Journal of
Clinical & Cellular Immunology

Molnar and Annaházi, J Clin Cell Immunol 2014, 5:4
DOI: 10.4172/2155-9899.1000253

Review Article Open Access

Pathogenesis of Ulcerative Colitis and Crohn's Disease: Similarities, Differences and a Lot of Things We Do Not Know Yet

Anita Annaházi[#] and Tamás Molnár[*#]

First Department of Medicine, University of Szeged, Szeged, Hungary

[#]Both authors drafted the article and revised and approved the final version

[*]**Corresponding author:** Tamár Molnár, First Department of Medicine, University of Szeged, Szeged, Korányi fasor 8-10., 6720, Hungary, Tel: + 3662545189; Fax: + 3662545185; E-mail: molnar.tamas@med.u-szeged.hu

Received date: June 18, 2014, **Accepted date:** August 23, 2014, **Published date:** September 01, 2014

Abstract

The pathogenesis of inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD) is complex, and our knowledge on the topic is constantly growing. The two disorders are distinct, yet overlap in their clinical manifestations and underlying causes. This review aims to provide a broad overview of the numerous pathogenetic factors that can lead to the development of IBD, focusing on novel findings and on the differences between UC and CD. Recent advances in genetics have identified new components in the pathogenesis, as an example, the importance of Th17 lymphocytes and the IL-17/IL-23 pathway have been highlighted in both diseases, apart from the previously known Th1-Th2 driven processes. Genetic background of increased permeability has been explored in UC, and the role of defective autophagy was recently described in CD. Genetic alterations can lead to an exaggerated immune response to the resident microbial flora. This microflora is altered in IBD patients, probably due to their reduced ability to stabilize its bacterial components and due to different environmental factors. An exhaustive exploration of environmental factors is particularly important, as they can be influential in many cases. The impact of smoking is the most established environmental factor, having deleterious effects in CD and protective in UC. Recent opinions on other factors, such as early appendectomy, diet, reduced vitamin D levels, the use of specific medications, breastfeeding, personal hygiene and psychological factors are also discussed. Epigenetics, a new field of research, links environmental factors to genetics. Understanding these factors is of great significance as changing lifestyles and improving life circumstances have started to increase the prevalence of IBD also in developing countries.

Keywords: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Pathogenesis

Abbreviations

CD: Crohn's disease; COX: Cyclooxigenase; DCs: Dendritic Cells; DR3: Death Domain-containing receptor 3; FUT: Fucosyltransferase; HLA: Human Leukocyte Antigen; HVEM: Herpes Virus Entry Mediator; IBD: Inflammatory Bowel Disease; IEC: Intestinal Epithelium Cell; IL: Interleukin; ILCs: Innate Lymphoid Cells; IF-γ: Interferon-gamma; IRF5: Interferon Regulatory Factor; Itga: α-integrin; JAK: Janus Kinase; NOD2: Nucleotide-binding Oligomerization Domain 2; NSAIDs: Non-steroid Antiinflammatory Drugs; NF: Nuclear Factor; OCTN: Organic Cation Transporters; PTPN: Protein Tyrosine Phosphatase Non-receptor type; PUFA: Polyunsaturated Fatty Acid; SLC9A: Solute Carrier family 9 member; STAT: Signal Transducer and Activator of Transcription; Th: T helper; TLR: Toll-like Receptor; TNF-α: Tumor Necrosis Factor- α; 25[OH]D: 25-hydroxi-D-vitamin; UC: Ulcerative Colitis; VDR: Vitamin D Receptor; XBP: X-box Binding Protein

Introduction

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic, remittent gastrointestinal disorders with a multifactorial pathogenesis (Figure 1). The most accepted explanation of their development is an inadequate immune response to the intestinal microbial flora, which appears in a genetically susceptible host [1]. This review aims to summarize recent updates in genetics and microbiota, complete with environmental factors and epigenetics. The role of environmental factors is supported by the fact that IBD is more prevalent in countries with a westernized lifestyle compared to developing countries. Western Europe is an outstandingly high-prevalence area, with twice as high annual incidence rates (CD: 6.3/100,000; UC: 9.8/100,000) than in Eastern Europe (CD: 3.3/100,000; UC: 4.6/100,000), although the availability of diagnostic tools is better in Eastern Europe in terms of the use of colonoscopies and diagnostic delay [2]. Methodological differences probably also exist, since incidence rates reported from Hungary, although situated in Eastern Europe, are similar to that of Western countries [3]. Interestingly, dietary risk factors of IBD were found higher among Eastern European patients, which may at least partly explain the rapid increase of the disease in this area in recent decades [4]. The increased occurrence of IBD among immigrants from developing countries to high incidence areas also provides evidence that this difference between industrialized and developing countries is not explained purely by genetics [5]. The growing incidence of IBD highlights the importance of understanding the role of environmental factors, and the use of this knowledge in the prevention and treatment of the disease.

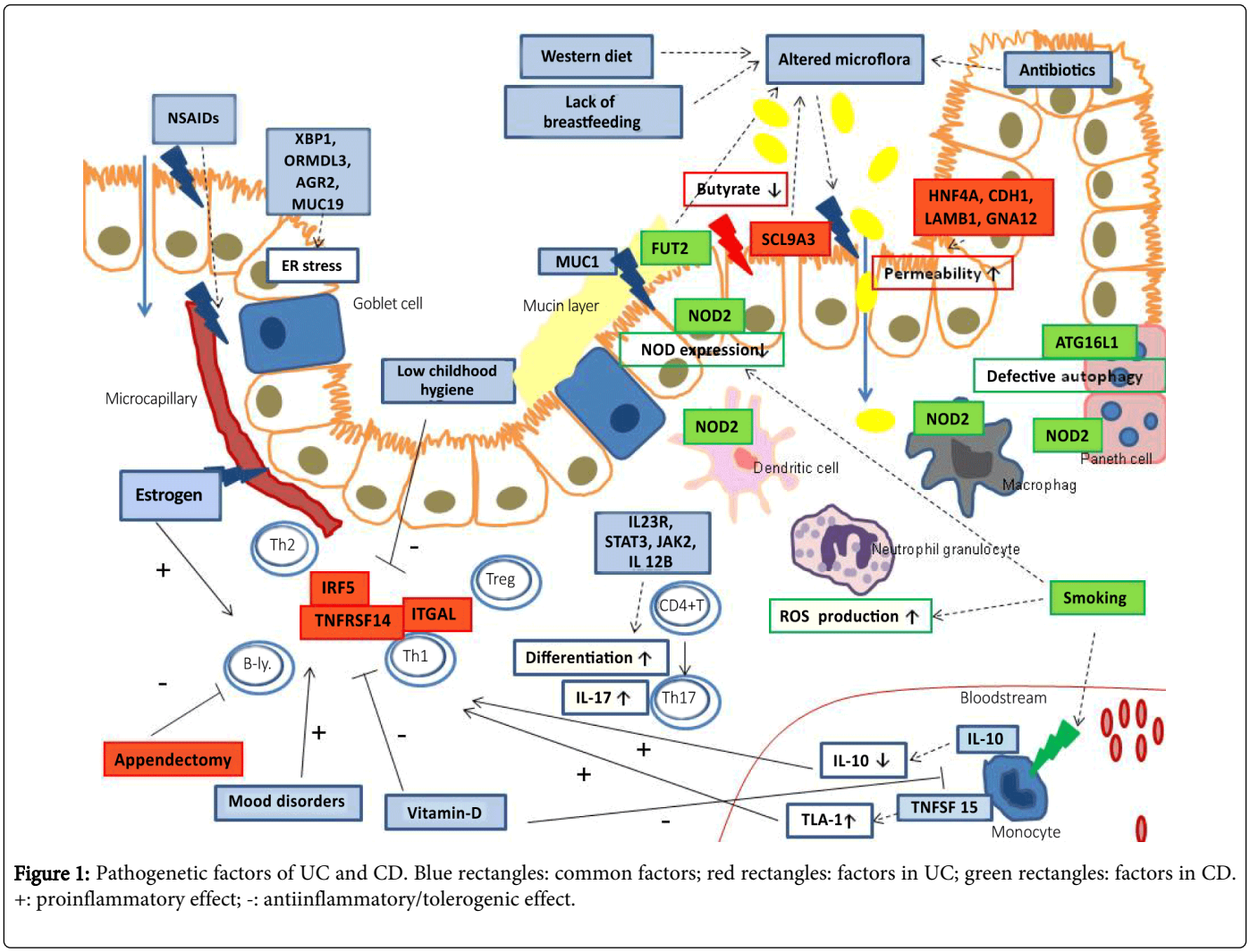

Figure 1: Pathogenetic factors of UC and CD. Blue rectangles: common factors; red rectangles: factors in UC; green rectangles: factors in CD. +: proinflammatory effect; -: antiinflammatory/tolerogenic effect.

UC and CD are separate entities, although they sometimes overlap. For instance, UC is usually present with diffuse continual mucosal inflammation involving the rectum and possibly extending more proximal in the colon, while in CD any part of the gastrointestinal tract can be affected with focal, discontinuous inflammation [6]. Endoscopically, UC presents with continous mucosal friability and small, superficial ulcers. Nevetheless, in CD, deep fissuring ulcers and aphtous lesions are seen. Histologically, the inflammation is limited to the mucosa in UC. To the contrary, in CD it is transmural, and typical histological features are the so-called granulomas. The occurrence of extraintestinal manifestations, mostly affecting the skin, the eye, the joints and the biliary system are more frequent among CD patients compared to UC patients [7]. Although the pathogenesis of UC and CD are similar in many aspects, important differences are also observed, which will be collected in this review.

The Intestinal Immune System

The immune system of the gastrointestinal tract differs from the systemic immune system in many ways. It has to create and maintain the equilibrium between the host and the large non-noxious microflora that inhabit the human intestines, and at the same time it must identify and overcome pathogens. The failure of the latter permits infections, while disturbances in the former may lead to the development of IBD [8].

The first line of defense is the intestinal epithelium cell (IEC) layer, a single, polarised columnar layer consisting of differentiated cell types such as enterocytes, Goblet cells, Paneth cells, enteroendocrine cells and M cells. Goblet cells secrete mucin glycoproteins (MUC2) that form the mucus layer, which limits colonisation to the outer, loose part, while the "inner" adherent mucus layer is almost free from microbes [9]. Paneth cells can be found in the base of small intestinal crypts, where they produce antimicrobial molecules, such as alpha-defensins, which are crucial mediators of host-microbe interactions. Paneth cells also contribute to epithelial cell renewal [10].

