Epigenetics in Paediatric Gastroenterology, Hepatology, and Nutrition: Present Trends and Future Perspectives

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ABSTRACT

Epigenetics can be defined as stable, potentially heritable changes in the cellular phenotype caused by mechanisms other than alterations to the underlying DNA sequence. As such, any observed phenotypic changes including organ development, aging, and the occurrence of disease could be driven by epigenetic mechanisms in the presence of stable cellular DNA sequences. Indeed, with the exception of rare mutations, the human genome-sequence has remained remarkably stable over the past centuries. In contrast, substantial changes to our environment as part of our modern life style have not only led to a significant reduction of certain infectious diseases but also seen the exponential increase in complex traits including obesity and multifactorial diseases such as autoimmune disorders. It is becoming increasingly clear that epigenetic mechanisms operate at the interface between the genetic code and our environment, and a large body of existing evidence supports the importance of environmental factors such as diet and nutrition, infections, and exposure to toxins on human health. This seems to be particularly the case during vulnerable periods of human development such as pregnancy and early life. Importantly, as the first point of contact for many of such environmental factors including nutrition, the digestive system is being increasingly linked to a number of ''modern'' pathologies. In this review article, we aim to give a brief introduction to the basic molecular principals of epigenetics and provide a concise summary of the existing evidence for the role of epigenetic mechanisms in gastrointestinal health and disease, hepatology, and nutrition.

Key Words: coeliac disease, epigenetics, hepatology, inflammatory bowel disease, necrotizing enterocolitis, nutrition

What Is Known

- Epigenetics defines biological mechanisms capable of changing phenotype and cellular function without causing alterations to the underlying DNA sequence.
- Epigenetic mechanisms operate at the interface between environment and the mammalian genome being able to mediate effect of external triggers into stable changes of cellular phenotype.

What Is New

- Increasing evidence suggests a major role for epigenetics in the pathogenesis of human diseases including those within the field of paediatric gastroenterology, hepatology, and nutrition—this review provides an introduction of the basic principals and summary of present evidence.

(*JPGN* 2016;62: 521–529)

The principal concept of epigenetics was first proposed by Conrad Hall Waddington in 1942. A developmental biologist, Waddington, coined the term ''epigenetic landscape'' as his vision of the way in which genes interact with the environment to ultimately produce a phenotype (1). Today, epigenetics can be defined as mechanism(s) that alters the phenotype without changing the underlying DNA sequence. These changes are potentially heritable, allowing the ''epigenetic code'' to be passed on to daughter cells during mitosis or even across generations (2). Robust evidence for the latter phenomenon, referred to as transgenerational epigenetic inheritance, remains limited in mammals. If proven to be true, epigenetics could, however, challenge the dogma of genetically determined inheritance proposed by Darwin and Mendel, and provide support for Lamarck theory, which suggested acquired phenotypic traits (ie, in response to environmental triggers or requirements) could be passed on to future generations. In fact, if this change were to occur without alterations in the DNA sequence, ''epigenetics'' would provide the biological mechanism behind Lamarck theory and, hence, open a new window on the principles of evolution (3). Given that a key feature of epigenetic mechanisms is their responsiveness to the environment (4), it is highly plausible that the evolutionary concept of adapting your phenotype to the environment (and passing this on) could well be mediated by such mechanisms. At this point, it is important to understand that epigenetic heritability is a much less stable and

Received October 22, 2015; accepted December 24, 2015.

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The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000001053

much more flexible concept compared with genetic heritability. In one of the most famous epidemiological studies, transgenerational effects of unique environmental factors were only documented over 2 or 3 generations (5). Similarly, a recent elegant experimental study in rodents could show that altered DNA methylation profiles in the germline of an exposed generation can be efficiently erased during the process of germline development in the subsequent generation (6). Thus, both the experimental and epidemiological data suggest that epigenetic marks are readily reprogrammed between 2 generations when the environmental stimulus is removed.

How does the above fit into the context of recent epidemiological information? Increasingly, common traits such as obesity, cardiovascular disease, along with the rapid rise of multifactorial, autoimmune/immune-mediated diseases, could all point to epigenetic mechanisms as the potential "missing link" in disease pathogenesis—either in terms of heritability or an intermediate translating alterations in the environment into phenotype changes. Specifically, the combination of recent rises in multifactorial/complex diseases, in the presence of a stable human genome, suggests that the recent dramatic changes to our environment/lifestyle may prove a major aetiological factor. Epigenetic mechanisms may indeed be responsible for translating environmental change into stable phenotypic changes, which in certain circumstances may ultimately lead to disease.

A large body of existing evidence supports the importance of environmental factors such as diet and nutrition, infections, and exposure to toxins (eg, smoking, alcohol) on human health. This is particularly the case during vulnerable periods of human development, for example, pregnancy and early life (7). Importantly, the digestive system is the first point of contact for many of such environmental factors, hence it is not surprising that an increasing number of "modern" pathologies are being linked to primary changes in the gastrointestinal (GI) tract, for example, diabetes, obesity, allergies.

