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DNA methylation changes in inflammatory bowel disease

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Abstract

The cause of inflammatory bowel disease, encompassing Crohn's disease and ulcerative colitis, remains a mystery but evidence is accumulating that complex interactions between the genetic background and the gut microbiota of the host and environmental factors associated with rapid industrialization and westernized life styles may underlie its pathogenesis. Recent epigenetic studies have suggested that interactions between environment and host DNA may play a leading role in the phenotypical expression of both diseases, explaining amongst others the differences in disease expression in monozygotic twins. DNA methylation is the most studied epigenetic modification and during the last decade its correlation to IBD pathogenesis has been well established. Genes from different molecular pathways have been studied but till now there is no standardized database of methylated genes in IBD. Thus, a thorough and in depth study of DNA methylation, its potential relation to IBD and its interaction with the available pharmaceutical armamentarium is of great interest.

Keywords Methylation, inflammatory bowel disease, inflammation, epigenetics

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Introduction

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing-remitting or continuously active diseases of the gastrointestinal tract and are occasionally associated with extra-intestinal manifestations [1]. The incidence of IBD has increased during the last decades in the developing world whereas it appears to be rather stable in northern Europe and North America [2,3]. Although the exact etiology of IBD remains unknown, current data converge on a dysregulation of the immune response against the intestinal flora in a genetically susceptible individual [1,4]. More specifically, IBD could be accounted as the result of interactions between the host and the environment, which encompass the intestinal microbiota, the immune system, the genetic composition of the host and specific environmental factors, such as the effect of smoking, breastfeeding, drugs, dietary products, etc. Regarding the interaction between environment and genome, epigenetic mechanisms and more specifically DNA methylation seem to be of great importance [5]. Epigenetic modifications include global DNA methylation, gene-specific DNA methylation, histone modifications, chromatin immunoprecipitation, non-coding RNA and microRNA hybridization. However, the two most widely studied forms of epigenetic modifications are the methylation of DNA [6-8] and the posttranslational modification of histone proteins [9,10], both of which have been functionally implicated in the regulation of gene expression in a variety of organisms. DNA methylation results from the transfer of a methyl group (CH_3) in the C5 site of cytosine, creating the 5-methyl-cytosine (5mC), by DNA methyltransferase (DNMT) enzymes. Methylation of the CpG dinucleotide is so frequent that 5mC has been referred to as the fifth nucleotide. Therefore, global DNA methylation is the analysis of the total 5mC content, whereas gene-specific DNA methylation refers to measurement of methylated CpG islands on a specific gene of interest. The attachment of methyl groups in the promoter region of a gene (hypermethylation) is associated with so-called "silencing" or inactivation of that gene. On the contrary, lower levels of methylation (hypomethylation) in the promoter region of a gene makes it transcriptionally active [11]. The role of DNA methylation is regulation of gene transcription, either by activating proteins which interfere with the suppression of gene transcription, or by inhibiting transcription factors binding to DNA. It is important to note that epigenetic silencing causes no structural alterations to DNA. Methylation and de-methylation occur normally in human cells in order to create a stable regulation pattern of gene transcription. The proper DNA methylation is essential for cell differentiation and embryonic development. Moreover, in some cases, methylation has been observed to play a key role in mediating gene expression. A number of studies have shown that methylation near gene promoters

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varies considerably depending on cell type. Moreover higher methylation of gene promoters has been correlated to low or no transcription [12]. The functional consequences of promoter methylation on chromatin configuration and transcriptional regulation are well documented. However the role of gene body methylation remains largely unresolved and recent data suggest that gene body methylation is a widespread, conserved form of DNA methylation, the ancestral role of which may have been the reduction of transcriptional noise [13]. Undoubtedly, the high-throughput sequencing is likely to uncover many genomic regions that exhibit disease-specific epigenetic alterations [14]. However, large scale genome analysis of DNA methylation is still a costly approach and large scale epigenetic biomarker development may become more feasible only when several methods with very different trade-offs between genomic coverage and per-samples costs are combined. These requirements are met by bisulfite sequencing of region-specifically enriched DNA, and by array-based epigenotyping assays [15]. Until present time, a lot of evidence had associated DNA methylation with cancer development and dysplasia, but only recently has DNA methylation been related to IBD pathogenesis *per se*. We searched PubMed using the terms: "DNA methylation", "Crohn's disease", "Ulcerative colitis" and "inflammatory bowel disease". The aim of the present review was to highlight all the existing data on DNA methylation and IBD, focusing only on the relationship of methylated genes to the pathogenesis of IBD and its clinical aspects. The majority of the studies focus on UC patients and mainly on methylation in tissue biopsy samples.

DNA methylation studies on intestinal tissue samples

DNA methylation changes and UC

UC represents one major sub-phenotype of human IBD. In past decades, UC displayed a remarkably steep rise in incidence, which cannot be explained only by genetic variants. Beyond germline DNA variants, epigenetic variants e.g. DNA methylation (DNAm) and histone modifications, could modulate disease-relevant gene function [16]. Methylation studies extend to different kinds of genes and genetic loci (cadherins, genes related to transcription, kinase inhibitors etc), trying to detect the possible correlation of their methylation to IBD. Indeed, according to the current literature, epigenetic modifications represent promising candidates for elucidating processes of disease manifestation beyond the identified risk loci.