The next step of innate immunity is represented by intestinal macrophages, which are unique in many respects. For instance, unlike other macrophages in the body, they do not express the lipopolysaccharide co-receptor CD14 and do not produce proinflammatory cytokines in response to toll like receptor (TLR) ligands [11]. Furthermore, they secrete antiinflammatory molecules including IL-10 and contribute to the differentiation of regulatory T (Treg) cells, while they suppress the secretion of IL-17 from dendritic cells (DCs). Based on their distinct characteristics, resident intestinal macrophages are largely anergic to inflammation but still exert active

defense function against pathogens [12]. DCs are specialized antigen-presenting cells, which can coordinate innate and adaptive immune responses [13]. For example, they initiate an immunoglobulin isotype switching to IgA, and induce the differentiation of T lymphocytes to either Treg or T helper (Th) 17 cells. A newly described lymphoid cell type is the innate lymphoid cells (ILCs). ILCs develop from the common lymphoid progenitor, but lack a specific antigen receptor [14]. ILCs are classified in three groups, and secrete similar cytokines to those of Th cells [15]. Indeed, group 1 ILCs produce Th1 type cytokines, and natural killer cells are thought to belong in this group. Group 2 ILCs secrete Th2 type cytokines, and play important roles in allergy and defense from parasites. Group 3 ILCs are mostly characterized by the secretion of Th17 cytokines, and are considered to play the most important role among ILCs in mucosal homeostasis and intestinal inflammation. A subset of group 3 ILCs, lymphoid tissue inducer cells, are essential in lymph node organogenesis and development of Peyer's patches, a specific lymphoid tissue of the small bowel [16]. Another subset, NCR+ILC3 cells are a major source of IL-22, a cytokine responsible for host defense and tissue repair, but also enhancing colonization of pathogens [17].

Specific aspects of the adaptive immune system in the gastrointestinal tract are also known. Treg cells are essential for the maintenance of tolerance for the resident microflora. They produce IL-10 and transforming growth factor β in response to microbial stimuli to suppress both T-cell mediated and T-cell independent intestinal inflammation [18]. A small population of DCs constitutively secrete IL-23 in response to microbial signals, which activates Th17 cells. Th17 cells have important homeostatic functions in the intestine, apart from their role of orchestration of neutrophils during inflammation [8]. Therefore the role of Th17 cells is very complex, and they secrete both pro- and antiinflammatory cytokines, which will be detailed below.

Genetics and the Immune System

The role of genetics in the development of IBD began to be outlined in the 1980s, based on studies showing increased number of cases among patients' family members [19]. However, only genome-wide association studies were capable of providing the basis for the identification of up to 163 loci in the human genome associated with IBD until now, significantly more than in any other complex disease [20]. Nevertheless, these loci account for only 13.6% disease variance in CD and 7.5% in UC, the rest being the result of other factors, such as the environment, or "hidden heritability" caused by genetic interactions or epigenetics [20,21]. Of the 163 identified loci, 110 are linked to both CD and UC, while 30 are specific for CD, and 23 are specific for UC [20]. This fact suggests that many, but not all, of the mechanisms playing a role in CD are somewhat involved also in UC. Several genes that are associated with both CD and UC play a role in the interleukin (IL)-23 signalling pathway (such as IL23R, Janus kinase [JAK] 2, signal transducer and activator of transcription [STAT] 3 and IL12B) [22,23]. STAT3 is also important in Th17 differentiation. IL12B encodes a subunit of the interleukins IL-12 and IL-23. The association of these genes with IBD is not surprising, as IL-23 is crucial in the induction of IL-17 by Th17 lymphocytes, an important step in the pathomechanism of autoimmune diseases. These recent findings have questioned the previous theory of a clear Th2-driven inflammation in UC and Th1-driven responses in CD, and have drawn attention to the role of Th17 lymphocytes in the pathogenesis of both disorders [15]. Indeed, both UC and CD patients have increased levels of IL-17 in the serum and in the inflamed colonic mucosa [24]. The role of IL-17 was at least partly elucidated by animal experiments, which have suggested that IL-17A protects from intestinal inflammation, while IL-17F promotes it [15]. Anti-IL-17A antibodies called secukinumab were challenged in CD patients, and found not only ineffective, but to cause higher rates of adverse events, further supplying the protective role of IL-17A in intestinal inflammation [25]. However, STAT3 and JAK2 are involved in the signal transduction of many other cytokines, thus an alteration in their function may have several distinct effects. For instance in murine models, the activation of STAT3 in innate immune cells enhance mucosal barrier function, while its activation in T-cells exacerbates colitis [23].

Another example of common susceptibility loci in UC and CD is the IL-10 gene [26]. IL-10 is a key anti-inflammatory cytokine produced by monocytes and lymphocytes, which is known to inhibit synthesis of pro-inflammatory cytokines within macrophages and Th1 cells, and to suppress antigen presenting cell activity. IL-10 or IL-10 receptor deficient mice have been long used as rodent models of IBD, as they spontaneously develop intestinal inflammation [27]. Furthermore, a low IL-10 producer genotype showed an association with steroid dependency in IBD patients [28]. IL-10 receptor gene polymorphism was associated with UC and CD in children, and two specific polymorphisms were associated with very early onset of UC [29].

The transcription factor X-box binding protein (XBP) 1, a key component of the endoplasmic reticulum (ER) stress response is also linked to both UC and CD [30,31]. XBP1 is required in the cell to increase its ability to process the load of unfolded proteins. In transgenic mice, XBP1 deletion in IECs caused dysfunction of the Paneth cells and over reactivity of the epithelium to bacterial flagellin and tumor necrosis factor-α (TNF-α), and consequently resulted in a spontaneous enteritis and increased susceptibility to induced colitis [31]. It is hypothesized that similar mechanisms also play a role in human IBD, based on the gene polymorphism of XBP1 and other ER stress linked genes, e.g. ORMDL3, AGR2 and MUC19. Indeed, abnormalities connected to ER stress have been detected in uninflamed colonic mucosa of UC patients [32].

TNFSF 15, a gene encoding TL1A, a recently discovered member of the TNF superfamily, is also associated with both UC and CD [33]. Haplotype B of the TNFSF15 gene represents an increased risk of CD and a more aggressive disease course in Jewish patients, but a decreased risk of CD in non-Jewish patients [34]. Monocytes from Jewish patients carrying this haplotype react with increased membrane bounded and soluble TL1A release in response to human IgG stimulation [35]. TL1A binds to the death domain-containing receptor DR3, and is a cofactor of increasing interferon-gamma (IF-γ) release from T lymphocytes and NK cells. TL1A levels correlate with the degree of inflammation in intestinal tissue specimens from CD patients, whereas the number of DR3-positive T lymphocytes in the intestinal lamina propria are increased in IBD patients [36]. In a rodent model of chronic colitis, anti-TL1A antibodies were able to prevent chronic inflammation and to attenuate established colitis by the down-regulation of Th1 and Th17 activation, which anticipates a possible therapeutic use of such antibodies in a selected population of IBD patients [37].

Genes of the human leucocyte antigen (HLA) complex are also involved in IBD. Products of these genes have important immunoregulatory roles and are required for healthy lymphocyte

function. Many alleles have been identified lately that increase the risk of developing IBD, mostly variations of the HLA-DR gene. This belongs to the classical class II. HLA genes, which encode cell-surface glycoproteins expressed on antigen presenting cells [38]. Some alleles, most notably DRB1*0103 and B*52, are associated with both UC and CD. While DRB1*0103 predicts overall susceptibility in UC, it is associated with extensive or severe disease forms in CD.

Another important susceptibility gene is MUC1, encoding the mucin 1 glycoprotein which is normally expressed at a low level on the luminal side of colonic epithelial cells, and is required for normal epithelial defense. MUC1 is also considered as a tumor associated antigen, and is overexpressed and hypoglycosylated in IBD patients [39]. Hypoglycosylated MUC1 has chemotactic properties for the cells of the innate immune system, and IBD patients have anti-MUC1 antibodies in their serum. Vaccination against MUC1 in MUC1$^+$/IL-10 deficient mice significantly attenuated the development of colitis and prevented colitis-associated colon cancer [40]. The prevention of cancer may be explained on one hand by a specific adaptive immunity that eliminates cells with abnormal mucin expression, but on the other hand, it may be linked to the changes in microenvironment. That is, in vaccinated mice, smaller mononuclear cells represented the major infiltrate in the inflamed colonic tissue, contrary to a neutrophil-dominant infiltrate seen in non-vaccinated animals. Although it is too early for strong conclusions, development of a similar vaccination therapy for humans could open new perspectives in IBD treatment and the prevention of colon cancer.

Interestingly, 70% of IBD associated loci are common with other complex diseases [20]. More than half of them are connected to other immune-mediated disorders, mostly to diabetes mellitus type I, ankylosing spondylitis, psoriasis, and primary immunodeficiencies [20]. The genes involved in both IBD and immunodeficiencies are those causing decreased levels of circulating T-cells, (e.g. ADA and CD40), or reduction in specific T-cell lines like Th17 (STAT3), memory (SP110), or regulatory T-cells (STAT5B).

Important gene loci in UC

Increased intestinal permeability has been well described in UC. However, whether it was a preceding condition or only the consequence of inflammation was not clear. Recent studies have clarified that defects of the epithelial barrier can indeed be determined by genetic alterations [41]. Thus, HNF4A, accounting for the regulation of multiple components of the cell-cell junction, such as adherens junctions, tight junctions and the desmosomes; CDH1, a gene which encodes E-cadherin, the key component of adherens junctions; and LAMB1 encoding laminin β1 subunit, a crucial component of the intestinal basement membrane have all been implicated in UC [41]. Interestingly, CDH1 is also associated with colorectal cancer, a severe complication of UC. Additionally, two years later GNA12, encoding a membrane-bound GTPase, which plays an important role in the tight junction integrity in epithelial cells, has been added to this palette [22]. Enhanced permeability may lead to colonic inflammation by increasing the contact of luminal antigens with the mucosal immune system, thereby creating the basis of an exaggerated immune response.

The solute carrier family 9 member (SLC9A) 3 gene, encoding an epithelial Na^+/H^+ exchanger, has also been implicated in UC, but not in CD [20]. SCL9A3 deficient mice spontaneously develop chronic distal colitis, which can be reduced by broad spectrum antibiotic treatment. Similarly, when these mice are kept in an ultraclean barrier facility, the symptoms of colitis are significantly reduced [42]. Recolonisation of SCL9A3 deficient mice with the conventional microflora reinduced colitis, while reduced number of Clostridia clusters IV and XIVa were detected in the colonic content. This observation is an elegant example of the connection between genetic alterations, disturbances in the microflora and consequent colitis.

Some UC-associated genes are involved in immune functions. The genetic deficiency of α-integrin (Itga) L, encoded by the gene ITGAL, blunt the adhesion of T cells to the stimulated colonic epithelium, and decrease T cell rolling behaviour [43]. In a recent study, the ITGAL gene locus has been found to be associated with erythema nodosum, an extraintestinal skin manifestation in IBD patients [44]. The UC-specific TNFRSF14 gene encodes herpes virus entry mediator (HVEM), a member of the tumour-necrosis factor receptor family, which is a key orchestrator of mucosal immunity [45]. The role of HVEM includes the augmentation of immune responses by inducing an optimal level of STAT3 activation, which then leads to the epithelial expression of genes required for host defense. HVEM deficient animals have shown an impaired response to bacterial infection of the colon, with increased bacterial burden and mortality. Likewise, polymorphisms of the TNFRSF14 gene predispose UC patients to Clostridium difficile infections [46]. The next gene implicated in immune responses is IRF5, encoding interferon regulatory factor 5, a transcription factor which regulates activity of type I interferons and induces proinflammatory cytokines such as IL-6, IL-12 and TNF-α [22]. Primary lymphocytes from control individuals with two copies of a specific polymorphism of the IRF5 gene showed increased proinflammatory IL-12p70 production [47]. This is in agreement with data showing the association of this haplotype with IBD in Jewish patients.