In this review article, we aim to give a brief introduction to the basic molecular principles of epigenetics and provide a concise summary of the existing evidence for the role of epigenetic mechanisms in GI health and disease, hepatology, and nutrition.

BASIC PRINCIPLES

Presently, the main epigenetic mechanisms in mammals are DNA methylation and hydroxymethylation, expression of noncoding RNAs (ncRNAs), and posttranslational modifications (PTM) of histone proteins. The most extensively studied epigenetic mechanism is "DNA methylation," which occurs primarily on the 5′ position of the pyrimidine ring of cytosines in the context of CpG dinucleotides (5-methylcytosine). Most CpGs in the human genome are known to be methylated, however, one major exception is the genomic areas containing a high proportion of CpG motifs; these are referred to as CpG islands (CGIs). CGIs are frequently located within promoter regions, where changes in DNA methylation have been shown to impact on gene transcription. Although our understanding of exact mechanisms is still incomplete, as a basic principle, hypermethylation of CGIs within promoter regions is associated with silencing of the associated gene, while hypomethylation favours gene expression. To date, exact mechanisms are not completely understood, but regulation of gene expression via DNA methylation is thought to occur through its effect on the binding of transcription factors, as well as its impact on chromatin structure (8). Chromatin is defined as a complex of DNA, RNA, and proteins. Among the latter, histones represent the major organisational components arranging 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/genomic areas (Fig. 1A). The chromatin state can be altered not only by DNA methylation, as mentioned above, but also through "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 (9). These PTMs can alter the degree of chromatin compaction and ultimately create chromatin structures favourable either for active transcription (ie, euchromatin) or repression (ie, heterochromatin) of genes (Fig. 1). For example, euchromatin is associated with high levels of acetylation and trimethylation of H3K4 (ie, methylation of lysine 4 of histone 3), H3K36, and H3K79, whereas heterochromatin is characterised by low levels of acetylation and high levels of H3K9 and H3K27 methylation. Nevertheless, it is important to realise that this is a simplified model and that interpretation of histone marks is often much more complicated and needs always to be done in correlation to gene expression and other factors (10). Expression of nonprotein coding RNA represents the third major epigenetic mechanism. Until recently, the fact that only about 2% of human RNA transcripts are processed into proteins led the remaining transcripts to be classified as "junk." It is, however, becoming increasingly clear that these ncRNA sequences play a major role in regulating gene expression and cellular function (11). Among the best-studied ncRNAs are the microRNAs (miRNAs), which are short RNA sequences (ie, 20–23 nucleotides), which usually prevent the translation of messenger RNA into protein by binding to a short complementarity region. Additionally, this binding process can activate a miRNA-induced silencing complex (miRISC), which includes an enzyme (ie, endoribonuclease) capable of cleaving double stranded RNA and therefore preventing translation of messenger RNA into protein (Fig. 1B).

Importantly, epigenetic mechanisms are all closely interconnected, representing a complex system of regulating gene expression and cellular function. It is therefore not surprising that epigenetic mechanisms play a critical role in several fundamental biological processes, for example, X chromosome inactivation, silencing of (retro) transposons, genomic imprinting, cellular differentiation, as well as regulating tissue/cell-type specific gene expression. The latter implies that in multicellular organisms, epigenetic mechanisms play a key role in defining cellular phenotype. In addition, unlike the human genome that remains largely stable throughout life, epigenetic profiles are more easily and rapidly influenced by, and can respond to, environmental factors (4). These environmentally induced epigenetic changes can then be passed on during cell division, resulting in a permanent alteration to the phenotype, which can be inherited by a future generation in readiness to deal with similar environmental challenges. Once again, it is important to highlight that this is a simplified view because actual processes are likely to be much more complex and the temporary relationship between environmental stimuli and epigenetic changes shows wide variations and may not easily be recognized. For example, in an individual changes in epigenetic signatures in early life may alter the developmental trajectory with permanent consequences even if the initial alterations in epigenetic signatures do not persist.

In the following text, we provide brief summaries of recent developments in the field of epigenetics, with reference to specific conditions in paediatric gastroenterology, hepatology, and nutrition.