The first evidence that DNA methylation, as an epigenetic mechanism, is related to UC pathogenesis and was reported in 1996 by Gloria *et al* [17], who found that incorporation of the 3^{H}-methyl groups into DNA was 10-fold higher in UC patients than controls and significantly higher in histologically active than inactive disease. Two years later, in a study conducted by Hsieh *et al* [18] 89 tissue samples from UC patients who underwent colectomy were collected and *p16INK4a* (a cyclin-dependent kinase inhibitor) methylation was observed in regions negative for dysplasia, dysplastic lesions and carcinomatous lesions. A progressive increase in methylation status (12.07%, 70%, and 100% respectively) was detected, evidencing the positive relation between DNA methylation and inflammation, as well as neoplastic progression in UC.

Another study on a different kind of gene, *E-cadherin* (a specific calcium ion-dependent cell adhesion molecule), proved that its promoter methylation was detected in 93% of dysplastic biopsy samples from long-standing UC in contrast to 6% of non-dysplastic biopsies. The result was confirmed by testing the level of *E-cadherin* synthesis, found to be reduced in samples with dysplasia and normal in samples without dysplasia, suggesting that long standing inflammation is related to hypermethylation of the gene promoter and that DNA methylation may be used as a biomarker for detecting high risk patients for developing colorectal cancer [19].

Estrogen receptor *(ER), MYOD, GSPG2* and *p16* gene exon 1, which are genetic loci all related to transcription, were evaluated in 33 samples from 23 patients; 12 UC patients with high-grade dysplasia or cancer, 6 UC patients without dysplasia or cancer and 5 non-UC controls. *ER, MYOD* and *p16* were found to be methylated higher in dysplastic than normal appearing epithelium, indicating that methylation precedes dysplasia development [20]. This result was also confirmative of the increased methylation of *p16* mentioned in the study by Hsieh *et al* [18]. Moreover a positive association between methylation of the aforementioned genes (*ER, MYOD, GSPG2, p16*) and increasing age was revealed. Saito *et al* [21] compared the methylation levels of four genes (*CDH1, GDNF, HPP1* and *MYOD1)* between tissue samples from actively inflamed mucosa and quiescent colon mucosa. The results showed that especially for *CDH1* (a calcium ion-dependent cell adhesion molecule) and *GDNF* (Glial cell Derived Neurotrophic Factor - a gene related to survival and differentiation of cells), active inflammation was independently related to higher DNA methylation. Moreover, longer disease duration and the total amount of steroids (>10 g) were important factors also related to higher DNA methylation of *GDNF*.

It is known that changes in *MDR1* function and/or expression contribute to the pathogenesis of inflammatory disorders of the gastrointestinal tract, including UC. *MDR1* promoter methylation has been studied in UC patients and found to be methylated in 61.4% of rectal inflammatory mucosal specimens. Also, it was methylated higher in mucosal biopsies from the rectum than from normal terminal ileum. *MDR1* was related to clinical phenotypes of UC; chronic continuously active colitis, extensive colitis, younger age of disease onset (<20 years old), higher number of hospitalizations, and severe disease phenotype were all independently related to *MDR1* hypermethylation [22]. An analogous study on protease-activated receptor (*PAR2*), which is a member of the large family of 7-transmembrane-region receptors that couple to guanine nucleotide-binding proteins, correlated higher *PAR2* methylation levels in rectal mucosa with steroid-dependent or steroid-refractory colitis. Moreover,

PAR2 methylation levels were higher in rectal biopsies from patients with extensive colitis than patients with proctitis [23]. Another study, conducted in 2008 also showed different levels of methylation between rectum and terminal ileum in ulcerative colitis. *p14* and *p16*, two promoters of *ER* gene, were methylated higher in rectal than in ileal mucosa but were also related to the type of the disease; ER methylation was higher in patients with relapsing-remitting disease than a single attack disease. The higher methylation was also found to be correlated to longer than 7 years of disease [24]. *ER-1* and tumor suppressor candidate 3 (*N-33*) were also found to be higher methylated even in macroscopically normal mucosa of patients with UC compared to controls (7.9% vs. 5.9%; P=0.015 and 66% vs. 9.3%; P<0.001 respectively) [25]. In this regard, the study by Moriyama *et al* [26] investigated the possible relation of hypermethylation of *p14* with high risk of dysplasia. Apart from the verification of the aforementioned hypothesis, hypermethylation of *p14 (ARF)* was also found to be related to longer duration of UC. Three years later Garrity-Park *et al* [27] proved that *p14* shows no methylation in normal mucosa from control patients and increased methylation in tissue samples from inflamed mucosa and colorectal cancer complicating UC. The *p14*, *p16* and *CDH1* promoters' methylation has also been studied in rectal mucosal biopsies from UC patients who were in clinical remission at endoscopy and displayed no dysplasia or neoplasia during the histopathological examination. Moreover CIHM (CpG Island Hyper Methylation) was detected in 45.2% for *p16*, 34.5% for *p14* and 53.6% for *CDH1*, showing higher frequency in non-neoplastic colonic mucosa in UC patients and higher than that in the study of Garrity-Park *et al* [27,28].

Death-associated protein kinase (*DAPK*) is the translational product of a tumor suppressor gene and its inactivation through methylation has been related to many different types of cancer, including inflammation associated tumors and their pre-malignant lesions such as chronic gastritis - gastric cancer and Barrett's esophagus - Barrett's adenocarcinoma [29-31]. Hypermethylation of *DAPK* was found to be associated to the inflammation of mucosa in UC patients and a gradient from lower to higher methylation was similar to the gradient from mild to severe inflammation. Moreover in unmethylated mucosa *DAPK* protein expression was proportional to the severity of inflammation, probably representing a protective role of *DAPK* during chronic inflammation in UC [32]. Another similar finding was shown by Cooke *et al* [33]. A large number of genes were tested for methylation (Table 1) in UC and CD and hypermethylation was detected in UC samples compared to controls. But the most important result was that in UC the hypermethylation is present only in inflamed mucosa and not in the normal appearing (non-inflamed) mucosa.