Autophagy is a pathomechanism in IBD that previously has been only implicated in CD, but the association of death associate protein (DAP), also with UC suggests a possible role of autophagy in both disorders [22]. It is known that DAP is a negative regulator of autophagy, and acts as a positive mediator of programmed cell death, but its role in UC has not yet been clarified. Among HLA genes, a specific haplotype in the Japanese population was significantly associated with an increased risk of UC, and a reduced risk of non-colonic CD, demonstrating a genetic difference between the two disorders [48]. Furthermore, an excess of a specific HLA-DRB1 allele (DR13) was associated with pancolitis, surgical resection, and extraintestinal manifestations in UC patients [49].

Important gene loci in CD

It is already known from twin studies that genetic heritability is more expressive in CD than in UC [50]. In the update of the first Swedish monozygotic twin study, disease concordance rates of 15% for UC and 27% for CD were found [51]. Location and behaviour of the disease in monozygotic CD patients was highly concordant both at diagnosis and 10 years later [50,51]. A recent study investigating all known 163 IBD risk gene loci in CD and UC patients has found that increased genetic burden was associated with early diagnosis of CD, but not of UC [52]. Ileal disease was also predicted by higher genetic burden in CD, however, in UC there was no association with disease phenotype. Accordingly, ileal CD is probably determined by genetic factors, while CD with colonic localisation can be explained more by environmental factors [51].

The most well-known gene associated with CD is CARD15, encoding nucleotide-binding oligomerization domain 2 (NOD2)

[53,54]. NOD2 plays a role in pattern recognition of pathogen microbiota, activates nuclear factor (NF)-κB in monocytes following lipopolysaccharide stimulation, and leads to the induction of proinflammatory cytokines. Three common and several rare NOD2 mutations have been identified so far, affecting almost exclusively the leucine-rich repeat region of NOD2, which is in the ligand-binding domain of the receptor [55]. NOD2 is expressed in Paneth cells, DCs, macrophages and IECs, but the mediating cell type and the mechanism by which NOD2 gene polymorphism leads to CD is still not clear. Animal studies have shown that NOD2 deficiency caused impaired intestinal barrier function and predisposed to intestinal bacterial infections [56]. Ileal CD patients have been demonstrated to present reduced levels of the antimicrobial peptide alpha-defensin originating from Paneth-cells [57]. This decrease was more pronounced in patients with a $NOD2_{1007fs}$ variant, and was suggested to alter the intestinal microbial flora and compromise innate immunity. However, this observation has been questioned by others, claiming that the reduced alpha-defensin expression is simply the consequence of inflammation and independent of NOD2 status [58]. Furthermore, in a recent study on C57BL/6 mice, NOD2 deficient animals presented with equivalent defensin levels and identical antimicrobial activity against commensal and pathogenic bacterial strains as their wild type littermates, and their gut microbial composition was also similar [59]. Nevertheless, other animal experiments have suggested distinct mechanisms connecting NOD2 deficiency to CD. In a murine model, NOD2 deficiency increased Toll-like receptor (TLR) 2-mediated activation of NF-κB, and resulted in enhanced Th1 responses, a characteristic of CD [60]. Additionally, chronic stimulation of NOD2 caused tolerance to a repeated induction by NOD2, TLR 2 or TLR 4 ligands, and decreased the production of the proinflammatory cytokines in macrophages from a large cohort of individuals, but not in macrophages with a mutant NOD2 variant [61]. This suggests that NOD2 polymorphism may predispose to CD by decreasing tolerance to the resident microbial flora. CD in patients carrying at least one major variant of CARD15 tends to show a more aggressive clinical course [62]. Interestingly, CD risk alleles of NOD2 were found significantly protective against UC [20].

A relatively new discovery in the pathogenesis of CD is the association of the disease with autophagy genes (ATG16L1 and IRGM). Autophagy is a catabolic process that ensures the breakdown and recyclation of damaged or unnecessary cellular components or intracellular bacteria via the lysosomes. Deficiency in autophagy related to a homozygous ATG16L1 allele causes impaired engulfment and degradation of the cytoplasmic content and microorganisms, defective presentation of bacterial antigens to $CD4^+$ T-cells, and increased inflammatory cytokine production by Paneth-cells [55]. NOD2 and ATG16L1 are involved in common processes, as bacterial cell wall components can stimulate autophagy and increase killing of Salmonella in human colonic epithelial cells via a NOD2 dependent pathway [63]. However, in cells transfected with CD-associated NOD2 variants, both autophagy and antimicrobial activity were defective. Furthermore, another CD associated gene, protein tyrosine phosphatase non-receptor type (PTPN) 22 controls NOD2 induced cytokine release and autophagy, and its deficiency causes an enhanced monocytic response towards bacteria [64]. Perturbation of bacterial killing and defective antimicrobial pathways may cause an uncontrolled inflammation and ultimately lead to CD [55].

The mucus layer, an important component of the innate protective barriers is also affected in CD [65]. Gene loci associated with mucus production (MUC1, MUC19) [30,66], and more specifically to CD, fucosyltransferase (FUT) 2, a gene encoding alpha 1,2 fucosyltransferase, are all implicated [65]. FUT2 regulates gastrointestinal mucosal expression of blood group A and B antigens, and 20% of Caucasians are non-secretors. It has been shown that the non-secretor state is associated with altered mucosal microbial flora, and susceptibility to different infections beside CD [67]. In non-secretors, the metabolism of luminal microbiota was also disturbed, accompanied by a subclinical inflammation of the intestinal mucosa [68]. The metabolism of the epithelium in CD may also be impaired, as the functional variants of genes encoding the organic cation transporters (OCTN) -1 and -2 are linked to the disease [69]. In the last decade, polymorphism of OCTN has been associated with increased risk [70] or perianal and penetrating forms of CD [71], and even with UC [72]. The impaired function of OCTN can reduce carnitine transport to cells, which may lead to defects in oxygen burst –mediated pathogen killing or altered fatty acid β-oxidation in the intestinal epithelium. Regarding HLA genes, a CD-specific haplotype has also been described. Indeed, HLA-DRB1*0401 is a susceptibility allele in CD and a protective allele in UC, although the effect seems weak and population-specific [38].

Intestinal Microbiota

The intestinal microbial flora, consisting of up to 15,000-36,000 different species of bacteria, numerous fungi, viruses and bacteriophages, is a crucial component in the pathogenesis of IBD. Animal models have demonstrated that the presence of a bacterial flora is essential for the development of colitis [73,74]. The main theory suggests that a dysregulated immune activation in a genetically susceptible individual leads to an excessive response to the normal microbial flora. However, others suggest that it is an altered microbial flora which provokes an abnormal response from the normal immune system in these patients. Our current understanding assumes that the reality is probably a combination of both hypotheses [15]. The dominant members of intestinal bacterial flora in healthy individuals are Bacteroidetes and Firmicutes, but Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia are also present [75]. Several data exist showing an altered microbial composition both in UC and CD, however, examinations have failed to identify a single bacterial strain specific to these diseases [76].

Dysbiosis in UC

The biodiversity of active bacteria is reduced in UC patients compared to controls [77]. A decreased number of gastrointestinal health-promoting bacteria, Bacteroidetes and Lachnospiraceae, were found in surgical specimens from UC patients by rRNA sequence analysis compared to controls [76]. Another study has shown a decrease of the beneficial Bifidobacteria in rectal biopsy specimens of UC patients, parallel to an increase of *Clostridia* and *E. coli* [78]. *E. coli* is also overrepresented among active fecal bacteria of UC patients, and is suggested to have a role in the development of colitis [77]. The number of *Faecalibacterium prausnitzii*, associated with mucosal protection, was also reduced in fecal samples of UC patients recovering from a relapse [79]. Similarly, in an *in vitro* dynamic gut model called M-SHIME, butyrate producing bacteria were less abundant and butyrate production was reduced among luminal microbiota from UC patients, which may cause energy deficiency in colonocytes [80].

Dysbiosis in CD

The intestinal bacterial profile in CD also differs from healthy controls [81]. The abundance of mucosa-protective *Faecalibacterium prausnitzii* is reduced, while the number of potentially harmful *Clostridia* and *Escherichia* are increased in CD patients. Patient-to-patient variations are greater than differences between healthy controls, suggesting that individuals predisposed to CD are less able to regulate the microbial composition of their intestines. Occasionally, opportunistic pathogens, such as *Enterobacter*, *Proteus*, *Haemophylus* and *Klebsiella*, were also found in the mucosal samples of these patients, but had never detected in the samples from healthy controls. The importance of *Faecalibacterium prausnitzii* has been underlined by the fact that a decreased number of these bacteria in the ileal mucosa at the point of surgery are associated with increased endoscopic recurrence rates 6 months after surgery in CD [82]. This observation is in accordance with animal studies showing that *Faecalibacterium prausnitzii* and its supernatant also displays anti-inflammatory properties in rodent colitis models [83].

Environmental Factors

Smoking

Smoking is the most studied environmental factor in IBD, and is also a particular factor where CD and UC clearly split up.

UC

The first report on non-smoking as a risk factor in UC was published in 1982, and several more have confirmed this observation [84]. Smoking is associated with lower onset risk, fewer hospitalizations, lower relapse rate and decreased need for colectomy in UC [85-87]. Quitting smoking increases the risk to develop UC in the following years, and this risk remains elevated over 20 years [88,89]. Passive smoking is not protective against UC, and it predisposes to ileal disease (pouchitis and backwash ileitis) compared to non-passive smokers [90]. The pathomechanism of the effect of smoking in IBD is not yet established. However, it is generally believed that the beneficial effect of smoking in UC is mostly related to nicotine [91]. Nicotine increases mucine secretion in the colon, provokes the release of NO, and has several anti-inflammatory properties [92-94]. IgA concentration is markedly decreased in the intestinal fluid of smoking UC patients compared to healthy non-smokers [95]. Smoking also increases the level of suppressor $CD8^+$ T cells, decreases the synthesis of proinflammatory molecules, such as Il-1β, IL-2, IL-8, TNF-α, and impairs phagocytosis, which may explain its positive effects in UC [91].

CD

Contrary to UC, passive and active smoking is both risk factors of developing CD, and this risk can be attenuated by cessation of smoking [89,90,96,97]. Several studies have found that steroid and immunosuppressive therapy are more frequently needed, recurrence rates are significantly higher and surgery is more often required in CD patients who are currently smoking [98-100], although these observations are questioned by others [101]. Deleterious effects of smoking in CD may be related to different mechanisms than described above, such as the increase of oxygen free radical production by polymorphonuclear leukocytes in smokers, smoke-related functional impairment of monocytes in CD patients, and reduced expression of NOD2 in intestinal epithelial cells by cigarette smoke [102-104].

Appendectomy

The appendix is an important lymphoid organ along the gastrointestinal tract, which helps to maintain the healthy gut microbial flora by modulating the epithelial regeneration and protecting from pathogen microbes via the production of biofilms and compounds such as mucin and IgA [105]. It may also regulate the immune response to host microflora by acting as a reservoir of enteric bacteria [106]. The possible role of the appendix in the development of UC and CD has been raised following recent epidemiological studies.