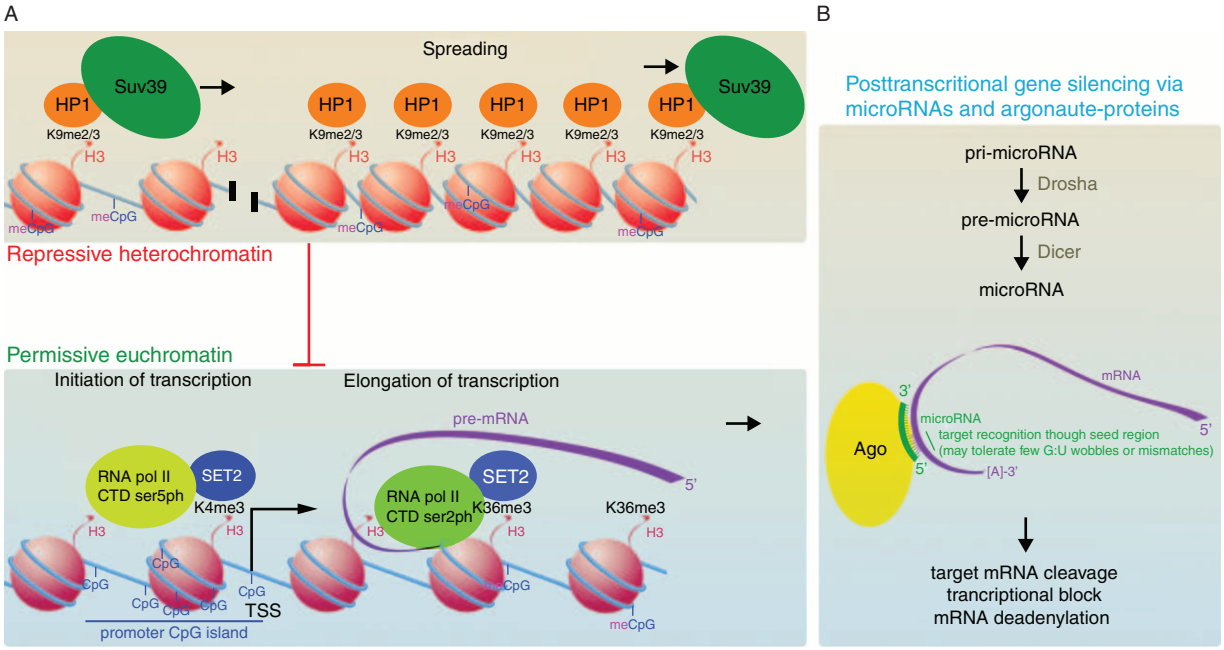

FIGURE 1. Basic principles of transcriptional regulation via epigenetic mechanisms. A, Cellular DNA is packaged into macromolecules called chromatin consisting of DNA, protein, and RNA. In heterochromatin, DNA is not accessible to the transcription machinery. This state is characterised by binding of HP1. In euchromatin, the chromatin structure is more permissive and allows binding of the transcription machinery (RNA Pol II) eventually leading to transcription and expression of the respective genes. DNA methylation at 5′ position of cytosine residues (CpG) together with PTMs provide a unique epigenetic signature that regulates chromatin organisation and gene expression. CpG methylation and di- or trimethylation at lysine 9 of histone protein 3 (K9me2/3) induce heterochromatin, whereas demethylation of CpGs and methylation at lysine 4 of histone protein 3 (K4me) induce euchromatin. B, Short ncRNAs represent the third epigenetic mechanism involved in regulating DNA expression. Among these, miRNAs are transcribed into primary miRNA transcripts (pri-miRNA) and then processed by the RNase III enzyme Drosha into pre-miRNA. The latter is then exported into the cytoplasm where it is cleaved by Dicer into a transient, ~22-nucleotide miRNA/miRNA∗ duplex intermediate. The antisense strand of this intermediate then complements with the target mRNA sequence, forming an A-form double-stranded helix within the miRISC. The matching process between miRNA and target mRNA allows for a small number of base missmatch (ie, wobbles), facilitating the coregulation of many genes by a single miRNA, which ultimately occurs through translational inhibition (main mechanism in mammals), RNA degradation (by de-adenylation) or endonucleolytic cleavage (mediated by Ago). CTD = carboxy-terminal domain of RNA polymerase II; HP1 = heterochromatin-protein 1; miRNA = microRNA; miRSC = multiprotein RNA-induced silencing complex; mRNA = messenger RNA; ncRNA = noncoding RNA; PTM = posttranslational modification; RNA Pol II = RNA polymerase II; SET1 = histone methyltransferase SET1; SET2 = histone methyltransferase SET2; Suv39 = histone lysine methyltransferase Suv39; TSS = transcription start site.

Epigenetics and Nutrition

Evidence is rapidly accumulating that nutritional exposure throughout life modulates key health outcomes via epigenetic effects. The literature is substantial and can only briefly be summarised here. It is essential that paediatricians in particular recognise that dietary modification provides an important opportunity to reduce the incidence of a range of noncommunicable chronic disease in later life. Osteoporosis, heart disease, stroke, and type II diabetes combine to represent the most important causes of global morbidity and mortality. Although maternal (as well as paternal and grandparent) nutritional status before conception is important, the strongest evidence to date for nutritional epigenetic modification relates to nutrient intakes during pregnancy and early postnatal life (7,12). This has led to the concept of "nutrition during the first 1000 days" representing the critically important 9 months of pregnancy and the first 2 years of infancy (13). Key nutritional exposures include differences in both macronutrient and micronutrient intake.