Finally, in the most recent methylation study, by Haesler *et al* [34], a three-layer epigenome-wide association study was conducted on biopsies from 10 monozygotic twin pairs with discordant manifestation of UC. After the identification and validation of Methylation Variable Positions (MVPs, which represent individual methylation events within the proximal promoter region of transcription start sites) and differentially methylated regions (DMRs, which represent group effects of linked MVPs), 61 disease-associated loci with different DNA methylation were detected. Ten of them showed the closest proximity between MVP/DMR and transcript location (Table 1).

All the above studies indicate that DNA methylation is a really common phenomenon in UC, especially in mucosal biopsies from inflamed tissue. Moreover, DNA methylation has been related to many different clinical aspects, such as disease severity, disease duration, disease phenotype, disease extent, steroid use, steroid dependence or refractoriness, age of onset, number of hospitalizations and finally active inflammation and dysplasia.

UC-associated neoplasia

There is an established increased relative risk for the development of colorectal neoplasia in UC patients [35]. DNA methylation has already been studied in sporadic adenomas and cancer but seems to be also related to dysplasia and cancer development in UC patients [36]. Tahara *et al* [37] have shown that CIHM status may be influenced by *CD14-159, IL-1β-31, p22PHOX242 and MBL2* codon54 single nucleotide polymorphisms in the rectal mucosa of UC patients and may therefore be substantially involved in UC-associated carcinogenesis. More recently, four genes (*TGFB2, SLIT2, HS3ST2* and *TMEFF2*) were evaluated for DNA methylation status in biopsies of four patient-groups: 60 patients with sporadic CRC, 32 patients with IBD-associated neoplasia, 85 patients with IBD without neoplasia and 28 healthy controls. The results showed a trend of increasing prevalence of DNA methylation from mucosal biopsies from healthy controls to mucosal biopsies from IBD patients without neoplasia, to adjacent nonneoplastic mucosa and finally to biopsies from IBD-associated neoplastic regions. Especially *SLIT2* and *TMEFF2* appeared to be more frequently methylated in the mucosa of IBD patients (15/20) at high risk of dysplasia or cancer than patients at low risk (32/63) [38]. Therefore, higher levels of DNA methylation could be related to an increased risk for cancer development. The most recent data from the same group of investigators, have added evidence for two more genes (*AGTR1, WNT2*) and confirmed the data for *SLIT2* gene. All three genes were methylated higher in neoplastic tissue from IBD-colorectal samples (50% for *AGTR1*, 58% for *WNT2* and 86% for *SLIT2*) than in adjacent non-neoplastic tissue (33%, 33% and 47% respectively) and tissue from healthy controls (5%, 3% and 5% respectively) [39].

DNA methylation and CD

On the contrary, limited data exists concerning the contribution of DNA methylation status in CD pathogenesis. Recently, Lin *et al* [40] compared normal to inflamed tissue from CD and UC patients and found significant differences in DNA methylation of seven CpG loci (*BGN_P333_R,*

SERPINA5_P156_F, TNFRSF1A_P678_F, AATK_P709_R, GABRA5_P862_R, MAPK10_E26_F, STAT5A_P704_R). Additionally, subtype specific DNA methylation changes were detected linking 25 CpG sites to CD and 13 CpG sites to UC. The aforementioned publication by Cooke *et al* [33] added evidence on the different level of DNA methylation between inflamed mucosa from CD/UC patients and controls for a large number of genes. It is important that the genes studied and found differentially methylated are involved in different pathways of IBD pathogenesis. *DOK2*, for example, acts as a scaffolding protein for the assembly of multi-molecular signaling complexes such as modulate the cellular proliferation induced by interleukin (IL)-4. Tap 1 is involved in the transport of antigens from the cytoplasm to the endoplasmic reticulum for association with MHC class I molecules. CD28 is a co-stimulatory molecule essential for CD4+ T cell proliferation, Th2 development and IL-2 production. *ICAM3* encodes an intercellular adhesion molecule that plays a key role in leukocyte adhesion and migration as well as signaling. Extremely interesting is also the evidence of increased DNA methylation of *CDH1*. This gene encodes *E-cadherin*, which plays a central role in epithelial cell-cell adhesion. As mentioned earlier in this review, *CDH1* has been reported to be down-regulated in areas of UC inflammation. It must be noticed that some of the genes tested, such as *THRAP2, FANCC* and *GBGT1* have been found methylated in inflamed mucosa of both UC and CD patients whereas others were only CD-specific (Table 1). Moreover, as stated earlier for UC, in CD the non-inflamed tissue shows no significant difference in DNA methylation levels when compared to controls.

Peripheral blood DNA methylation studies

One of the first studies comparing mucosal (lamina propria - LP) to peripheral blood (PB) DNA methylation, showed that *INFG* DNA methylation in PB T cells from healthy individuals or IBD patients is higher compared to LP T-cells from healthy individuals or IBD patients respectively (35% and 38%, P<0.001 and P<0.001-0.03 respectively). Moreover no relationship was found between DNA methylation level and patient gender or age, anatomic location of disease, or concomitant therapy. When the DNA methylation index of *IFNG* was examined in three different regions of the gene (*CNS-22*, proximal promoter, transcribed region), the *CNS-22* region was inversely correlated to DNA methylation of *IFNG* promoter and the transcribed region positively correlated. Regardless of disease status, *IFNG* CpG DNA methylation differs in LP T cells compared to peripheral blood (PB) T cells across all the aforementioned regions [41].