UC

Large number of studies have found a highly significant negative association between appendectomy and UC [106]. Indeed, previous appendectomy, especially before the age of 20, protects from UC, delays the onset of the disease, and is associated with a milder disease course [107]. Previous observations have highlighted a crucial role of the humoral immune system and B-lymphocytes in UC, therefore the removal of the appendix rich in B-lymphocytes in a genetically susceptible individual may be sufficient to prevent or ameliorate the disease [107]. Another hypothesis claims no causal relationship between appendectomy and UC, but assumes that appendectomized individuals probably represent a distinct group in the population in terms of genetic or environmental risk factors. This is supported by observations that appendectomy prevents UC only if performed on an inflamed appendix or in the case of mesenterial lymphadenitis, and before the age of 20, but not in cases of non-specific abdominal pain [108].

CD

The link between appendectomy and CD are controversial. Although most studies report an increased risk, protective or no effect was also found in some cases [107,109]. The fact that the increased risk of CD returns to normal levels 5 years after appendectomy suggests that it is probably related to diagnostic problems in incipient CD, and is not a real causative factor [75]. That is, the misleading right lower quadrant pain incite clinicians to perform appendectomy in a notable amount of cases, before the diagnosis of CD is established.

Diet

Dietary intake has dramatically shifted to a high-fat, high-carbohydrate diet in Western countries in the last decades, which is thought to increase the risk of several chronic illnesses. However, obesity as measured by the body mass index by itself is not associated with the development of UC or CD [110]. A systematic review of the literature has revealed that high intake of saturated fats, total polyunsaturated fatty acids (PUFAs), omega-6 fatty acids and meat predisposes to the development of both CD and UC, whereas dietary fiber and fruit consumption decreases the risk of CD, and high vegetable intake reduces the risk of UC [111]. Another study has confirmed that low intake of fruits and/or vegetables were associated with an increased onset risk of IBD, and grain consumption may have a protective role in CD [112]. High intake of sugar was also identified as a risk factor of the development of IBD. Furthermore, regular consumption of fast food was associated with an increased risk [113] and a younger age at diagnosis [4] of both UC and CD. IBD patients,

especially those suffering from CD, are clearly convinced of the importance of diet; 70% of them rating it to be as important as medical therapy [114]. Further, 86% of patients could identify drinks and specific food as provocative factors for a relapse, mostly alcoholic beverages, soda, coffee and strong spices.

Recent evidence has made it clear that changes in diet can induce significant modifications in the components of the colonic microbial flora. In germ-free mice colonized with human fecal microbiota, switching from a low-fat, plant polysaccharide-rich diet to a high-fat, high-sugar "Western" diet altered microbiota composition after only one day, together with changes in methabolic pathways and microbiome gene expression [115]. The mechanism of such microbial alteration was explored in a recent study, where a diet rich in high-saturated fat (derived from milk) and low in PUFA promoted taurine-conjugation of hepatic bile acids, which then increased the availability of organic sulfur, inducing the proliferation of the sulfite-reducing *Bilophila wadsworthia* [116]. These changes were followed by a Th1 immune activation and increased incidence of colitis in genetically susceptible IL-10$^{-/-}$, but not in wild type mice. In humans, probably one single dietary factor can not be responsible for the development of IBD. Nevertheless, modification of the microbial flora by a favorable diet or by dietary supplements could help to induce and maintain remission [117].

Vitamin-D

Vitamin-D deficiency in IBD could be the consequence of the disease itself, due to insufficient intake, malabsorption, reduced sunlight exposure and low physical activity. Nevertheless, lower levels of vitamin-D are observed already in newly diagnosed patients, suggesting a possible role in the pathogenesis [118]. In a prospective study on female nurses, higher predicted 25-hydroxi-D-vitamin (25[OH]D) plasma levels significantly reduced the risk for the onset of CD and nonsignificantly reduced the risk for UC [119]. Serum 25-(OH)D was also inversely associated with disease activity in CD [120]. Further, human genome mapping has revealed recently that vitamin D receptor (VDR) binding sites are significantly enriched near PTPN2, a gene associated with Crohn's disease [121]. In the last decade new data has been collected on the effects of vitamin D on human health apart from calcium and phosphate homeostasis and bone mineralization, which could explain its role in the pathogenesis of IBD [122]. Vitamin D influences both the innate and the adaptive immune system by several pathways [122]. It is required for the antibacterial responses via toll-like receptor (TLR) activation and plays a role in the regulation of autophagy [123]. Two different forms of vitamin D, 1,25(OH)2D and 25(OH)D(3) dose-dependently inhibit lipopolysaccharide-induced production of IL-6 and TNF-α in human blood monocytes [124]. Vitamin D modifies the function of both T and B lymphocytes, prompting them to shift from a proinflammatory to a more tolerogenic immune status [122,123]. Experimental data also supports the role of vitamin D-deficiency in intestinal inflammation. In IL-10 KO mice, vitamin D-deficiency significantly accelerated the development of spontaneous enterocolitis, and mortality was dramatically increased compared to animals with sufficient vitamin D levels [125]. Intestine-specific VDR KO mice were more susceptible to experimental colitis than controls [126]. These observations can explain the pathogenic role of vitamin D-deficiency in autoimmune diseases, and underlines the importance of optimal vitamin D levels in IBD patients.

Medications

Some medications are suspected to participate in the development and in relapses of IBD [127]. First of all, previous antibiotic use has been suggested to increase the risk of IBD, particularly CD, by altering the intestinal flora. Antibiotic use in the first year of life was associated with increased risk of IBD–mostly CD-in childhood [128,129]. Furthermore, in several studies on adult patients, antibiotic treatment 2-5 years preceding the diagnosis was associated with CD [130,131] or with both CD and UC [132]. Out of the different types of antibiotics, the strongest correlation was found with metronidazol and the weekest with penicillin, while clindamycin showed no correlation [132]. Another study has noted a high association of doxycycline with CD 84, confirmed by recent observations in Australians, when deployement to a developed country and doxycyclin use was found as a risk factor of IBD onset [133]. Although this data shows an increased antibiotic use preceding the diagnosis of IBD, the causative link may be questioned. That is, it can be speculated that antibiotic treatment in childhood may be necessary for bacterial infections more easily acquired in future IBD patients based on their genetic background, while in adults high rate of antibiotic use preceding the diagnosis may be the consequence of a misinterpretation of the initial abdominal symptoms.

The next group of medications implicated in the pathogenesis of IBD are the non-steroid anti-inflammatory drugs (NSAIDs). Many of them are widely prescribed in the general practice, therefore it is crucial for clinicians to be aware of the risk of these medications, particularly in case of known IBD patients. Numerous studies have found a positive correlation between the use of NSAIDs and the onset of UC [134], CD [135] or both [136,137]. This can be mediated via several mechanisms, most notably, NSAIDs inhibit the cyclooxigenase (COX) enzyme, thereby decreasing the synthesis of prostaglandins, which are important to mucosal integrity and function [127]. They also alter mucosal microcirculation and cause direct damage to the epithelium, resulting in a permeability increase. The use of selective COX-2 inhibitors are considered somewhat safer than classical NSAIDs. Nevertheless, experimental and clinical data are not sufficient, and their prescription in IBD patients is suggested with caution [138].

Oral contraceptives can be capable of provoking IBD by the proinflammatory properties of estrogen and its effects on the mucosal microcirculation [127]. Several publications have reported an increased risk of CD [97,139] or both CD and UC [140] among women using oral contraceptives. Similarly, a recent prospective cohort study has found that oral contraceptive use increased the risk of CD, but elevated risk of UC was only observed in women with a history of smoking [141]. Postmenopausal hormone therapy has also been associated with the onset of UC [142] or CD [140]. Other studies have found no link between oral contraceptives and disease relapse [143]. Altogether the results are insufficient and controversial, therefore the use of oral contraceptives is still accepted in IBD, as accurate family planning is particularly important in these patients.

Anti-TNF-α monoclonal antibodies, namely infliximab and adalimumab are effectively used in IBD, unlike etanercept, a soluble TNF-α receptor, which was ineffective in moderate to severe CD patients [144]. Anti-TNF-α therapy is also applied in inflammatory rheumatic diseases, where it was associated with the paradoxical development of IBD in some cases. A retrospective study conducted in France has identified four new-onset cases of IBD during anti-TNF-α treatment in 296 patients with spondyloarthropathy, three receiving etanercept and one infliximab [145]. Another report from France

collected data from 16 patients with *de novo* IBD treated with etanercept (14 cases) or infliximab (2 cases) for inflammatory rheumatic diseases [146]. Among these patients, eight were identified as CD, six as CD-like, one as UC and one unclassified. On one hand, the explanation of these paradoxical adverse events may be that spondyloarthropathies are associated with IBD, and the doses applied in rheumatic diseases are simply not sufficient to prevent the development of an incidental extraskeletal manifestation [145]. On the other hand, etanercept is associated with increased production of TNF-α and INF-γ in $CD4^+$ T-cells, contrary to the downregulation seen in infliximab treatment, which may explain its property to induce a new-onset IBD [147].

Breastfeeding

The role of breastfeeding at childhood is still a question of debate in the pathogenesis of IBD [148]. Some studies have found that breastfeeding is a risk factor for CD [149] or has no effect on the development of either CD or UC [150]. However, a large meta-analysis has concluded that breastfeeding is protective against UC and even more protective against CD [151]. Based on this data, the recent revisions of the Nordic Nutrition Recommendations have stated that breastfeeding has probable evidence against IBD [152]. Nevertheless, they also admit that most studies were retrospective, and sufficient data based on prospective studies are missing. Breastfeeding with normal milk (from wild-type mothers) has significantly reduced TNF-α and IFN-γ secretion and prevented development of colitis in IL-10 deficient mice, with normal levels of colonic adherent bacteria [153]. The reason for this beneficial effect is probably based on the development of a more advantageous intestinal flora rich in *Bifidobacterium* and *Lactobacillus* in the pups, stimulated by oligosaccharides found in the milk. Similarly, in human infants, the nature of early nutrition determines the constitution of the microbial flora, and influences the development of the immune system, which, if defective, may lead to the onset of IBD by an excessive immune response to the normal flora [154].

The "hygiene hypothesis"

Early postnatal and childhood infections can shape the immune system. It is well established that high sanitary standards and low exposure to infectious agents in childhood increases the risk of atopic diseases [155]. The lower incidence of IBD in developing countries suggests that these factors also predispose to the development of CD and UC. Nevertheless, studies aiming to clarify the association between childhood exposure to infectious agents and the risk of IBD have been inconclusive so far. For example, having a pet in childhood was shown to increase the risk of CD [156], while regular contact with farm animals or a cat in infancy was indeed protective against both CD and UC [157]. However, clear evidence was found on the beneficial effect of *Helicobacter pylori* infection and helminthiasis in IBD [158,159]. Less clear is the validity of the "cold chain hypothesis", suggesting that the development of CD is in association with the use of food refrigeration, which increases the possibility of particular infections such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium botulinum* and *Bacillus cereus*. The evidence supporting a causative role of in utero or perinatal measles in the development of CD is also insufficient. Furthermore, results of well-designed studies did not support a link between vaccination against measles and the risk of of IBD. Similarly, the role of infectious gastroenteritis in childhood is also controversial. Some studies have shown an increased risk of CD, while others have claimed a decreased risk of both CD and UC [160]. Several studies have reported that higher domestic hygiene, such as hot water tap and higher socioeconomic status, are associated with the development of CD, but not with UC, by preserving the individual from contact with infectious agents, and predisposing to an inappropriate immune response, when the exposure occurs later [157]. Although hygiene practices and bathroom facilities have markedly improved in the last decades, and they therefore tend to lose their influence, a recent study has still identified higher social status, higher educational level of parents and living in urban areas as important risk factors for both UC and CD [160]. Novel experimental data have suggested that the background of these observations may lie at least partly on the ability of early gut microbial exposure to regulate the expression of chemokine receptor CXCR6 on mucosal invariant natural killer cells, modulating the accumulation and function of these cells in the colon and lungs, and consequently altering inflammation [161].