Dietary intake of nutrients that act as methyl donors include folic acid, choline, and betaine, as well as the amino acids methionine (essential) and serine (nonessential), but may involve a range of other micronutrients. These nutrients interact through a variety of overlapping metabolic pathways involved in DNA methylation, as well as other epigenetic mechanisms such as histone acetylation. Methyltransferases (DNA and histone) involved in epigenetic modification use the common substrate s-adenosyl-methionine formed from metabolic pathways involving methyl-donor nutrients, with the levels of s-adenosyl-methionine directly relating to dietary intake (14,15). Classic experiments feeding pseudo-Agouti pregnant mice diets enriched in methyl donors demonstrated permanent changes in gene expression affecting body size, tail kinking, and coat colour when compared with a normal diet (Fig. 2) (16,17). Although this shows how nutritionally induced epigenetic changes can alter the phenotype, it also demonstrates that genetic differences between individuals have important effects on dietary requirements, because wild-type Agouti mice are not influenced by the methyl-donor status of their mother's diet during pregnancy. This means that a dietary deficiency of methyl donors leads to disease in some individuals while having no measurable effect in others. These findings also underline the fact that the "one size fits all" approach to dietary intervention is flawed.

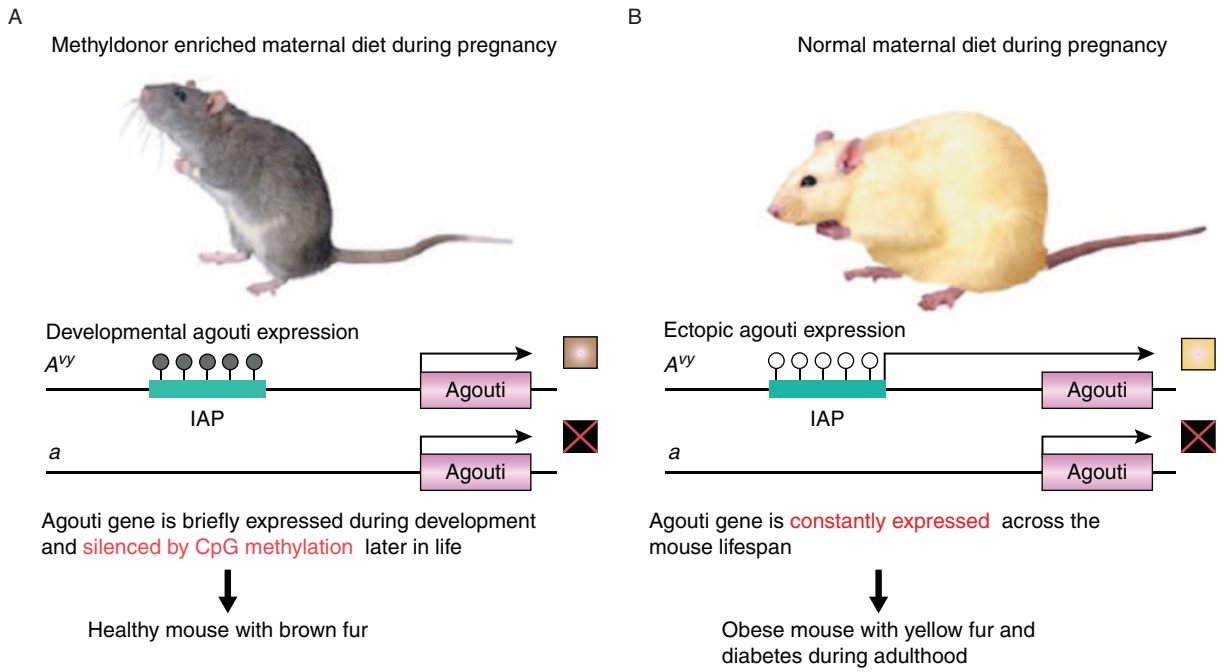

FIGURE 2. Simplified principle of epigenetic gene regulation in the agouti mouse model. In this mouse model, one of the agouti gene alleles is mutated (ie, allele a—dark box with red cross). Expression of the second allele, that is, A^{vy} can be epigenetically regulated according to maternal diet. In a healthy, wild type mouse, the A^{vy} allele of the agouti gene is only briefly expressed during early life. During normal development, the IAP is methylated resulting in silencing of the allele and a healthy mouse with brown coat colour (A). Alternatively, if the IAP remains unmethylated, the gene is ectopically expressed resulting in obese mice with yellow coat colour (B). Methylation at the IAP in the A^{vy}/a mice is substantially influenced by maternal diet during pregnancy. IAP = intracisternal A particle.

Macronutrient intake has also been implicated in epigenetic regulation of growth, both during pregnancy and in early infancy. The complexity of potential gene-nutrient interactions means it is difficult to identify the precise metabolic processes involved, but there is accumulating evidence to show that growth patterns and macronutrient intakes during early life have long-term impacts on metabolic outcomes (18). The timing of these exposures during early life is critical. In general, addressing these questions in studies on humans is extremely challenging in terms of a longitudinal approach and inclusion of metabolically affected controls that make the identification of causality difficult.

