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Diet, Gut Microbiome and Epigenetics: Emerging Links with Inflammatory Bowel Diseases and Prospects for Management and Prevention

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Abstract

Inflammatory bowel diseases (IBD) represent a growing public health concern due to increasing incidence worldwide. The current notion on the pathogenesis of IBD is that genetically susceptible individuals develop intolerance to dysregulated gut microflora (dysbiosis) and chronic inflammation develops as a result of environmental triggers. Among the environmental factors associated to IBD, diet plays an important role in modulating the gut microbiome, influencing epigenetic changes and, therefore, could be applied as a therapeutic tool to improve the disease course. Nevertheless, the current dietary recommendations for disease prevention and management are scarce and of weak evidence. This review summarizes the current knowledge on the complex interactions among diet, microbiome and epigenetics in IBD. Whereas over-abundance of calories and some macronutrients increases gut inflammation, several micronutrients have the potential to modulate it. Immunonutrition has emerged as a new concept putting forward the importance of vitamins such as vitamins A, C, E, D, folic acid and beta-carotene and trace elements such as zinc, selenium, manganese and iron. However, when assessed in clinical trials, specific micronutrients exerted a limited benefit. Beyond nutrients, anti-inflammatory dietary patterns as a complex intervention approach have become popular over the recent years. Hence, exclusive enteral nutrition in pediatric Crohn's disease is the only nutritional intervention currently recommended as a first-line therapy. Other nutritional interventions or specific diets including the Specific Carbohydrate Diet, the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyol diet and most recently the Mediterranean diet have shown strong anti-inflammatory properties and provide a promise for improving disease symptoms. Definitely, more work is required to evaluate the role of individual food compounds and complex nutritional interventions with potential to decrease inflammation as means for prevention and management of IBD.

Keywords

diet, gut microbiota, epigenetics, inflammatory bowel diseases

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Introduction

The inflammatory bowel diseases (IBD) - Crohn's disease (CD) and ulcerative colitis (UC) - are two diseases characterized by chronic relapsing inflammation of the gastrointestinal tract that represent an increasing public health concern while an etiological enigma due to unknown causal factors. Despite suggested differences in pathology, both diseases are believed to share common etiology. The strongest IBD risk factor identified to date is the family history of IBD [1]. The current notion on the pathogenesis of IBD is that genetically susceptible individuals develop intolerance to dysregulated gut microflora (dysbiosis) and chronic inflammation develops as a result of environmental triggers [2]. Current research in the field of IBD largely focused on establishing the role of causal variants on gene expression and various pathological pathways have been already uncovered [3]. However, still the genetic risk loci identified to date only explain a small part of genetic variance in disease risk and more factors need to be taken into account to understand the IBD multifactorial pathology [4]. Impaired immune response that occurs in genetically susceptible individuals as the result of a complex interaction among disturbed immune responses, impaired intestinal barrier function and dysfunctional microbe-host interactions has been therefore suggested as major unifying etiological background [5]. While the identification of IBD environmental risk factors remains a subject of intensive research, diet remains one of the most putative candidates. Diet participates in the regulation of intestinal inflammation, either directly or indirectly by modifying the gut microbiota [6,7] A greater understanding of the contribution of dietary factors to dysbiosis is therefore critical having the pivotal role of a healthy microbiome in preventing the development of IBD and its complications [8]. Most recently, the fast evolving field of epigenetics offers new explanations on the mechanisms by which environmental changes induce pathological gene expression and determine cell phenotype and function in IBD [9]. The pathogenic mechanisms for IBD could be largely imposed by gene-

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85 environmental interactions switching on a cascade of induced effects on the microbiota, the
86 immune system and the mucosal barrier (Figure 1). Here, we review the recent developments
87 in understanding the role of gut microbiome, epigenetics and dietary factors in IBD that
88 outline directions for disease management and prevention.