Personality traits

IBD was long considered to be psychosomatic disorder, and over 70% of patients still believe that the development of the disease is related to stress or their own personality [162]. Significant personality differences have been found in IBD patients compared to their healthy siblings, summarized as a fixation of dependency and lack of autonomy [163]. These characteristic personality traits in IBD could result from the longstanding chronic illness. However, a study examining a group of patients previous to an established diagnosis has shown that increased levels of neuroticism and introversion were highly prevalent, even before the diagnosis was established [164]. Further characteristics are perfectionism, and alexithymia (difficulty in recognizing, regulating and expressing emotions), which can lead patients to show distress by developing somatic symptoms instead of verbal communication [165,166].

Stress and mood disorders

Several studies have concluded that anxiety and depression are common in IBD patients, and their severity parallels the disease activity [167-170]. These psychological conditions may not simply be the consequences of the chronic illness. In a retrospective study both depression and anxiety preceded UC, but not CD, significantly more often than expected by chance [171]. Mood disorders may initiate and deteriorate inflammation by enhancing the release of proinflammatory mediators and by disturbing the immunoregulatory circuits [172,173]. The central nervous system is strongly connected with the enteric nervous system by several pathways, creating the so-called brain-gut axis. It is well known that stress induces central and peripheric secretion of corticotropin-releasing factor, which has been shown to inhibit upper GI motility and stimulate colonic motility, induce colonic mast cell degranulation, and increase colonic permeability in animal studies [174,175]. Increased permeability may lead to inflammation by altering host-microbial interactions, and consequently result in visceral hypersensitivity [176]. Stress also provokes the release of pro-inflammatory Th1 cytokines, such as TNF-α and neuropeptides, such as tachykinins [177]. In animal experiments, colitis can be exacerbated by early life stress (neonatal maternal deprivation) in genetically susceptible IL-10 KO mice, but not in wild type mice, probably through impaired intestinal barrier function [178]. Although most patients consider stressful life events important in the development of their disease, one study focusing on

life events occurring no more than six months prior to the onset of diagnosis failed to find any correlation in UC patients compared to controls [179]. In CD patients, life events occured more frequently in the past 6 month preceding diagnosis, however, the association was no more significant when other factors were also taken into account. A metaanalysis pointed out that stress in UC and depressive symptoms in CD play roles in the disease course, but it also could not find evidence that they contribute to disease onset [180]. Recently it was also shown that job strain, indicating work-related stress, did not correlate with the onset of either CD or UC [181]. Taken together, stress and psychological issues are clearly important in the disease course and in the management of IBD patients, but their role in the pathogenesis could not be convincingly established so far.

Epigenetics

Environmental factors may lead to the development of IBD by various mechanisms, which are detailed above. However, a new field of research, namely epigenetics, which links environmental factors to genetics in IBD has been evolving. Epigenetics describe mitotically heritable modifications in gene expression not explained by changes in the DNA sequence [21]. Important epigenetic mechanisms are DNA methylation, histone modification, RNA interference, and the positioning of nucleosomes. Epigenetic changes are likely to occur in the prenatal and early postanatal periods, and animal studies have shown that DNA methylation links transient nutritional influences to a permanent phenotype modification [182]. A clearly visible example of this phenomenon is observed, when Avy (viable yellow Agouti) mice during pregnancy receive oral methyl-donor supplementation, which induces DNA methylation of the Agouti gene in the offspring, resulting in a decreased gene expression and brown-coloured offspring instead of yellow [183]. Recent data suggest that similar processes may work in humans, linking under nutrition during gestation and infancy to a higher risk of childhood infections and death by differential methylation at genes associated with defense against infection and immune response [184]. Methyl-donors are abundant in human maternal dietary supplements. In mice, maternal methyl-donor supplementation augmented susceptibility to colitis in offspring, parallel to colonic mucosal DNA methylation and expression changes, followed by a sustained effect of the diet on colonic mucosal microbiota [185]. Although similar data in humans is missing, a study on ileal CD patients has found that several of the known CD risk gene loci were enriched in methylation compared to healthy controls, including the Th17 pathway and NOD2 [186]. More studies are needed to elucidate the effects of periconceptional and prenatal nutrition on DNA methylation in humans, and to assess its possible role in the pathogenesis of IBD.

Conclusions

Knowledge of the pathogenesis of IBD has been rapidly growing in the past decades. Previously considered as a "psychosomatic disorder", IBD has now evolved to an excessive immune response to the intestinal microflora in genetically susceptible individuals, influenced by several environmental factors. In our review we have summarized current knowledge of genetic and environmental factors participating in the development of IBD, with special focus on differences between UC and CD. Recent progress in the field of genetics has allowed us to understand new mechanisms participating in the pathogenesis, such as the involvement of Th17 cells in both UC and CD, or the role of autophagy in CD. These genetic discoveries create the basis for developing novel biological therapies or the expansion of existing therapies, which were originally developed for other diseases, to IBD [187]. For example based on the recently described pathway of IL-23/Th17 in IBD, ustekinumab (Janssen-Cilag), a human anti-interleukin-12/-23 monoclonal antibody, which was previously applied in psoriasis, has been successfully tested in moderate to severe CD [188]. Nevertheless, a randomized clinical trial in active CD patients with everolimus (Novartis), an inducer of autophagy, was terminated in the enrollment phase due to the lack of efficacy [189]. This underlines the role of carefully designed studies in the future, where patients are genetically selected and only patients with defective autophagy are enrolled [187]. Such studies would be milestones on the road leading to personalized therapy, based on each patient's genetic profile. Another possible use of genetic profiling would be to predict the disease course, and thus individualize therapy intensity. As mentioned above, some genetic variations, such as CARD15 mutations or specific HLA alleles are associated with more extensive disease or aggressive disease course, but they are not frequent or not significant enough to be used in the clinical practice. Genome-wide association studies have highlighted several common pathways among UC and CD, but differences are also present, which may explain the overlapping, yet usually distinguishable clinical entities. As these genetic variations are rare, developing a combined test based on different genetic markers specific to either UC or CD, such as NOD2 or some HLA alleles could be useful in the differential diagnosis in problematic cases, e.g. in children. Nonetheless, genetic screening of the general population to predict the onset of IBD would probably not be successful, as in most patients, genetic background in association with environmental factors leads to the onset of the disease. Thorough understanding of the role of environmental factors is extremely important, as they are easier to modify in order to prevent the onset or the relapse of IBD (Table 1). Nevertheless, in the case of many environmental factors, large-scale studies are missing, and results are controversial probably due to methodological differences, making interpretation difficult. It seems clear that dietary factors, reduced vitamin-D levels, and specific medications can participate in the development of IBD. These factors can be surveyed and adjusted in IBD patients to minimize the number of relapses. Furthermore, they also can be controlled in predisposed individuals, e.g. in a patient's family members to reduce the risk of developing IBD. Patients tend to avoid specific foods that they believe provoke their symptoms, and a diet rich in fruits and vegetables and low in fat and sugar is advisable for many reasons beside IBD. Optimal vitamin-D level can be achieved in IBD patients. Among specific medications, it is generally believed that excessive prescriptions of antibiotics should be avoided to prevent the development of bacterial resistance and to preserve the normal colonic flora. Relapses in IBD, however, often require the use of antibiotics. NSAID consumption should be minimized in IBD patients to avoid relapses. Smoking is a strong risk factor of CD and a protective factor of UC. Nevertheless, it is advisable that UC patients stop smoking, considering the several well-known harmful and carcinogenic effects of smoking on other organs of the body. Appendectomy is protective in UC, while breastfeeding seems beneficial against both UC and CD, but inverse results also exist. The role of childhood infections is controversial. However, high hygienic standards in the early ages may predispose to IBD by reducing contact with infectious agents during development of the immune system. Specific personality traits may facilitate the development of IBD. Stress and psychosomatic disorders affect the disease course. Therefore patients with psychological issues should be encouraged to see a specialist for treatment, which can result in longer remissions.

Environmental factors may contribute to IBD in the prenatal and early postnatal period by mitotically heritable modifications in gene expression, such as DNA-methylation. Further research can shed light on the nature and importance of such factors, for example the possible role of maternal dietary supplements in epigenetic changes. Improving life standards and propagation of Western lifestyle promotes the increasing prevalence of IBD all over the world, including in developing countries. Therefore more large-scale, prospective studies are needed to firmly establish knowledge on the contributing factors to help us in the prevention and to open new perspectives in IBD therapy.

Factor	UC	CD
smoking	---	+++
appendectomy	---	+/0
dietary factors		
high fat	+	+
high dietary fiber	-	-
high fruit/vegetable	-	-
high sugar	+	+
fast food	+	+
vitamin D deficiency	+	+
medications		
antibiotics	+	+
NSAIDs	+	+
oral contraceptives/hormone therapy	+	+
anti-TNF-α therapy	+	+
breastfeeding	-/?	-/?
hygiene hypothesis		
pet in childhood/farm environment	-/?	?
Helicobacter pylori	-	-
helminthiasis	-	-
measles	?	?
vaccination against measles	?	?
"cold chain"	0	+/?
infectious gastroenteritis in childhood	?	?
urban environment	+	+
stress	+/?	+/?
"-": decreased risk; "+": increased risk; "0": no effect; "?": question of debate		

Table 1: Environmental risk factors of UC and CD.