Despite these limitations, some of the strongest data come from long-term follow-up of the "Dutch Hunger Winter" cohort, where periconceptional famine conditions resulted in less DNA methylation of the imprinted *IGF2* gene, compared with famine later in gestation, when the same methylation effects were not seen (19). In the same cohort, using a comprehensive genome-scale approach, 181 differentially methylated regions (DMRs) were identified to be related to prenatal malnutrition. Interestingly, methylation at the 6 DMRs with the most distinct differences revealed a strong interaction between the start of pregnancy and famine exposure. Genes mapping to these 6 DMRs are involved in developmental processes including eye development, forebrain formation, growth, and sustaining early pregnancy (20). Observational studies in infants show strong association between more rapid weight gain in early postnatal life and adverse later metabolic outcomes, whereas controlled trials show that faster growth in preterm infants is adversely associated with measures of insulin resistance and vascular health in adolescents (21,22). In animal models, a low protein diet in utero was found to modify a promoter–enhancer interaction at the Hnf4a locus through alterations in histone marks possibly at least partly explaining the development of pancreatic β-cell dysfunction and subsequent type 2 diabetes in these animals (23). All of these processes are likely to involve epigenetic mechanisms, although detailed studies remain largely confined to animal models. In summary, a large number of modifiable nutritional exposures in early life can act as key regulators of epigenetic mechanisms, which in turn may be implicated in determining our health and disease in later life, and possibly even that of subsequent generations.

EPIGENETICS IN INFLAMMATORY BOWEL DISEASES

The incidence of inflammatory bowel diseases (IBDs) such as Crohn disease and ulcerative colitis (UC) has been increasing for more than 5 decades (24,25). Particularly, the number of patients diagnosed during childhood has risen sharply, presently accounting for almost 25% of all of the patients. Another interesting change in recent decades is the fact that IBD is being increasingly recognised in developing countries, particularly those adopting a more Western life style, for example, China and India (26). Together these findings strongly point toward a major role of environmental factors in the rise of IBD. Moreover, large-scale genome-wide association studies confirmed that disease predisposing genetic changes may only be present in 15% to 20% of patients with IBD (27). Taken together, it seems plausible to hypothesise that environmental changes leading to an increase in disease incidence, in the absence of changes to the underlying DNA sequence, are likely to be mediated, at least in part, by epigenetic mechanisms. A number of excellent reviews have summarised this concept in great detail and are recommended to the interested reader (27–29).

Despite the plausibility that epigenetic mechanisms provide a logical framework to explain IBD disease pathogenesis, evidencing this concept is difficult. Although novel methodological approaches allow the investigation of epigenetic mechanisms on a genome wide or even whole genome level, there are a number of crucial hurdles that first need to be overcome. In clear contrast to former genome-wide association studies, which only require genomic DNA extraction from blood or available tissue, the success of epigenetic studies relies on the careful selection and purification of disease relevant tissue/cell types. Because all epigenetic mechanisms are highly cell-type specific, performing analysis on mixed-cell tissues (eg, mucosal biopsies or blood samples) carries the major risk of results being confounded by variation of cell type, particularly when analysing inflamed tissue samples (30). Studies reporting the potential involvement of epigenetic mechanisms in IBD disease pathogenesis are still scarce. There are presently no noteworthy reports on the role of histone modification in IBD. In contrast, a number of studies have investigated DNA methylation profiles in mucosal biopsy samples and peripheral blood mononuclear cells obtained from patients with IBD and controls (31–33). Results of these studies are promising, because authors report disease-specific DNA methylation changes present in patients with IBD. Moreover, genes with alterations in their methylation profile seem to be enriched for disease-relevant immune pathways (31). Given that all of these studies have been performed on mixed-cell tissue samples (ie, peripheral blood mononuclear cells or mucosal biopsies), their results may be, however, substantially confounded by cellular composition. This is likely to be particularly prevalent when comparing inflamed and noninflamed tissue. Nevertheless, disease-specific DNA methylation profiles may prove to be useful diagnostic and/or disease prognostic biomarkers in the future, given their responsiveness and cell specificity. A recent study by Kraiczy et al (34) demonstrates significant changes in the DNA methylation profile between purified intestinal epithelium obtained from human fetal gut and healthy paediatric biopsy samples, highlighting the important role of this epigenetic mechanism in regulating epithelial cell function during GI development. Interestingly, the authors observed significant differences in the DNA methylation profile of purified colonic intestinal epithelium derived from children newly diagnosed with IBD compared with healthy controls (34).