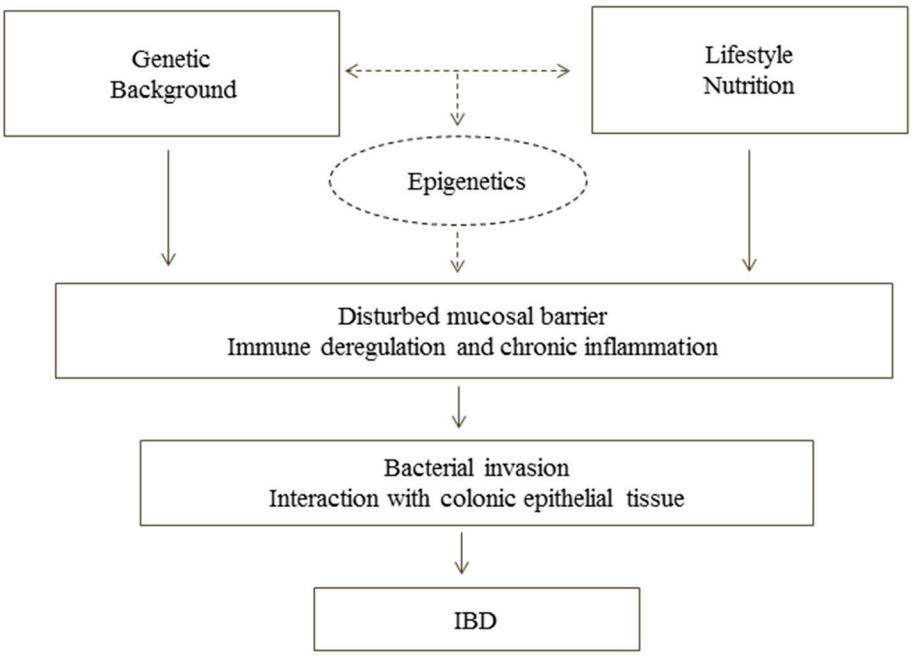

Figure 1. Proposed patho-physilogical mechanisms for inflammatory bowel disease (IBD)

Complex interaction between genetic and lifestyle factors and the putative role of epigenetics on the interplay between microbiota, the immune system and the mucosal barrier

89

90 **Epidemiology of IBD and environmental exposures**

91 IBDs occur worldwide with differences in epidemiology, exposures to risk factors and
92 phenotype between regions. The prevalence of IBD is higher in industrialized (Western
93 Europe, United States of America, Canada, Australia and New Zealand) than in developing
94 countries (Asia, Middle East, South America and Africa) [10]. The incidence of IBD steadily
95 increased in industrialized countries during the 20th century and, although some studies

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suggested that a plateau was reached in some regions during the 21st century [10], recent reports point that it could be still increasing in these countries [11-13]. In developing countries, traditionally considered low incidence areas, an increasing incidence is being described since the beginning of the 21st century [8-10,14]. These observed increases in the incidence rates could be partly accounted for by pragmatic reasons such as the media coverage and increased health awareness in both developed and developing countries, improved access to medical technology and health care providers, and the development of sophisticated disease surveillance systems [15,16]. However, the parallel of higher incidence rates with the Westernization of affected societies points to the potential important role of the environment [15,16]. In that regard, environmental factors (also known as "exposome") have been thoroughly studied and many of the factors related to Westernization have been associated to the risk of developing IBD . The list of putative factors includes environmental pollution, medication, stress level, infections, and lifestyle. Certain differences in environmental risk for CD and UC have been reported, such as that cigarette smoking was shown to increase the risk of developing CD, whilst smoking is less common in those who develop UC [17]. Studies of migrant populations moving from regions of low to high IBD incidence point to early life as a key time for environmental triggers [18]. In these populations, it is the second generation (those born in the high incidence region) with higher IBD incidence rates than their parents. Early life environmental exposures have been also implicated in IBD risk, but, except for having been breastfed, few factors have been shown to alter the risk of developing IBD. However, an important obstacle for identifying the role of environmental factors in IBD is the lack of methodological standardization among studies [19].