References

1. Xavier RJ, Podolsky DK (2007) Unravelling the pathogenesis of inflammatory bowel disease. Nature 448: 427-434.
2. Burisch J (2014) Crohn's disease and ulcerative colitis. Occurrence, course and prognosis during the first year of disease in a European population-based inception cohort. Dan Med J 61: B4778.
3. Lakatos L, Mester G, Erdélyi Z, Balogh M, Szipócs I, et al. (2003) Epidemiology of inflammatory bowel diseases in Veszprém county of Western Hungary between 1977 and 2001. Orv Hetil 144: 1819-1827.
4. Burisch J, Pedersen N, Cukovic-Cavka S, Turk N, Kaimakliotis I, et al. (2014) Environmental factors in a population-based inception cohort of inflammatory bowel disease patients in Europe--an ECCO-EpiCom study. J Crohns Colitis 8: 607-616.
5. Ko Y, Butcher R, Leong RW (2014) Epidemiological studies of migration and environmental risk factors in the inflammatory bowel diseases. World J Gastroenterol 20: 1238-1247.
6. Bousvaros A, Antonioli DA, Colletti RB, Dubinsky MC, Glickman JN, et al. (2007) Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. J Pediatr Gastroenterol Nutr; 44: 653-674.
7. Vavricka SR, Brun L, Ballabeni P, Pittet V, Prinz Vavricka BM, et al. (2011) Frequency and risk factors for extraintestinal manifestations in the Swiss inflammatory bowel disease cohort. Am J Gastroenterol 106: 110-119.
8. Cader MZ, Kaser A (2013) Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. Gut 62: 1653-1664.
9. Kim YS, Ho SB (2010) Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr Gastroenterol Rep 12: 319-330.
10. Clevers HC, Bevins CL (2013) Paneth cells: maestros of the small intestinal crypts. Annu Rev Physiol 75: 289-311.
11. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol 8: 1086-1094.
12. Smith PD, Smythies LE, Shen R, Greenwell-Wild T, Gliozzi M, et al. (2011) Intestinal macrophages and response to microbial encroachment. Mucosal Immunol 4: 31-42.
13. Rescigno M (2010) Intestinal dendritic cells. Adv Immunol 107: 109-138.
14. Spits H, Cupedo T (2012) Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annu Rev Immunol 30: 647-675.
15. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ (2014) Immunopathology of inflammatory bowel disease. World J Gastroenterol 20: 6-21.
16. Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, et al. (2004) An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 5: 64-73.
17. Behnsen J, Jellbauer S, Wong CP, Edwards RA2, George MD3, et al. (2014) The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. Immunity 40: 262-273.
18. Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, et al. (2003) CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. J Exp Med 197: 111-119.
19. Lashner BA, Evans AA, Kirsner JB, Hanauer SB (1986) Prevalence and incidence of inflammatory bowel disease in family members. Gastroenterology 91: 1396-1400.
20. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, et al. (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 491: 119-124.

21. Ventham NT, Kennedy NA, Nimmo ER, Satsangi J (2013) Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics. Gastroenterology 145: 293-308.
22. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, et al. (2011) Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 43: 246-252.
23. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, et al. (2008) Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40: 955-962.
24. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, et al. (2003) Increased expression of interleukin 17 in inflammatory bowel disease. Gut 52: 65-70.
25. Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, et al. (2012) Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut; 61: 1693-1700.
26. Lv H, Jiang Y, Li J, Zhang M, Shang Z, et al. (2014) Association between polymorphisms in the promoter region of interleukin-10 and susceptibility to inflammatory bowel disease. Mol Biol Rep 41: 1299-1310.
27. Shouval DS, Ouahed J, Biswas A, Goettel JA, Horwitz BH2, et al. (2014) Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans. Adv Immunol 122: 177-210.
28. Castro-Santos P, Suarez A, Lopez-Rivas L, Mozo L, Gutierrez C. (2006) TNFalpha and IL-10 gene polymorphisms in inflammatory bowel disease. Association of -1082 AA low producer IL-10 genotype with steroid dependency. Am J Gastroenterol 101: 1039-1047.
29. Moran CJ, Walters TD, Guo CH, Kugathasan S, Klein C, et al. (2013) IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm Bowel Dis 19: 115-123.
30. Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, et al. (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42: 1118-1125.
31. Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, et al. (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 134: 743-756.
32. Treton X, Pedruzzi E, Cazals-Hatem D, Grodet A, Panis Y, et al. (2011) Altered endoplasmic reticulum stress affects translation in inactive colon tissue from patients with ulcerative colitis. Gastroenterology 141: 1024-1035.
33. Kakuta Y, Kinouchi Y, Negoro K, Takahashi S, Shimosegawa T (2006) Association study of TNFSF15 polymorphisms in Japanese patients with inflammatory bowel disease. Gut 55: 1527-1528.
34. Picornell Y, Mei L, Taylor K, Yang H, Targan SR, et al. (2007) TNFSF15 is an ethnic-specific IBD gene. Inflamm Bowel Dis 13: 1333-1338.
35. Michelsen KS, Thomas LS, Taylor KD, Yu QT, Mei L, et al. (2009) IBD-associated TL1A gene (TNFSF15) haplotypes determine increased expression of TL1A protein. PLoS One 4: e4719.
36. Bamias G, Martin C 3rd, Marini M, Hoang S, Mishina M, et al. (2003) Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. J Immunol 171: 4868-4874.
37. Takedatsu H, Michelsen KS, Wei B, Landers CJ, Thomas LS, et al. (2008) TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. Gastroenterology 135: 552-567.
38. Ahmad T, Marshall SE, Jewell D (2006) Genetics of inflammatory bowel disease: the role of the HLA complex. World J Gastroenterol 12: 3628-3635.
39. Furr AE, Ranganathan S, Finn OJ (2010) Aberrant expression of MUC1 mucin in pediatric inflammatory bowel disease. Pediatr Dev Pathol 13: 24-31.
40. Beatty PL, Narayanan S, Gariepy J, Ranganathan S, Finn OJ (2010) Vaccine against MUC1 antigen expressed in inflammatory bowel disease and cancer lessens colonic inflammation and prevents progression to colitis-associated colon cancer. Cancer Prev Res (Phila) 3: 438-446.
41. UK IBD Genetics Consortium, Barrett JC, Lee JC, Lees CW, Prescott NJ, et al. (2009) Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. Nat Genet 41: 1330-1334.
42. Larmonier CB, Laubitz D, Hill FM, Shehab KW, Lipinski L, et al. (2013) Reduced colonic microbial diversity is associated with colitis in NHE3-deficient mice. Am J Physiol Gastrointest Liver Physiol 305: G667-677.
43. Chidlow JH, Jr., Glawe JD, Alexander JS, Kevil CG (2010) VEGF(1)(6)(4) differentially regulates neutrophil and T cell adhesion through ItgaL- and ItgaM-dependent mechanisms. Am J Physiol Gastrointest Liver Physiol 299: G1361-1367.
44. Weizman A, Huang B, Berel D, Targan SR, Dubinsky M, et al. (2014) Clinical, serologic, and genetic factors associated with pyoderma gangrenosum and erythema nodosum in inflammatory bowel disease patients. Inflamm Bowel Dis 20: 525-533.
45. Shui JW, Larange A, Kim G, Vela JL, Zahner S, et al. (2012) HVEM signalling at mucosal barriers provides host defence against pathogenic bacteria. Nature 488: 222-225.
46. Ananthakrishnan AN, Oxford EC, Nguyen DD, Sauk J, Yajnik V, et al. (2013) Genetic risk factors for Clostridium difficile infection in ulcerative colitis. Aliment Pharmacol Ther 38: 522-530.
47. Gathungu G, Zhang CK, Zhang W, Cho JH (2012) A two-marker haplotype in the IRF5 gene is associated with inflammatory bowel disease in a North American cohort. Genes Immun 13: 351-355.
48. Okada Y, Yamazaki K, Umeno J, Takahashi A, Kumasaka N, et al. (2011) HLA-Cw*1202-B*5201-DRB1*1502 haplotype increases risk for ulcerative colitis but reduces risk for Crohn's disease. Gastroenterology 141: 864-871.
49. Annese V, Piepoli A, Latiano A, Lombardi G, Napolitano G, et al. (2005) HLA-DRB1 alleles may influence disease phenotype in patients with inflammatory bowel disease: a critical reappraisal with review of the literature. Dis Colon Rectum 48: 57-64.
50. Halfvarson J, Bodin L, Tysk C, Lindberg E, Järnerot G (2003) Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology 124: 1767-1773.
51. Halfvarson J (2011) Genetics in twins with Crohn's disease: less pronounced than previously believed? Inflamm Bowel Dis 17: 6-12.
52. Ananthakrishnan AN, Huang H2, Nguyen DD, Sauk J, Yajnik V, et al. (2014) Differential effect of genetic burden on disease phenotypes in Crohn's disease and ulcerative colitis: analysis of a North American cohort. Am J Gastroenterol 109: 395-400.
53. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, et al. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411: 599-603.
54. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411: 603-606.
55. Muzes G, Tulassay Z, Sipos F (2013) Interplay of autophagy and innate immunity in Crohn's disease: a key immunobiologic feature. World J Gastroenterol 19: 4447-4454.
56. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, et al. (2005) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307: 731-734.
57. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, et al. (2005) Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A 102: 18129-18134.
58. Simms LA, Doecke JD, Walsh MD, Huang N, Fowler EV, et al. (2008) Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. Gut 57: 903-910.
59. Shanahan MT, Carroll IM, Grossniklaus E, White A, von Furstenberg RJ, et al. (2014) Mouse Paneth cell antimicrobial function is independent of Nod2. Gut 63: 903-910.