A substantial number of studies have been published during the past 5 years on the topic of miRNAs and their potential role in IBD pathogenesis. For example, Zikusoka et al (35) observed decreased levels of the anti-inflammatory miR-192 in the mucosa of patients diagnosed with UC. Ghorpade et al (36) were able to demonstrate NOD2-driven inflammation to be regulated via altered expression of nitric oxide-responsive miR-146a using a dextran sulfate sodium colitis murine model. Importantly miR-146a is known to activate Sonic hedgehog signalling, which is an essential pathway associated with gut development and maintenance of gut homeostasis. Impaired expression of miR-146a resulted in unrestricted Sonic hedgehog signalling and expression of inflammatory genes such as IL-12, TNF-α, IL-6, CCL-5, and CXCL-9 (36). One of the very few studies performed on paediatric patients identified reduced levels of miR-124 in the colon of children diagnosed with UC (37). With miR-124 known to regulate expression of STAT3 and hence inflammatory responses, the authors suggest that altered miR-124 expression may be implicated in the pathogenesis of paediatric UC (37). Recently, miR-595 targeting the neural cell adhesion molecule-1 and miR-1246 that activates the proinflammatory nuclear factor of activated T cells have been found to be biomarkers of active Crohn disease and UC. They may also become potential targets for therapeutic intervention in the treatment of inflammatory bowel disease (38).

Epigenetics in Necrotizing Enterocolitis

In modern neonatology necrotizing enterocolitis (NEC) is the most devastating acute gastrointestinal disorder in premature infants. It remains a high priority for research in the absence of a clear aetiology and a static incidence for more than the past 2 decades. Until recently, epigenetic mechanisms have not been considered relevant in its disease pathogenesis. It is clear that NEC develops primarily in premature infants and that the presence of gut bacteria is essential. Moreover, prenatal treatment with glucocorticoids has been shown to decrease the incidence of NEC (39). Present evidence suggests that the primary factor leading to NEC may be exaggerated and excessive, but immature, host intestinal immune response (40). More specifically, the innate immune pattern recognition receptor toll-like-receptor 4 (TLR4) seems to play a key role, with C3H/HeJ mice carrying defective TLR4 being protected against the development of NEC (41). Activation of TLR4 by bacterial products induces inflammatory gene expression in intestinal epithelial cells (IECs) (42); in addition, it leads to reduced enterocyte proliferation and an impaired vascular response to hypoxia both in vitro, and in an in vivo mouse model (43,44). Nevertheless, it still remains unclear why TLR4 has such a detrimental effect and whether it is "constitutively" upregulated in the immature gut or if other factors are required for its activation. Genome-wide expression analysis on intestinal samples derived from premature human infants diagnosed with NEC found no differential expression of TLR4 when compared with premature infants with focal intestinal perforation (45). In addition, genetic defects have been only rarely associated with NEC and to date no mutations in TLR4 have been reported (46). A plausible explanation for the observed/reported modulation of TLR4 expression in the absence of genetic alterations could be provided by epigenetic mechanisms. In fact, a study by Takahashi et al (47,48) demonstrated that TLR4 expression in IECs is regulated via DNA methylation and histone deacetylation in response to the presence/absence of gut microbes, resulting in TLR4 unresponsiveness. Specifically, the highest levels of DNA methylation at the *TLR4* gene were observed in IECs of the large intestine in a germ-free milieu. The authors concluded that commensal bacteria contribute to the maintenance of intestinal symbiosis (eg, downregulation of TLR4) by controlling epigenetic modification of host genes in the large intestine. Similarly, expression of intestinal epithelial human β-defensin 2, an antimicrobial peptide with potent bactericidal activity, requires histone H3 tri-methylation at Lys4 as well as DNA methylation, which together control expression in response to local microbial flora (49). Substantial evidence derived from a preterm piglet model also suggests that early postnatal enteral nutrition plays a crucial role in driving the functional development of the intestinal epithelium, and therefore significantly influences the risk of developing NEC (50). These findings would be in keeping with the observed protective effect of breast milk in human premature infants (51) and the fact that introduction of formula feeds increases the incidence of NEC (52). Importantly, recent evidence using the same model shows that formula feeds induce subclinical inflammation in the premature intestine, corresponding with a more open chromatin structure in key inflammatory genes such as *TLR4* and *IL8* (53). Thus, it seems very likely that epigenetic signatures orchestrate early adaptive processes in the intestine. This adds to the hypothesis that environmental factors such as nutrition and microbiota induce changes in chromatin structure and CpG signalling, destabilizing intestinal homeostasis, and thus creating the basis for an inappropriate immune response on exposure to certain bacterial antigens. Epigenetics may thus provide a further missing piece of the jigsaw in understanding the pathophysiology of NEC.

Epigenetics in Coeliac Disease

Coeliac disease is the most common food-induced chronic disease described to date. The incidence of this condition has been rising dramatically for more than the past 50 years and is presently affecting between 1 in 100 and 1 in 40 individuals in European countries (54). Part of this rapid increase in incidence is due to the introduction of more sensitive and reliable testing methods, as well as increased screening of at-risk populations.