As a continuum of this study, Gonsky *et al* [42] showed that INF-γ secretion differs between mucosal specimens and PB T cells. Whereas DNA methylation of *interferon* (*IFN*)-*γ* gene is decreased in mucosal compartments and thus *IFN-γ* expression increased, PB T cells from IBD patients show decreased *IFN-γ* production as a result of increased *IFN-γ* gene DNA methylation.

Lin *et al* [43] complemented their previous study [40] by comparing the DNA methylation status of many different genes in B cells from PB. IBD-associated changes in DNA methylation were detected in 11 B cell line CpG cites from 18 IBD patients. Disease subtype-specific changes were found in 14 CpG sites from CD patients and 24 UC patients. Important notices are: a) several of the methylated loci, such as *BCL3*, *STAT3, OSM* and *STAT5*, are involved in the regulation or downstream signaling in the IL-23 pathway and b) both *IL-12* and *IL-23* share the same disease-associated methylated gene, p40, as a common subunit.

The DNA methylation status of 27,578 CpG sites, extracted from 21 CD patients, was analyzed by Nimmo *et al* [44]. Two groups were created: patients with well defined phenotype (adult-onset female nonsmokers with inactive ileal disease, not receiving immunomodulatory therapy at the time of investigation) and a sex-matched cohort of healthy controls. The CpG sites that showed the most significant differential DNA methylation include those at the genes *MAPK13, FASLG, PRF1, S100A13, RIPK3, IL-21R;* all plausible candidates in CD pathogenesis. Notably, the data suggests that also *IL-27, IL-19*, tumor necrosis factor *(TNF)-α* and *NOD2* are subject to DNA methylation changes.

A recent research study by Harris *et al* [45] on peripheral blood DNA samples, examined 6 genomic loci (*chr13:20771319-20771319, chr20: 60915009-60915009, chr6: 31803832-31803832, chr2: 34719565-34719565, chr1: 44466601-44466601, chr10: 126360669-126360669*). These loci were studied in two different groups: a) concordant (CD=4, UC=3) and discordant (CD=4, UC=7) monozygotic twin pairs; and b) discordant monozygotic twin pairs (CD=3,UC=3), treatment-naïve IBD pediatric cases (CD=14, UC=8) and controls (n=14). No IBD-specific peripheral blood leukocyte DNA methylation association was found, possibly indicating that microarray proofing of DNA methylation from peripheral blood leukocyte (PBL) is less likely to yield positive results in IBD. Despite the inconclusive results, a further investigation was made on DNA extracted from peripheral blood mononuclear cells (PBMCs) after the exclusion of neutrophil granulocytes. *TEPP* (testis prostate placenta) gene showed a CD-associated increase in DNA methylation in this specific subpopulation of peripheral cells, which was absent after treatment.

Balasa *et al* [46] conducted a study on the *IRF5* promoter DNA methylation in PBL DNA samples: 87 samples of PBL DNA obtained from 49 healthy controls, 18 CD and 20 UC patients were assessed for *IRF5* promoter DNA methylation and results showed insignificant differences in the average DNA methylation, indicating that the dysregulation of *IRF5* promoter is unlikely to be associated to IBD.

A recent Korean study investigated the DNA methylation of signal transducer and activator of transcription 4 (*STAT4*) in colonic mucosa and PBMCs of IBD patients and its relation to specific polymorphisms. In both specimens, IBD patients showed higher expression of *STAT4* than did the healthy controls and a correlation of DNA methylation status at -172 of the *STAT4* promoter and risk alleles was observed [47].

Table 1 All known genes whose methylation has been related to inflammatory bowel disease (IBD), Crohn's disease (CD) or ulcerative colitis (UC)