60. Watanabe T, Kitani A, Murray PJ, Strober W (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 5: 800-808.
61. Hedl M, Li J, Cho JH, Abraham C (2007) Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc Natl Acad Sci U S A 104: 19440-19445.
62. Annese V, Lombardi G, Perri F, D'Incà R, Ardizzone S, et al. (2005) Variants of CARD15 are associated with an aggressive clinical course of Crohn's disease--an IG-IBD study. Am J Gastroenterol 100: 84-92.
63. Homer CR, Richmond AL, Rebert NA, Achkar JP, McDonald C. (2010) ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. Gastroenterology 139: 1630-1641.
64. Spalinger MR, Lang S, Vavricka SR, Fried M, Rogler G, et al. (2013) Protein tyrosine phosphatase non-receptor type 22 modulates NOD2-induced cytokine release and autophagy. PLoS One 8: e72384.
65. McGovern DP, Jones MR, Taylor KD, Marciante K, Yan X, et al. (2010) Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. Hum Mol Genet 19: 3468-3476.
66. Kumar V, Mack DR, Marcil V, Israel D, Krupoves A, et al. (2013) Genome-wide association study signal at the 12q12 locus for Crohn's disease may represent associations with the MUC19 gene. Inflamm Bowel Dis 19: 1254-1259.
67. Rausch P, Rehman A, Künzel S, Häsler R, Ott SJ, et al. (2011) Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. Proc Natl Acad Sci U S A 108: 19030-19035.
68. Tong M, McHardy I2, Ruegger P3, Goudarzi M4, Kashyap PC5, et al. (2014) Reprograming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. ISME J .
69. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, et al. (2004) Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 36: 471-475.
70. Török HP, Glas J, Tonenchi L, Lohse P, Müller-Myhsok B, et al. (2005) Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. Gut 54: 1421-1427.
71. Vermeire S, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, et al. (2005) Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. Gastroenterology 129: 1845-1853.
72. Waller S, Tremelling M, Bredin F, Godfrey L, Howson J, et al. (2006) Evidence for association of OCTN genes and IBD5 with ulcerative colitis. Gut 55: 809-814.
73. Dianda L, Hanby AM, Wright NA, Sebesteny A, Hayday AC, et al. (1997) T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. Am J Pathol 150: 91-97.
74. Rath HC, Herfarth HH, Ikeda JS, Grenther WB, Hamm TE, Jr., et al. (1996) Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. J Clin Invest 98: 945-953.
75. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. (2005) Diversity of the human intestinal microbial flora. Science 308: 1635-1638.
76. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, et al. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 104: 13780-13785.
77. Sokol H, Lepage P, Seksik P, Doré J, Marteau P (2006) Temperature gradient gel electrophoresis of fecal 16S rRNA reveals active Escherichia coli in the microbiota of patients with ulcerative colitis. J Clin Microbiol 44: 3172-3177.
78. Mylonaki M, Rayment NB, Rampton DS, Hudspith BN, Brostoff J (2005) Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm Bowel Dis 11: 481-487.
79. Varela E, Manichanh C, Gallart M, Torrejón A, Borruel N, et al. (2013) Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Aliment Pharmacol Ther 38: 151-161.
80. Vermeiren J, Van den Abbeele P, Laukens D, Vigsnaes LK, De Vos M, et al. (2012) Decreased colonization of fecal Clostridium coccoides/Eubacterium rectale species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment. FEMS Microbiol Ecol 79: 685-696.
81. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil LJ (2006) Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflamm Bowel Dis 12: 1136-1145.
82. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, et al. (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 105: 16731-16736.
83. Martin R, Chain F, Miquel S, Lu J, Gratadoux JJ, et al. (2014) The commensal bacterium Faecalibacterium prausnitzii is protective in DNBS-induced chronic moderate and severe colitis models. Inflamm Bowel Dis; 20: 417-430.
84. Harries AD, Baird A, Rhodes J (1982) Non-smoking: a feature of ulcerative colitis. Br Med J (Clin Res Ed) 284: 706.
85. Höie O, Wolters F, Riis L, Aamodt G, Solberg C, et al. (2007) Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. Am J Gastroenterol 102: 1692-1701.
86. Lakatos PL, Vegh Z, Lovasz BD, David G, Pandur T, et al. (2013) Is current smoking still an important environmental factor in inflammatory bowel diseases? Results from a population-based incident cohort. Inflamm Bowel Dis; 19: 1010-1017.
87. Odes HS, Fich A, Reif S, Halak A, Lavy A, et al. (2001) Effects of current cigarette smoking on clinical course of Crohn's disease and ulcerative colitis. Dig Dis Sci 46: 1717-1721.
88. Aldhous MC, Drummond HE, Anderson N, Baneshi MR, Smith LA, et al. (2007) Smoking habit and load influence age at diagnosis and disease extent in ulcerative colitis. Am J Gastroenterol 102: 589-597.
89. Higuchi LM, Khalili H, Chan AT, Richter JM, Bousvaros A, et al. (2012) A prospective study of cigarette smoking and the risk of inflammatory bowel disease in women. Am J Gastroenterol 107: 1399-1406.
90. van der Heide F, Dijkstra A, Weersma RK, Albersnagel FA, van der Logt EM, et al. (2009) Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 15: 1199-1207.
91. Bastida G, Beltrán B (2011) Ulcerative colitis in smokers, non-smokers and ex-smokers. World J Gastroenterol 17: 2740-2747.
92. Finnie IA, Campbell BJ, Taylor BA, Milton JD, Sadek SK, et al. (1996) Stimulation of colonic mucin synthesis by corticosteroids and nicotine. Clin Sci (Lond) 91: 359-364.
93. Green JT, Richardson C, Marshall RW, Rhodes J, McKirdy HC, et al. (2000) Nitric oxide mediates a therapeutic effect of nicotine in ulcerative colitis. Aliment Pharmacol Ther 14: 1429-1434.
94. AlSharari SD, Akbarali HI, Abdullah RA, Shahab O, Auttachoat W, et al. (2013) Novel insights on the effect of nicotine in a murine colitis model. J Pharmacol Exp Ther 344: 207-217.
95. Srivastava ED, Barton JR, O'Mahony S, Phillips DI, Williams GT, et al. (1991) Smoking, humoral immunity, and ulcerative colitis. Gut 32: 1016-1019.
96. Mahid SS, Minor KS, Stromberg AJ, Galandiuk S (2007) Active and passive smoking in childhood is related to the development of inflammatory bowel disease. Inflamm Bowel Dis 13: 431-438.
97. Corrao G, Tragnone A, Caprilli R, Trallori G, Papi C, et al. (1998) Risk of inflammatory bowel disease attributable to smoking, oral contraception and breastfeeding in Italy: a nationwide case-control study. Cooperative Investigators of the Italian Group for the Study of the Colon and the Rectum (GISC). Int J Epidemiol 27: 397-404.

98. Cosnes J, Beaugerie L, Carbonnel F, Gendre JP (2001) Smoking cessation and the course of Crohn's disease: an intervention study. Gastroenterology 120: 1093-1099.
99. Breuer-Katschinski BD, Holländer N, Goebell H (1996) Effect of cigarette smoking on the course of Crohn's disease. Eur J Gastroenterol Hepatol 8: 225-228.
100. Cosnes J, Carbonnel F, Beaugerie L, Le Quintrec Y, Gendre JP (1996) Effects of cigarette smoking on the long-term course of Crohn's disease. Gastroenterology 110: 424-431.
101. Zabana Y, Garcia-Planella E, Van Domselaar M, Mañosa M, Gordillo J, et al. (2013) Does active smoking really influence the course of Crohn's disease? A retrospective observational study. J Crohns Colitis 7: 280-285.
102. Kalra J, Chaudhary AK, Prasad K (1991) Increased production of oxygen free radicals in cigarette smokers. Int J Exp Pathol 72: 1-7.
103. Bergeron V, Grondin V, Rajca S, Maubert MA, Pigneur B, et al. (2012) Current smoking differentially affects blood mononuclear cells from patients with Crohn's disease and ulcerative colitis: relevance to its adverse role in the disease. Inflamm Bowel Dis; 18: 1101-1111.
104. Aldhous MC, Soo K, Stark LA, Ulanicka AA, Easterbrook JE, et al. (2011) Cigarette smoke extract (CSE) delays NOD2 expression and affects NOD2/RIPK2 interactions in intestinal epithelial cells. PLoS One 6: e24715.
105. Scaldaferri F, Gerardi V, Lopetuso LR, Del Zompo F, Mangiola F, et al. (2013) Gut microbial flora, prebiotics, and probiotics in IBD: their current usage and utility. Biomed Res Int 2013: 435268.
106. Ng SC, Bernstein CN, Vatn MH, Lakatos PL, Loftus EV Jr, et al. (2013) Geographical variability and environmental risk factors in inflammatory bowel disease. Gut 62: 630-649.
107. Radford-Smith GL, Edwards JE, Purdie DM, Pandeya N, Watson M, et al. (2002) Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. Gut 51: 808-813.
108. Andersson RE, Olaison G, Tysk C, Ekbom A (2001) Appendectomy and protection against ulcerative colitis. N Engl J Med 344: 808-814.
109. Kaplan GG, Jackson T, Sands BE, Frisch M, Andersson RE, et al. (2008) The risk of developing Crohn's disease after an appendectomy: a meta-analysis. Am J Gastroenterol 103: 2925-2931.
110. Chan SS, Luben R, Olsen A, Tjonneland A, Kaaks R, et al. (2013) Body mass index and the risk for Crohn's disease and ulcerative colitis: data from a European Prospective Cohort Study (The IBD in EPIC Study). Am J Gastroenterol 108: 575-582.
111. Hou JK, Abraham B, El-Serag H (2011) Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol 106: 563-573.
112. Spooren CE, Pierik MJ, Zeegers MP, Feskens EJ, Masclee AA, et al. (2013) Review article: the association of diet with onset and relapse in patients with inflammatory bowel disease. Aliment Pharmacol Ther 38: 1172-1187.
113. Persson PG, Ahlbom A, Hellers G (1992) Diet and inflammatory bowel disease: a case-control study. Epidemiology 3: 47-52.
114. Farkas K, Szepes Z, Nagy F, Bálint A, Bor R, et al. (2014) Letter: role of diet in the onset and relapse of inflammatory bowel disease from the patients' perspective. Aliment Pharmacol Ther 39: 340.
115. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, et al. (2009) The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 1: 6ra14.
116. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, et al. (2012) Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature 487: 104-108.
117. Leone V, Chang EB, Devkota S (2013) Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. J Gastroenterol 48: 315-321.
118. Leslie WD, Miller N, Rogala L, Bernstein CN (2008) Vitamin D status and bone density in recently diagnosed inflammatory bowel disease: the Manitoba IBD Cohort Study. Am J Gastroenterol 103: 1451-1459.
119. Ananthakrishnan AN, Khalili H, Higuchi LM, Bao Y, Korzenik JR, et al. (2012) Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. Gastroenterology 142: 482-489.
120. Jørgensen SP, Hvas CL, Agnholt J, Christensen LA, Heickendorff L, et al. (2013) Active Crohn's disease is associated with low vitamin D levels. J Crohns Colitis 7: e407-413.
121. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, et al. (2010) A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 20: 1352-1360.
122. Prietl B, Treiber G, Pieber TR, Amrein K (2013) Vitamin D and immune function. Nutrients 5: 2502-2521.
123. Mouli VP, Ananthakrishnan AN (2014) Review article: vitamin D and inflammatory bowel diseases. Aliment Pharmacol Ther 39: 125-136.
124. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, et al. (2012) Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 188: 2127-2135.
125. Cantorna MT, Munsick C, Bemiss C, Mahon BD (2000) ,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 130: 2648-2652.
126. Kim JH, Yamaori S, Tanabe T, Johnson CH, Krausz KW, et al. (2013) Implication of intestinal VDR deficiency in inflammatory bowel disease. Biochim Biophys Acta 1830: 2118-2128.
127. Dubeau MF, Iacucci M, Beck PL, Moran GW, Kaplan GG, et al. (2013) Drug-induced inflammatory bowel disease and IBD-like conditions. Inflamm Bowel Dis 19: 445-456.
128. Shaw SY, Blanchard JF, Bernstein CN (2010) Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am J Gastroenterol 105: 2687-2692.
129. Hviid A, Svanström H, Frisch M (2011) Antibiotic use and inflammatory bowel diseases in childhood. Gut 60: 49-54.
130. Card T, Logan RF, Rodrigues LC, Wheeler JG (2004) Antibiotic use and the development of Crohn's disease. Gut 53: 246-250.
131. Margolis DJ, Fanelli M, Hoffstad O, Lewis JD (2010) Potential association between the oral tetracycline class of antimicrobials used to treat acne and inflammatory bowel disease. Am J Gastroenterol 105: 2610-2616.
132. Shaw SY, Blanchard JF, Bernstein CN (2011) Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. Am J Gastroenterol 106: 2133-2142.
133. Lee TW, Russell L, Deng M, Gibson PR (2013) Association of doxycycline use with the development of gastroenteritis, irritable bowel syndrome and inflammatory bowel disease in Australians deployed abroad. Intern Med J 43: 919-926.
134. Gleeson MH, Davis AJ (2003) Non-steroidal anti-inflammatory drugs, aspirin and newly diagnosed colitis: a case-control study. Aliment Pharmacol Ther 17: 817-825.
135. Chan SS, Hart AR (2011) Aspirin use and development of inflammatory bowel disease: confounding or causation? authors' reply. Aliment Pharmacol Ther 34: 1351.
136. Ananthakrishnan AN, Higuchi LM, Huang ES, Khalili H, Richter JM, et al. (2012) Aspirin, nonsteroidal anti-inflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. Ann Intern Med 156: 350-359.
137. Felder JB, Korelitz BI, Rajapakse R, Schwarz S, Horatagis AP, et al. (2000) Effects of nonsteroidal antiinflammatory drugs on inflammatory bowel disease: a case-control study. Am J Gastroenterol 95: 1949-1954.
138. Kefalakes H, Stylianides TJ, Amanakis G, Kolios G (2009) Exacerbation of inflammatory bowel diseases associated with the use of nonsteroidal anti-inflammatory drugs: myth or reality? Eur J Clin Pharmacol 65: 963-970.
139. Godet PG, May GR, Sutherland LR (1995) Meta-analysis of the role of oral contraceptive agents in inflammatory bowel disease. Gut 37: 668-673.