The genetic component of coeliac disease is well established and the vast majority of patients will carry at least 1 disease-specific genotype within their human leukocyte antigen HLA (ie, HLA-DQ). The responsible HLA-DQ2 and -DQ8, however, have a high prevalence of almost 25% in whites, but only a small proportion of these individuals (1%–4%) ultimately develop frank disease. In part, this may be explained by the reported differences in the repertoire of T-cell receptors between individuals with similar HLA types, which seem to restrict the recognition of gliadin fragments presented on HLA-DQ2 and -DQ8 (55). Nevertheless, the disease phenotype (ie, age of disease onset, severity of symptoms, associated pathologies including dermatitis herpetiformis) varies substantially between affected individuals, suggesting that in addition to genetic predisposition additional factors are responsible for modifying the phenotype. Such factors include environmental exposures.

The timing, quality, as well as the amount of gluten exposure have been previously suggested to play a major role in determining the presentation of the disease (56). Although more recent data do not seem to fully support these earlier findings (57–59), enteric infections (viral and bacterial), as well as specific alterations to the gut microbiota, have been proposed as potential environmental factors contributing to the onset and/or variations in the phenotype of coeliac disease (60,61).

Given we do not understand why only a tiny proportion of genetically predisposed individuals manifest the disease, and why there is such variation in disease phenotype, the environment's impact on epigenetic mechanisms could well provide an explanation to some of these key questions.

To date, limited published data is available reporting potential epigenetic alterations in patients with coeliac disease. Capuano et al investigated miRNA expression patterns in the small intestine of children diagnosed with active coeliac disease, coeliac disease on a gluten-free diet, as well as a cohort of matched healthy children. Interestingly, the authors found expression of about 20% of miRNAs tested in the small intestine to differ significantly between children with coeliac disease and controls, irrespective of whether the disease was active or not. This suggests expression of these miRNAs may represent an underlying epigenetic alteration that leads to gut inflammation on exposure to gluten (62). A recent study performed by Magni et al (63) also reported changes in miRNA expression in the duodenal mucosa of patients with coeliac disease. Moreover, Vaira et al even report a correlation between clinical phenotype and expression of 7 miRNAs in the duodenal mucosa of patients with coeliac disease and controls. The authors were also able to demonstrate altered miRNA expression of in vitro cultured fibroblasts in response to gliadin exposure (64). Based on their findings, the authors suggest that their model may develop into a useful tool optimising the clinical management of patients with coeliac disease in the future. In addition to miRNAs, a study performed by Fernandez-Jimenez et al (65) investigated the potential impact of DNA methylation in regulating the NFκB pathway in the intestine of patients with coeliac disease. The authors demonstrated constitutive alterations in the activation of the NFκB pathway in mucosal tissue samples obtained from patients with coeliac disease and controls. Moreover, a subset of these genes also displayed changes in DNA methylation, which seemed to be partially reversible. Based on their findings, the authors propose that a complex interplay between genetic, epigenetic, and environmental factors all contribute to the development and maintenance of the observed chronic inflammatory phenotype in patients with coeliac disease.

Epigenetics and Hepatology

The liver can be considered as the most metabolically active organ, providing a huge range of functions, all of which require adjustment to deal with the organism's present environment. In particular, controlling glycogen storage to provide sufficient glucose, and glycogenesis to deal with fasting. These long- and short-term adaptive processes are known to be orchestrated by epigenetic mechanisms. Because the associations between nutrition, epigenetics, metabolism, and metabolic disease are now firmly established, it is not surprising that epigenetic changes are increasingly implicated in the pathophysiology of many liver disorders, including nonalcoholic steatohepatitis (NASH), chronic hepatitis B and C, progression to fibrosis, and hepatocellular carcinoma (HCC). Analysis of 45,000 CpG sites on liver biopsies obtained from NASH patients, as well as from healthy obese and lean controls, revealed altered CpG methylation in at least 8 obesity-related differentially regulated genes (66). Importantly, following weight loss after bariatric surgery, the gene encoding protein-tyrosine phosphatase epsilon, a negative regulator of insulin signalling, became hypermethylated and transcriptionally downregulated (66). Moreover, about 100 miRNAs have been found to be differentially expressed in NASH (67). Among those, miRNA-122 plays an important regulatory role in lipid and cholesterol metabolism and is closely linked to the circadian rhythm controlled by the CLOCK machinery (68). Mice with dysfunctional CLOCK machinery are hyperphagic, obese, and develop NASH (69). Given human epidemiological data, this may at least partly explain the increased risk of metabolic disorders in humans with a disturbed circadian rhythm, eg, shift workers (70). Apart from metabolic diseases, HCC, particularly related to chronic hepatitis B and C virus (HBV; HCV) infection, represents another important liver disorder for which epigenetic changes have been described. Combined transcriptome and CpG methylation analyses on cancerous compared with normal adjacent liver tissue samples identified 322 genes that were hypermethylated and underexpressed—most of them mapped to pathways involved in apoptosis and cell cycle progression. In addition, most of the 230 hypomethylated and overexpressed genes in HCC were within pathways driving cellular differentiation and transformation (71). More specifically investigating tumour suppressor genes, Nishida et al found that 8 genes were hypermethylated in early stages of HCC. Patients with chronic hepatitis C displaying these epigenetic changes had a significantly shorter-time-to-HCC occurrence (72). Such reprogramming, mediated by chromatin-modifying mechanisms, has also been reported in chronic HBV infection. As an example, enrichment of the HBV X protein (HBx) seems to be associated with elevated DNA methyltransferases levels in infected patients (73–75). A study reports global histone hypoacetylation as a structural consequence of an increased sirtuin deacetylase activity in nontransformed HBV-infected hepatocytes. This leads to a more condensed chromatin structure and thus reduced chromatin accessibility. This clearly shows that epigenetic mechanisms are involved in HBV-mediated reprogramming and transforming of hepatocytes (76).