Disease type	Gene or polymorphism [Ref.]	
IBD	BGN_P333_R (Biglycan) [40]	SERPINA5_P156_F (Serpin peptidase inhibitor) [40]
	TNFRSF1A_P678_F (Tumor necrosis factor lingant superfamily) [40]	AATK_P709_R (Apoptosis-associated tyrosine kinase) [40]
	GABRA5_P862_R (Gamma-aminobutyric acid receptor) [40]	MAPK10_E26_F (Mitogen-activated protein kinase) [40]
	STAT5A_P704_R (Signal transducer and activator of transcription) [40]	TJP2_P330_R (Tight junction protein) [43]
	BCL3_E71_F (B-cell leukemia) [43]	SMAD2_P708_R (Mothers against Decapentaplegic Drosophila, homolog 2) [43]
	PPARG_P693_F (Peroxisome proliferator activated receptor Gamma) [43]	HCK_P46_R (Hemopoietic cell kinase) [43]
	LMTK2_P1034_F (Lemur tyrosine kinase2) [43]	IL12B_P392_R (Interleukin 12) [43]
	CASP2_P192_F (Caspase2, apoptosis related cysteine protease) [43]	SOX1_P1018_R (Sry-box1) [43]
	COL18A1_P494_R (Collagen,type XVIII, alpha-1) [43]	LMO1_P169_F (Lim domain only 1) [43]
	STAT4 (Signal transducer and activator of transcription) [47]	
CD	IL 18BP (Interleukin 18) [40]	LTB4R (Leukotriene B4 receptor) [40]
	PDGFRB (Platelet derived growth factor receptor) [40]	PECAM1(Platelet-endothelial cell adhesion molecule1) [40]
	FABP3(Fatty acid binding protein) [40]	FGF2(Fibroblast growth Factor) [40]
	S 100A4 (S100 Calcium binding protein) [40]	SPARC(Secreted protein acidic cysteine rich) [40]
	LAT (Linker for activation of T cells) [40]	DLC1(deleted in liver cancer1) [40]
	AFF3 (AF4/FMR2 family, member 3) [40]	NEU1(Neuraminidase1) [40]
	TJP2 (Tight junction protein 2) [40]	MFAP4(Microfibrillar-associated protein4) [40]
	GNAS (Guanine nucleotide-binding protein) [40]	RHOH(Ras homolog gene family member H) [40]
	MEG3 (Maternally expressed gene 3) [40]	KRT13(Keratin 13) [40]
	APOC2 (Apolipoprotein C-II) [40]	NOTCH4 (NOTCH drosophila, homolog of, 4) [40]
	MPL (Myeloproliferative leukemia virus oncogene) [40]	MPO (Myeloperoxidase) [40]
	SPI1 (Spleen focus forming virus proviral integration oncogene) [40]	THRAP2 (Thyroid hormone receptor-associated protein 2) [33]
	FANCC (Fanconi anemia complementation group C) [33]	TCOF1 (Treacher Collins syndrome) [33]
	B3GALT2 (Beta-1,3-N-acetylgalactosaminyltransferase 2) [33]	PTRF (RNA polymeprase 1 and transcript release factor) [33]
	ULK1 (UNC51-like Kinase 1) [33]	HK2 (Hexokinase 2) [33]
	GFPT1 (Glutamine:Fructose-6-phosphate aminotransferase 1) [33]	FOLR1(Folate receptor 1) [33]
	C15orf48 (Normal mucosa of esophagus-specific gene 1) [33]	PHYH (Phytanoyl-CoA hydroxylase) [33]
	HSPB6 (Heat-shock 27-KD Protein6) [33]	MYL5 (Myosin light chain 5) [33]
	GBGT1 (Globoside alpha-1,3-N-acetylgalactosaminyltransferase) [33]	COG8 (Component of oligomeric golgi complex 8) [33]
	DOK2 (Docking protein2) [33]	TAP1 (Transporter, ATP-binding cassette) [33]
	FLJ32065 [33]	POR (cytochrome P450 oxidoreductase) [33]
	TNFSF4 (Tumor necrosis factor ligand superfamily member 4) [33]	ZP (Zonna pellucida glycoprotein) [33]
	MYOM2 (Myomesin 2) [33]	PSMB8 (Proteasome subunit beta-type 8) [33]
	SLC2A9 (Solute carrier family 2, member 9) [33]	BMX (Bone Marrow Kinase, X-linked) [33]
	IL9 (Interleukin 9) [33]	GCET2 (Germinal center-expressed transcript 2) [33]
	CDC5L (Cell division cycle 5) [33]	PIPOX (Prolactin-Inducible Protein) [33]
	U2AF1L3 (U2 Small nuclear RNA auxiliary factor 1) [33]	SEPT5_P464_R (Septin 5) [43]
	GUCY2F P255 F (Guanylate cyclase 2F, retinal) [43]	CLDN4_P1120_R (Claudin 4) [43]
	SEFPINE1_P519_F (Serpin peptidase inhibitor, Clade F, Member 1) [43]	LMTK2_P1034_F (Lemur tyrosine kinase 2) [43]
	MST1R_P87_R (Macrophage stimulating 1) [43]	BGN_P333_R (Biglycan) [43]
	MXI1_P1269_F (Max interacting protein 1) [43]	IL16_P93_R (Interleukin 16) [43]
	GADD45A_P737_R (Growth arrest and DNA damage inducible gene) [43]	EPHA2_P203_R (Ephrin receptor 2) [43]
	RHOH_P953_R (Ras homolog gene family) [43]	LIF_P383_R (Leukemia inhibitory factor) [43]
	IL10_P348_F (Interleukin 10) [43]	MAPK13 (Mitogen activated protein kinase) [44]
	FASLG (FAS ligand) [44]	PRF1 (Perforin) [44]
	S100A13 (S100 Calcium-binding protein) [44]	RIPK3 (Rceptor interacting serine/threonine kinase 3) [44]
	IL-21R (Interleukin 23) [44]	IL-27 (Interleukin 27) [44]
	IL-19 (Interleukin 19) [44]	TNF (Tumor necrosis factor) [44]
	MST1 (Macrophage stimulating 1) [44]	NOD2 (Nucleotide binding oligomerization) [44]
	TEPP (Testis prostate placenta expressed protein) [45]	