140. García Rodríguez LA, González-Pérez A, Johansson S, Wallander MA (2005) Risk factors for inflammatory bowel disease in the general population. Aliment Pharmacol Ther 22: 309-315.
141. Khalili H, Higuchi LM, Ananthakrishnan AN, Richter JM, Feskanich D, et al. (2013) Oral contraceptives, reproductive factors and risk of inflammatory bowel disease. Gut 62: 1153-1159.
142. Khalili H, Higuchi LM, Ananthakrishnan AN, Manson JE, Feskanich D, et al. (2012) Hormone therapy increases risk of ulcerative colitis but not Crohn's disease. Gastroenterology 143: 1199-1206.
143. Zapata LB, Paulen ME, Cansino C, Marchbanks PA, Curtis KM (2010) Contraceptive use among women with inflammatory bowel disease: A systematic review. Contraception 82: 72-85.
144. Sandborn WJ, Hanauer SB, Katz S, Safdi M, Wolf DG, et al. (2001) Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology 121: 1088-1094.
145. Fouache D, Goëb V, Massy-Guillemant N, Avenel G, Bacquet-Deschryver H, et al. (2009) Paradoxical adverse events of anti-tumour necrosis factor therapy for spondyloarthropathies: a retrospective study. Rheumatology (Oxford) 48: 761-764.
146. Toussirot É, Houvenagel É, Goëb V, Fouache D, Martin A, et al. (2012) Development of inflammatory bowel disease during anti-TNF-α therapy for inflammatory rheumatic disease: a nationwide series. Joint Bone Spine 79: 457-463.
147. Zou J, Rudwaleit M, Brandt J, Thiel A, Braun J, et al. (2003) Up regulation of the production of tumour necrosis factor alpha and interferon gamma by T cells in ankylosing spondylitis during treatment with etanercept. Ann Rheum Dis 62: 561-564.
148. Ponder A, Long MD (2013) A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin Epidemiol 5: 237-247.
149. Baron S, Turck D, Leplat C, Merle V, Gower-Rousseau C, et al. (2005) Environmental risk factors in paediatric inflammatory bowel diseases: a population based case control study. Gut 54: 357-363.
150. Khalili H, Ananthakrishnan AN, Higuchi LM, Richter JM, Fuchs CS, et al. (2013) Early life factors and risk of inflammatory bowel disease in adulthood. Inflamm Bowel Dis 19: 542-547.
151. Klement E, Cohen RV, Boxman J, Joseph A, Reif S (2004) Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. Am J Clin Nutr 80: 1342-1352.
152. Hörnell A, Lagström H, Lande B, Thorsdottir I (2013) Breastfeeding, introduction of other foods and effects on health: a systematic literature review for the 5th Nordic Nutrition Recommendations. Food Nutr Res 57.
153. Madsen KL, Fedorak RN, Tavernini MM, Doyle JS (2002) Normal Breast Milk Limits the Development of Colitis in IL-10-Deficient Mice. Inflamm Bowel Dis 8: 390-398.
154. Ogra PL, Welliver RC, Sr. (2008) Effects of early environment on mucosal immunologic homeostasis, subsequent immune responses and disease outcome. Nestle Nutr Workshop Ser Pediatr Program 61: 145-181
155. Bresciani M, Parisi C, Menghi G, Bonini S (2005) The hygiene hypothesis: does it function worldwide? Curr Opin Allergy Clin Immunol 5: 147-151.
156. Amre DK, Lambrette P, Law L, Krupoves A, Chotard V, et al. (2006) Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study. Am J Gastroenterol 101: 1005-1011.
157. Radon K, Windstetter D, Poluda AL, Mueller B, von Mutius E, et al. (2007) Contact with farm animals in early life and juvenile inflammatory bowel disease: a case-control study. Pediatrics 120: 354-361.
158. Koloski NA, Bret L, Radford-Smith G (2008) Hygiene hypothesis in inflammatory bowel disease: a critical review of the literature. World J Gastroenterol 14: 165-173.
159. Halme L, Rautelin H, Leidenius M, Kosunen TU (1996) Inverse correlation between Helicobacter pylori infection and inflammatory bowel disease. J Clin Pathol 49: 65-67.
160. López-Serrano P, Pérez-Calle JL, Pérez-Fernández MT, Fernández-Font JM, Boixeda de Miguel D, et al. (2010) Environmental risk factors in inflammatory bowel diseases. Investigating the hygiene hypothesis: a Spanish case-control study. Scand J Gastroenterol 45: 1464-1471.
161. Olszak T, An D, Zeissig S, Vera MP, Richter J, et al. (2012) Microbial exposure during early life has persistent effects on natural killer T cell function. Science 336: 489-493.
162. Sajadinejad MS, Asgari K, Molavi H, Kalantari M, Adibi P (2012) Psychological issues in inflammatory bowel disease: an overview. Gastroenterol Res Pract 2012: 106502.
163. McMahon AW, Schmitt P, Patterson JF, Rothman E (1973) Personality differences between inflammatory bowel disease patients and their healthy siblings. Psychosom Med 35: 91-103.
164. Robertson DA, Ray J, Diamond I, Edwards JG (1989) Personality profile and affective state of patients with inflammatory bowel disease. Gut 30: 623-626.
165. Flett GL, Baricza C, Gupta A, Hewitt PL, Endler NS (2011) Perfectionism, psychosocial impact and coping with irritable bowel disease: a study of patients with Crohn's disease and ulcerative colitis. J Health Psychol 16: 561-571.
166. Porcelli P, Taylor GJ, Bagby RM, De Carne M (1999) Alexithymia and functional gastrointestinal disorders. A comparison with inflammatory bowel disease. Psychother Psychosom 68: 263-269.
167. Kovács Z, Kovács F (2007) Depressive and anxiety symptoms, dysfunctional attitudes and social aspects in irritable bowel syndrome and inflammatory bowel disease. Int J Psychiatry Med 37: 245-255.
168. Bokemeyer B, Hardt J, Hüppe D, Prenzler A, Conrad S, et al. (2013) Clinical status, psychosocial impairments, medical treatment and health care costs for patients with inflammatory bowel disease (IBD) in Germany: an online IBD registry. J Crohns Colitis 7: 355-368.
169. Mittermaier C, Dejaco C, Waldhoer T, Oefferlbauer-Ernst A, Miehsler W, et al. (2004) Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. Psychosom Med 66: 79-84.
170. Walker JR, Ediger JP, Graff LA, Greenfeld JM, Clara I, et al. (2008) The Manitoba IBD cohort study: a population-based study of the prevalence of lifetime and 12-month anxiety and mood disorders. Am J Gastroenterol 103: 1989-1997.
171. Kurina LM, Goldacre MJ, Yeates D, Gill LE (2001) Depression and anxiety in people with inflammatory bowel disease. J Epidemiol Community Health 55: 716-720.
172. Ghia JE, Blennerhassett P, Collins SM (2008) Impaired parasympathetic function increases susceptibility to inflammatory bowel disease in a mouse model of depression. J Clin Invest 118: 2209-2218.
173. Taché Y, Bernstein CN (2009) Evidence for the role of the brain-gut axis in inflammatory bowel disease: depression as cause and effect? Gastroenterology 136: 2058-2061.
174. Larauche M, Kiank C, Tache Y (2009) Corticotropin releasing factor signaling in colon and ileum: regulation by stress and pathophysiological implications. J Physiol Pharmacol 60 Suppl 7: 33-46.
175. Williams CL, Peterson JM, Villar RG, Burks TF (1987) Corticotropin-releasing factor directly mediates colonic responses to stress. Am J Physiol 253: G582-586.
176. Wallon C, Söderholm JD (2009) Corticotropin-releasing hormone and mast cells in the regulation of mucosal barrier function in the human colon. Ann N Y Acad Sci 1165: 206-210.
177. Niess JH, Mönnikes H, Dignass AU, Klapp BF, Arck PC (2002) Review on the influence of stress on immune mediators, neuropeptides and hormones with relevance for inflammatory bowel disease. Digestion 65: 131-140.
178. Lennon EM, Maharshak N, Elloumi H, Borst L, Plevy SE, et al. (2013) Early life stress triggers persistent colonic barrier dysfunction and exacerbates colitis in adult IL-10-/- mice. Inflamm Bowel Dis 19: 712-719.

179. Lerebours E, Gower-Rousseau C, Merle V, Brazier F, Debeugny S, et al. (2007) Stressful life events as a risk factor for inflammatory bowel disease onset: A population-based case-control study. Am J Gastroenterol 102: 122-131.

180. Maunder RG, Levenstein S (2008) The role of stress in the development and clinical course of inflammatory bowel disease: epidemiological evidence. Curr Mol Med 8: 247-252.

181. Heikkilä K, Madsen IE2, Nyberg ST, Fransson EI3, Ahola K, et al. (2014) Job strain and the risk of inflammatory bowel diseases: individual-participant meta-analysis of 95,000 men and women. PLoS One 9: e88711.

182. Kellermayer R (2012) Epigenetics and the developmental origins of inflammatory bowel diseases. Can J Gastroenterol 26: 909-915.

183. Cooney CA, Dave AA, Wolff GL (2002) Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 132: 2393S-2400S.

184. Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S, et al. (2012) Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. Hum Mol Genet 21: 2086-2101.

185. Schaible TD, Harris RA, Dowd SE, Smith CW, Kellermayer R (2011) Maternal methyl-donor supplementation induces prolonged murine offspring colitis susceptibility in association with mucosal epigenetic and microbiomic changes. Hum Mol Genet 20: 1687-1696.

186. Nimmo ER, Prendergast JG, Aldhous MC, Kennedy NA, Henderson P, et al. (2012) Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. Inflamm Bowel Dis 18: 889-899.

187. Festen EA, Weersma RK2 (2014) How will insights from genetics translate to clinical practice in inflammatory bowel disease? Best Pract Res Clin Gastroenterol 28: 387-397.

188. Sandborn WJ, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, et al. (2008) A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. Gastroenterology 135: 1130-1141.

189. Reinisch W, Panés J, Lémann M, Schreiber S, Feagan B, et al. (2008) A multicenter, randomized, double-blind trial of everolimus versus azathioprine and placebo to maintain steroid-induced remission in patients with moderate-to-severe active Crohn's disease. Am J Gastroenterol 103: 2284-2292.

This article was originally published in a special issue, entitled: **"Inflammatory Bowel Disease"**, Edited by Dr. Nancy Louis, Emory University, USA and Dr. Ostanin Dmitry Vladimirovich, Louisiana State University Health Sciences Center, USA