In chronic HCV infection, miRNA-122 facilitates HCV replication by binding to multiple sites in the 5′ untranslated region

of HCV RNA genome, upregulating translation of the viral proteins and protecting HCV RNA against nucleolytic degradation (77). More important, mice with deleted miRNA-122 develop spontaneous hepatosteatosis and inflammation, which then progress to fibrosis and HCC (78,79). It therefore seems that the pathology of liver disease, liver fibrosis, and carcinogenesis, in particular, follows a more generic pattern of substantial changes in epigenetic signatures. Clinically, free miRNAs released from cells, or exosome-associated-miRNA derived from blood or plasma, may be used as biomarkers for liver injury or fibrosis. They are released by an injured liver and in due course could serve as prognostic indicators of disease activity or treatment response (80).

SUMMARY AND FUTURE PERSPECTIVES

We have shown increasingly strong evidence that epigenetic mechanisms modify phenotypes in human gastrointestinal health and disease. The recent and continually rising incidence of several gastrointestinal and liver diseases cannot be explained by alterations in DNA sequence alone. It is a reasonable hypothesis that the dramatic environmental changes that have occurred over the last century, however, induce epigenetic changes and subsequent differential gene expression. Such epigenetic modifications may then become heritable and possibly influence gene expression even in future generations.

Despite this attractive conceptual framework, the question remains how could the study of ''epigenetics'' impact on improving human health, specifically within paediatric gastroenterology, hepatology, and nutrition? Aside from improving our understanding of disease pathophysiology, with potential benefits to subsequent clinical care, a better understanding of epigenetic mechanisms may lead to therapeutic intervention. Given that epigenetic changes are potentially reversible, there is considerable potential to develop novel drugs and molecules that target the epigenetic machinery of specific cell types or tissues. Indeed, novel drugs are already being developed mainly for the treatment of several oncologic disorders such as acute myeloid leukaemia (81). They include histone deacetylase inhibitors, DNA methyltransferase inhibitors, and inhibitors of bromodomains. Furthermore, the use of epigenetic profiles as disease diagnostic and prognostic biomarkers is rapidly increasing particularly in the field of complex, multifactorial diseases. Clinical application of novel epigenetic biomarkers can therefore be expected to reach clinical practice within the next 5 to 10 years.

Finally, given the crucial role of early life events in programming the epigenome, children are likely to be critical in helping to unravel this fundamental biology that influences all of us in adult life. It is tempting to speculate that specific early life interventions on the basis of epigenetic profiles may someday become a reality. Because the GI tract remains one of the major interfaces between us and our environment, it is highly likely that the study of epigenetics in gut health and disease still has much of interest to reveal.

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Seasonal Diarrhea

Thomas Sydenham's (1624–1689) pupil, Walter Harris (1647–1732), in 1689 wrote a Latin text entitled *De Morbis Acutis Infantum*, in which he articulated for the first time in modern medical literature the challenge to physicians that pediatric care requires an enormous number of skills to glean from the *infans* (''the voiceless'') all of the signs and symptoms necessary for diagnosis and treatment. The book contained no original descriptions of disease, yet in the absence of any proof of microbes, Harris was the first to note the predictable epidemiologic nature of seasonal diarrhea in children:

> From the Middle of July to about the Middle of September, the Epidemical Gripes of Children are so rife every Year that more of them usually die in one Month, than in three or four at any other Time...

In North America, rotavirus seasonality is from December to June and norovirus from November to April, so it is possible that Harris was referring to the bacterial causes of diarrhea we associate with outdoor summer eating. Given the known sanitary squalor of 17th century London and the 50% mortality rate for children younger than 2 years, however, the exact nature of ''Epidemical Gripes of Children'' remains a mystery.


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DE
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Accessere
Observationes Medicæ de Morbis
aliquot gravioribus,
ET
Quædam de Luis Venereæ origine,
naturâ & curatione;
Nec non
VINCENTIUS KETELAER
DE
APHTHIS.
Editio quarta auctior & emendatior.
AMSTELÆDAMI,
Apud JANSSONIO-WAESBERGIOS.
M DCC XXXVI.

Contributed by Angel R. Colón, MD