Disease type	Gene or polymorphism [Ref.]	
UC	TNFSF8 (TNF ligand superfamily) [40]	PIK3R1 (Phosphatidylinositol 3kinase regulatory subunit 1) [40]
	PADI4 (Peptidylarginine deiminase) [40]	CLDN4 (Claudin 4) [40]
	CDH17 (Cadherin 17) [40]	HLA-DQA2 [40]
	HOXB2 (Homeobox B2) [40]	FRK (Fyn-Related Kinase) [40]
	AATK (Apoptosis associated tyrosine kinase) [40]	MEST (Mesoderm-specific transcript) [40]
	EPHX1 (Epoxide hydrolase 1) [40]	NOTCH3 (Notch,Drosophila,Homolog of,3) [40]
	FMR1(Fragile X mental retardation protein) [40]	TCOF1 [33]
	FANCC (Fanconi anemia complementation group C) [33]	THRAP2 (Thyroid hormone receptor-associated protein 2) [33]
	GFPT1(Glutamine:Fructose-6-phophate amidotransferase) [33]	GBGT1(Globoside Alpha-1,3-N-Acetylgalactosaminyltransferase) [33]
	ANKRD9 (Ankyrin repeat domain-containing protein 2) [33]	PAQR6(Progestin and Adipoq Receptor Family) [33]
	TNFSF12-TNFSF13 (TNF ligand superfamily) [33]	DOK2 (Docking protein) [33]
	C18orf14 (Chromosome 18 open reading frame 14) [33]	FUT7 (Fucosyltransferase 7) [33]
	TMEM116 (Transmembrane protein 116) [33]	SPATA22 (Spermatogenesis associated protein) [33]
	RNF113B (Ring finger protein) [33]	MS4A4A (Membrane spanning 4 domains, subfamily A, member 4a) [33]
	CYP2D6 (Cytochrome P450, subfamily IID, polypeptide 6) [33]	COG8 (Component of oligomeric Golgi complex 8) [33]
	CHML (CHM-like) [33]	VWF (Von willebrand factor) [33]
	TNFSF4 (TNF ligand superfamily) [33]	FCGBP (Fc fragment of IgG) [33]
	KCNK4 (Potassium channel, subfamily K, member 4) [33]	AKAP2 (A-kinase anchor protein 2) [33]
	CD28 [33]	PITPNM2 (Phosphatidylinositol transfer protein, membrane associated2) [33]
	SPI1 (Spleen focus forming virus proviral Integration oncogene) [33]	ICAM3 (Intercellular adhesion molecule 3) [33]
	MMRN2 (Multimerin 2) [33]	GCET2 (Germinal center-expressed transcript 2) [33]
	RBM13(RNA-binding motif protein) [33]	EYA4_P794_F (Eyes absent 4) [43]
	DHCR24_P652_R (24-Dehydrocholesterol Reductase) [43]	EPS8_P437_F (Epidermal growth factor receptor pathway substrate 8) [43]
	TBX1_P520_F(T-Box1) [43]	TEK_E75_F (Tyrosine kinase, endothelial) [43]
	PPAT_E170_R (Phosphoribosylpyrophosphate aminotransferase) [43]	BAX_E281_R(BCL2-associated X protein) [43]
	SEPT9_P58_R (Septin 9) [43]	IL128_P392_R(Interleukin 12) [43]
	SMARC81_P220_R (SWI/SNF-related,matrix associated,actin dependent regulator of chromatic, Subfamily C,Member 2) [43]	PPARG_P693_F (Peroxisome proliferator-activated receptor-gamma) [43]
	CD81_P272_R [43]	ARHGDIB_P148_R (RHO GDP-Dissociation inhibitor beta) [43]
	MUC1_E18_R (Mucin 1 transmembrane) [43]	PLAT_E158_F (Plasminogen activator tissue) [43]
	HLA-DO8_E432_R [43]	ELK3_P514_F (ETS-Domain Protein) [43]
	ERN1_P809_R (Endoplasmic reticulum to nucleus signaling 1) [43]	MOS_E60_R (V-MOS moloney murine sarcoma viral oncogene homoog) [43]
	LIG3_P622_R (Ligase III,DNA, ATP-dependent) [43]	P2RX7_P119_R(Purigenic Receptor P2X,ligand-gated ion channel,7) [43]
	IL16_P226_F (Interleukin 16) [43]	PCMC_P400_R (Pericentriolar material) [43]
	PECAM1_P135_F (Platelet endothelial cell adhesion molecule 1) [43]	CFI (Complement factor1) [34]
	FLNA (Filamin A) [34]	HKDC1 [34]
	IGHG1 (IgG Heavy chain locus) [34]	MT1H (Metallothionein 1H) [34]
	PTN (Pleiotrophin) [34]	SLC7A7(Solute carrier family 7) [34]
	SPINK4 (Serine protease inhibitor) [34]	THY1 (THY-1 T-cell antigen) [34]
	TK1 (Thymidine Kinase) [34]	p16INK4a (Cyclin dependent kinase inhibitor 2A-CDKN2A)
	E-cadherin [18]	ERN1_P809_R (Endoplasmic reticulum to nucleus signaling) [20]
	MYOD (Myogenic differentiation antigen) [20]	p16 gene exon 1 [20]
	CHDH1 (Chromodomain helicase DNA-binding protein1) [21]	GDNF (Glial cell line derived neurotrophic factor) [21]
	HPP1 (Hyperplastic polyposis gene 1) [21]	MYOD1(Myogenic differentiation antigen) [21]
	MDR1 (Multidrug resistance 1) [22]	PAR2 (Proteinase activated receptor 2) [23]
	P14 (ER) [24]	P16 (ER) [24]
	ESR-1(Estrogen receptor 1) [25]	N-33 (Tumor suppressor candidate 3) [25]
	p14 (ARF) [26]	P14 [28]
	P16 [28]	CDH1 (Cadherin 1) [28]
	DAPK(Death associated protein kinase) [32]	IFN-γ (Interferon-γ) [41,42]

Concluding remarks

DNA methylation constitutes a well understood epigenetic mechanism which regulates the expression of human genes. Though some biochemical tests, such as CRP and calprotectin, and clinical scoring systems – Crohn's disease activity index, Harvey-Bradshaw – help the clinician estimate the activity of IBD, none of them is adequate for the efficient clinical management of these patients [48,49]. In the struggle for developing a new 'ideal marker', which should be specific, clinically useful, non-interventional and able of detecting high-risk patients, the field of epigenetics and specially DNA methylation is really promising.

