected. (b) In familial disease, there is incomplete resetting of epimutations and disease is inherited to future generations (transgenerational epigenetic inheritance). Possible causes for incomplete epigenetic reprogramming include aberrant chromatin configurations as illustrated by DNA loops. Continuing failure to correct or reset epigenetic marks can lead to epigenetic anticipation with disease becoming more severe and occurring at an earlier age (illustrated as increasing numbers of red keys). Modified with permission from [41].

studies have used such tools to perform epigenetic profiling of tissue samples (i.e. blood and intestinal biopsies) obtained from IBD patients [50,51]. Although reported results are intriguing, there are a number of limitations to these early studies, which will have to be addressed in the design of future research [52▪]. For example, as outlined above, unlike DNA sequences, epigenetic signatures are highly cell-type specific. Hence, purification of individual cell types from mixed cell or tissue samples is required to avoid detecting signals (e.g. differential methylation) that only reflect the difference in cellular composition (e.g. inflamed versus noninflamed tissue). Although this may pose some difficulties particularly to large-scale studies, as fresh material has to be processed immediately, it is the only way to obtain meaningful data. Additionally, given that several cell types have been suggested to be implicated in IBD disease pathogenesis, an additional challenge for future epigenetic studies will be to select relevant cell type(s) in which changes are most likely to occur.

Given the major impact of environmental factors (including diet, age and medical treatment) to the epigenome, patient selection, detailed phenotyping and appropriate matching of controls are of paramount importance. Finally, in the absence of clean data about actual differences in epigenetic signatures implicated in disease, required sample sizes to demonstrate significant effects remains speculative. However, if epigenetics proves to be a truly crucial factor, necessary cohorts might be much smaller than those seen in previous GWAS studies.

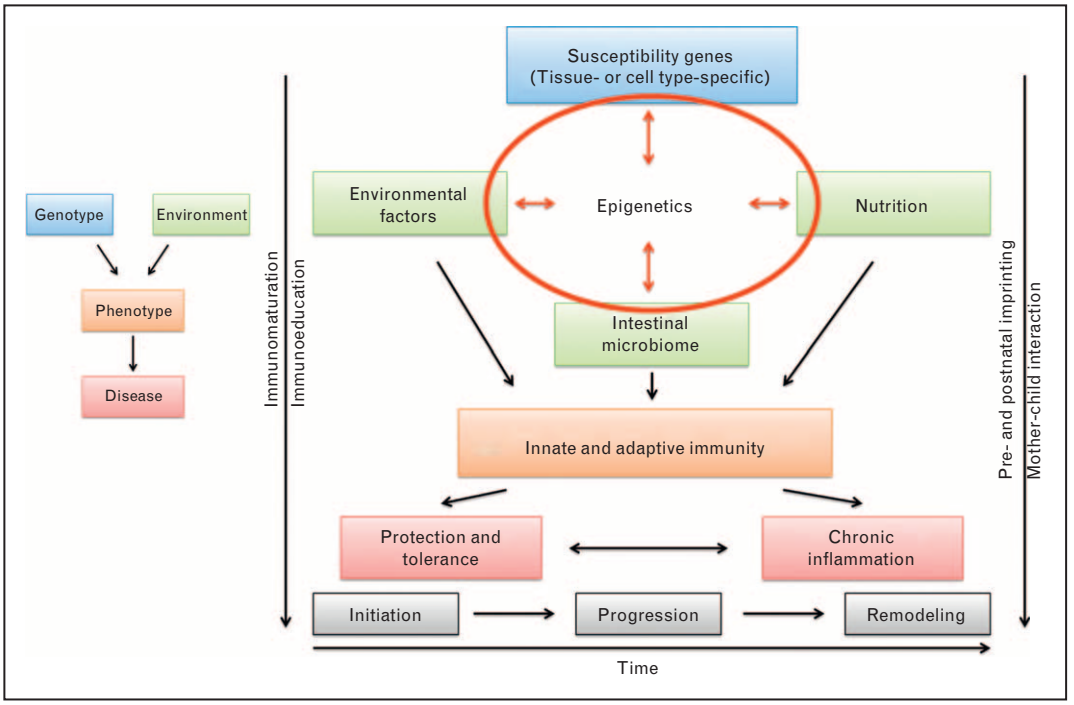

FIGURE 3. Revised model of inflammatory bowel disease (IBD) pathogenesis. (a) Traditional simplistic paradigm of IBD pathogenesis. (b) Revised, more complex model of IBD pathogenesis in which epigenetics plays a central role in mediating effects of the key cornerstones: genetic predisposition, environmental factors, nutrition and the intestinal microbiome. Modified with permission from [53].

CONCLUSION

Until now, our understanding of IBD pathogenesis is incomplete and a link for several important aspects remains missing. In this review, we tried to highlight how, in theory and based on early evidence, epigenetics could provide a novel and unifying framework with the potential to answer some of the key questions in IBD. Figure 3 [53] outlines a revised model of IBD pathogenesis, in which epigenetics plays a central role in mediating the individual aspects including genetic predisposition, environmental triggers and the intestinal microbiota (Fig. 3 [53]). Despite the fact that epigenetic research of complex diseases is still in its infancy and hard evidence is only just beginning to appear, the biological plausibility of the concept is raising expectations that this field should provide significant advances for the understanding of IBD in the near future.

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Conflicts of interest

There are no conflicts of interest.

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