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Intestinal microbiome in the pathogenesis of IBD

The gut microbiome interacts with the host in a symbiotic way and performs a variety of beneficial functions: digestion of substrates and production of nutrients; development, maturation and regulation of the immune system (both the local and the systemic response) and prevention of the growth of harmful microorganisms [20]. It has been described that human microbiota can be classified into clusters of well-balanced, defined microbial community compositions, known as "enterotypes" [21]. These enterotypes are stable, and environmental factors (i.e. diet) can influence microbiome composition but without affecting the enterotype identity [22,23]. IBD is associated with alterations in the composition of the intestinal microbiota characterized by decreased diversity, reduced proportions of Firmicutes, and increased proportions of Proteobacteria and Actinobacteria [6]. Some of the bacterial species enriched in patients with IBD (Escherichia, Fusobacterium) may potentiate the disease, and some of the species with anti-inflammatory properties (Faecalibacterium, Roseburia) are reduced in IBD [6]. The microbiota of active IBD patients is different from that of patients in remission, as confirmed by a recent meta-analysis: patients with active IBD had lower abundance of Clostridium coccoides, Clostridium leptum, Faecalibacterium prausnitzii and Bifidobacterium [24]. Prospective studies on microbiome changes during the disease course are scarce. A Dutch study on 10 CD and 9 UC patients assessed in remission and in subsequent relapse found patient-specific shifts in microbial composition, but could not demonstrate general changes in microbial composition or diversity [25] A Spanish study followed-up 18 UC patients during 1 year; in those who remained in remission Faecalibacterium prausnitzii increased steadily, while in those who relapsed it remained low [26].

Several factors can influence the microbiome composition. The intestinal colonization begins immediately after birth, and beyond genetic predisposition, is influenced very early in life by

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the route of delivering, the infant diet (breast- or formula-feeding) and hygiene conditions. Apart from environmental factors such as drugs, stress and toxics (i.e. tobacco), diet was suggested to play a decisive role in modulating microbiome composition [6,20,27]. Dietary composition was shown to affect the microbiota balance; therefore, it is conceivable that altering the diet can impact the inflammatory response [28]. For example, diets rich in saturated fats are shown to induce damage of the intestinal epithelial cell layer leading to loss of barrier function. In contrast, diets high in fiber predispose short-chain fatty acids production by microbiota and lead to improved energy expenditure [29]. Thus, a balanced low-fat and high-fiber diet may be important in preventing dysbiosis and preserving the immune system [30]. Targeting microbiota through nutritional interventions could represent a promising therapeutic approach. So far, several nutritional interventions have been evaluated such as dietary supplementation with prebiotics and contrabiotics, phosphatidylcholine and use of genetically modified bacteria [31]. Among these, therapies such as prebiotics and probiotics that selectively manipulate the intestinal microbiota have been evaluated as an attractive therapeutic option with few side effects [32]. For example, Clostridium coccodies and C. leptum have been shown to exert protective effects against IBD [30]. The multispecies products VSL#3 and E. coli Nissle have been revealed as particularly effective in maintaining remission in UC [29]. Probiotic yogurt intake was associated with significant anti-inflammatory effects that paralleled the expansion of peripheral pool of putative T(reg) cells in IBD patients and with few effects in controls [33]. However, any hope for long-term benefits from probiotics may be limited by the need for dietary modification. Furthermore, pre/probiotic administration may not be useful out of the context of an overall healthy diet. Even harmful effects of probiotic supplementation holding the potential to become pathogenic when exposed to an unhealthy diet was suggested in mouse models [34]. Probiotics and other commercial interventions such as tea or berry extracts are unlikely to counteract unhealthy nutritional behaviour. Much work remains before the understanding of the effects of dysbiosis

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in humans reaches that of mice, however while definitive statements may be lacking, the preponderance of current evidence strongly suggests that the gut microbiome is a major contributor to human health and disease [30].