Until now and according to the aforementioned articles, DNA methylation has been well correlated to IBD and more specifically to some special clinical characteristics of the patients, such as the duration of disease, the severity of inflammation, the phenotype of disease (single or rare flares vs. chronic active vs. relapse-remitting disease, steroid-dependent/refractory disease, etc), the extent of colitis, the age of onset, the number of hospitalizations, the risk of dysplasia and neoplasia, and finally the applied therapies, including the total dose of steroids. Moreover it has been clearly shown that DNA methylation status can effectively distinguish the inflamed mucosa in IBD patients from non-inflamed mucosal specimens. It is also important to note that DNA hypermethylation is one of the biggest epigenetic changes, and gene hypermethylation has been reported to be related with colorectal carcinogenesis. Therefore, the evaluation of hypermethylation has the potential to contribute to early diagnosis of IBD-related colorectal cancer [50]. All the above are important clinical parameters and their relation to DNA methylation of specific genes promises a possible use for DNA methylation as a non-invasive biomarker.

Another important clinical use is the relation between DNA methylation, disease prognosis and treatment. Is the total DNA methylation status altered after therapy? Can the induction of remission reduce the hypermethylation of specific loci? Is the level of DNA methylation or the DNA methylation itself related to the severity and prognosis of the disease? According to the current literature, DNA methylation has been correlated only to the total dose of steroids and the steroid dependent colitis. Apart from steroids all other treatments used, such as anti-TNF, immunosuppressive and methotrexate remain to be studied. Especially methotrexate, which inhibits dihydrofolate reductase and therefore reduces folic acid level (an important mediator of biological methylation, including DNA methylation), should be anticipated to reduce DNA methylation. For example, treatment with methotrexate has already been shown to increase the level of DNA methylation in PBMCs of patients suffering from arthritis [51].

Recent advances in our understanding of IBD-associated DNA methylation underlie many promising clinical applications such as molecular biomarkers for diagnosis and prognosis of the disease as well as prediction of treatment outcomes. Even if the results from different studies are encouraging, limitations have thus far prevented wide-spread clinical application. One possible reason could be that DNA methylation frequency of many candidate genes is not high enough to achieve the sensitivity needed for clinical use. Technical advantages in the near future are expected to reduce these problems, and DNA methylome is expected to play a key role in the development of personalized medicine.

References

1. Kaser A, Zeissiq S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010;**28**:573-621.
2. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;**142**:46-54.
3. Shanahan F, Bernstein CN. The evolving epidemiology of inflammatory bowel disease. *Curr Opin Gastroenterol* 2009;**25**:301-305.
4. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science* 2001;**293**:1068-1070.
5. Bird AP, Wolffe AP. Methylation-induced repression--belts, braces, and chromatin. *Cell* 1999;**99**:451-454.
6. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003;**33**:245-2454.
7. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006;**31**:89-97.
8. Zemach A, McDaniel IE, Silva P, et al. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* 2010;**328**:916-919.
9. Kharchenko PV, Alekseyenko AA, Schwartz YB, et al. Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. *Nature* 2011;**471**:480-485.
10. Suganouma T, Workman JL. Signals and combinatorial functions of histone modifications. *Annu Rev Biochem* 2011;**80**:473-499.
11. Barnett M, Bermingham E, McNabb W, et al. Investigating micronutrients and epigenetic mechanisms in relation to inflammatory bowel disease. *Mutat Res* 2010;**690**:71-90.
12. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 2008;**9**:465-476.
13. Huh I, Zeng J, Park T, et al. DNA methylation and transcriptional noise. *Epigenetics Chromatin* 2013;**6**:9.
14. Irizarry RA, Ladd-Acosta C, Wen B, et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009;**41**:178-186.
15. Ball MP, Li JB, Gao Y, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* 2009;**27**:361-368.
16. Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature* 2010;**465**:721-727.
17. Glória L, Cravo M, Pinto A, et al. DNA hypomethylation and proliferative activity are increased in the rectal mucosa of patients with long-standing ulcerative colitis. *Cancer* 1996;**78**:2300-2306.
18. Hsieh CJ, Klump B, Holzmann K, et al. Hypermethylation of the p16INK4a promoter in colectomy specimens of patients with long-standing and extensive ulcerative colitis. *Cancer Res* 1998;**58**:3942-3945.
19. Azarschab P, Porschen R, Gregor M, et al. Epigenetic control of the E-cadherin gene (CDH1) by CpG methylation in colectomy samples of patients with ulcerative colitis. *Genes Chromosomes Cancer* 2002;**35**:121-126.
20. Issa JP, Ahuja N, Toyota M, et al. Accelerated age-related CpG

island methylation in ulcerative colitis. *Cancer Res* 2001;**61**:3573-3577.
21. Saito S, Kato J, Hiraoka S, et al. DNA methylation of colon mucosa in ulcerative colitis patients: correlation with inflammatory status. *Inflamm Bowel Dis* 2011;**17**:1955-1965.
22. Tahara T, Shibata T, Nakamura M, et al. Effect of MDR1 gene promoter methylation in patients with ulcerative colitis. *Int J Mol Med* 2009;**23**:521-527.
23. Tahara T, Shibata T, Nakamura M, et al. Promoter methylation of protease-activated receptor (PAR2) is associated with severe clinical phenotypes of ulcerative colitis (UC). *Clin Exp Med* 2009;**9**:125-130.
24. Wang F-Y, Arisawa T, Tahara T, et al. Aberrant DNA methylation in ulcerative colitis without neoplasia. *Hepatogastroenterology* 2008;**55**:62-65.
25. Arasaradnam RP, Khoo K, Bradburn M, et al. DNA methylation of ESR-1 and N-33 in colorectal mucosa of patients with ulcerative colitis (UC). *Epigenetics* 2010;**5**:422-426.
26. Moriyama T, Matsumoto T, Nakamura S, et al. Hypermethylation of p14 (ARF) may be predictive of colitic cancer in patients with ulcerative colitis. *Dis Colon Rectum* 2007;**50**:1384-1392.
27. Garrity-Park MM, Loftus EV Jr, Sandborn WJ, et al. Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. *Am J Gastroenterol* 2010;**105**:1610-1619.
28. Tahara T, Shibata T, Nakamura M, et al. Association between polymorphisms in the XRCC1 and GST genes, and CpG island methylation status in colonic mucosa in ulcerative colitis. *Virchows Arch* 2011;**458**:205-211.
29. Eads CA, Lord RV, Kurumboor SK, et al. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. *Cancer Res* 2000;**60**:5021-5026.
30. Kang GH, Lee HJ, Hwang KS, et al. Aberrant CpG island hypermethylation of chronic gastritis, in relation to aging, gender, intestinal metaplasia, and chronic inflammation. *Am J Pathol* 2003;**163**:1551-1556.
31. Kang GH, Shim YH, Jung HY, et al. CpG island methylation in premalignant stages of gastric carcinoma. *Cancer Res* 2001;**61**:2847-2851.
32. Kuester D, Guenther T, Biesold S, et al. Aberrant methylation of DAPK in long-standing ulcerative colitis and ulcerative colitis-associated carcinoma. *Pathol Res Pract* 2010;**206**:616-624.
33. Cooke J, Zhang H, Greger L, et al. Mucosal genome-wide methylation changes in inflammatory bowel disease. *Inflamm Bowel Dis* 2012;**18**:2128-2137.
34. Häsler R, Feng Z, Bäckdahl L, et al. A functional methylome map of ulcerative colitis. *Genome Res* 2012;**22**:2130-2137.
35. Rogler G. Chronic ulcerative colitis and colorectal cancer. *Cancer Lett* 2013;S0304-3835(13)00552-00551.
36. Hartnett L, Egan LJ. Inflammation, DNA methylation and colitis-associated cancer. *Carcinogenesis* 2012;**33**:723-731.
37. Tahara T, Shibata T, Nakamura M, et al. Host genetic factors, related to inflammatory response, influence the CpG island methylation status in colonic mucosa in ulcerative colitis. *Anticancer Res* 2011;**31**:933-938.
38. Azuara D, Rodriguez-Moranta F, de Oca J, et al. Novel methylation panel for the early detection of neoplasia in high-risk ulcerative colitis and Crohn's colitis patients. *Inflamm Bowel Dis* 2013;**19**:165-173.
39. Koizumi K, Alonso S, Miyaki Y, et al. Array-based identification of common DNA methylation alterations in ulcerative colitis. *Int J Oncol* 2012;**40**:983-994.
40. Lin Z, Hegarty JP, Cappel JA, et al. Identification of disease-associated DNA methylation in intestinal tissues from patients with inflammatory bowel disease. *Clin Genet* 2011;**80**:59-67.
41. Gonsky R, Deem RL, Targan SR. Distinct Methylation of IFNG in the Gut. *J Interferon Cytokine Res* 2009;**29**:407-414.
42. Gonsky R, Deem RL, Landers CJ, et al. Distinct IFNG methylation in a subset of ulcerative colitis patients based on reactivity to microbial antigens. *Inflamm Bowel Dis* 2011;**17**:171-178.
43. Lin Z, Hegarty JP, Yu W, et al. Identification of disease-associated DNA methylation in B cells from Crohn's disease and ulcerative colitis patients. *Dig Dis Sci* 2012;**57**:3145-3153.
44. Nimmo ER, Prendergast JG, Aldhous MC, et al. Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. *Inflamm Bowel Dis* 2012;**18**:889-899.
45. Harris RA, Nagy-Szakal D, Pedersen N, et al. Genome-wide peripheral blood leukocyte DNA methylation microarrays identified a single association with inflammatory bowel diseases. *Inflamm Bowel Dis* 2012;**18**:2334-2341.
46. Balasa A, Gathungu G, Kisfali P, et al. Assessment of DNA methylation at the interferon regulatory factor 5 (IRF5) promoter region in inflammatory bowel diseases. *Int J Colorectal Dis* 2010;**25**:553-556.
47. Kim SW, Kim ES, Moon CM, Kim TI, Kim WH, Cheon JH. Abnormal genetic and epigenetic changes in signal transducer and activator of transcription 4 in the pathogenesis of inflammatory bowel diseases. *Dig Dis Sci* 2012;**57**:2600-2607.
48. Ricanek P, Brackmann S, Perminow G, et al. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical and fecal markers. *Scand J Gastroenterol* 2011;**46**:1081-1091.
49. Meuwis MA, Vernier-Massouille G, Grimaud JC, et al. Serum calprotectin as a biomarker for Crohn's disease. *J Crohns Colitis* 2013;**7**:e678-e683.
50. Maeda O, Ando T, Watanabe O, et al. DNA hypermethylation on colorectal neoplasms and inflammatory bowel disease: a mini review. *Inflammopharmacology* 2006;**14**:204-206.
51. Kim YI, Logan JW, Mason JB, et al. DNA hypomethylation in inflammatory arthritis: reversal with methotrexate. *J Lab Clin Med* 1996;**128**:165-172.