Epigenetics and IBD

Research over the recent years has largely contributed to an improved understanding of the role of epigenetic modifications – i.e. non-coding RNAs and DNA methylation - in defining the molecular basis of IBD [35,36]. Such research has been largely driven by observations that genetics alone cannot explain onset of IBD. Thus, a meta-analysis of GWAS studies estimated that susceptibility loci for UC explained only 16% of UC heritability [37]. In this regard, gene-environment interactions was suggested to play an important role in IBD pathogenesis and this is where epigenetics could offer new insights beyond genetic research [35,38]. Epigenetic factors were therefore suggested to mediate interactions between the environment and the genome, thereby providing new insights into the pathogenesis of IBD [39]. Earlier studies have reported on the differential expression of specific microRNAs in the colonic mucosa samples of IBD patients compared to the mucosa of control patients [36]. miRNAs identified in peripheral blood were additionally suggested as new biomarkers of disease. More recently, DNA methylation signatures for UC and CD have been also described. However, whether changes in DNA methylation systematically correlate with gene expression is not clear [40]. In addition, it remains challenging to identify etiologically significant epigenetic alterations since epigenetic modification of DNA may differ between tissues, time of development within the same tissue and environmental influences. Initial evidence arising from epigenetic research is sometimes hard to be proven in clinical practice. An example is the identified role of cytokines and subsequent development of biologicals which fail to prove important role in disease control. Thus, dysregulation of cytokine genes and increased mRNA levels of cytokines, including Interleukin1-beta and Tumor necrosis factor-αlpha (TNFα) have been reported in IBD patients compared with controls in the late

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90-s [41-43]. This has led to introducing anti-TNFα therapies in IBD patients. However, achieving adequate response levels still remains elusive as stand-alone anti-TNFα therapies have not been proven completely useful at predicting disease progression and drug response [44]. Recently, animal models suggested that the lack of response could be related to differences in gut microbiome prior to and after disease initiation. Thus, alternative strategies are needed that account for the interplay between immunity, epigenetics and dietary factors. Diet is known to influence epigenetic changes associated with disease and to modify gene expression patterns in a state of disturbed immunity [38]. The poor dietary choices are encoded into human gut, genetic make-up, and are transferred to the offspring. A number of nutrients have the ability to modulate immune response and counter inflammatory processes. Immune cells are rapidly dividing and have increased sensitivity to impaired DNA replication. Dietary factors act differently to modulate immune response, but all appear to have the potential to modulate inflammation [45]. Furthermore, active immunization against the outer membrane protein of bacteria present in the gut was recently shown to enhance local and systemic immune control via apoE-mediated immune-modulation [46]. Immunonutrition was therefore suggested as a less invasive alternative to immunotherapy in protection against chronic inflammation predisposing IBD [45].

Diet, immunity and IBD

Western diet is characterized by an over-consumption of refined sugars, salt, and saturated fat and overall low food variability. New features of human nutrition in the modern society include artificial sweeteners, gluten, and genetically modified foods. Western societies seem to have dealt with micro- and macronutrient deficiencies, however over-abundance of calories and the macronutrients pose the new challenges of increased inflammation, infection susceptibility, and increased risk for auto-inflammatory disease such as IBD [47,48]. Several micronutrients are especially important for immunonutrition among which vitamins such as

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vitamins A, C, and E, D, folic acid and beta-carotene and trace elements such as zinc, selenium, manganese and iron have gained much interest in research. Deficiencies in zinc and vitamins A and D may reduce natural killer cell function, whereas supplemental zinc or vitamin C may enhance their activity [49,50]. Vitamin D has been shown to play a role in intestinal defense by suppressing the microbial invasion into the epithelium. Vitamin D deficiency was identified in 82% of IBD patients compared to the 31% national average and has been linked to defective epithelial processes. Therapy targeting vitamin D3 signaling was suggested to provide new approaches for infectious and inflammatory diseases by affecting both innate and adaptive immune functions. However, the impact of the vitamins on IBD is still not well understood. So far, only two randomized clinical trials were conducted to evaluate vitamin D supplementation on IBD outcome. In a Danish study, 94 patients were randomized to receive oral vitamin D3 or placebo; patients receiving vitamin D3 had a non-significant reduced risk of relapse [51]. A more recent Iranian study conducted among 108 IBD patients reported that oral supplementation with vitamin D3 reduced serum TNF-alpha level though not substantially [52]. More studies with larger samples would be beneficial to assess effects of vitamin supplementation in IBD. Trace elements represent another important avenue for research in prevention and control of inflammatory diseases. Zinc is involved in the control of DNA replication and transcription and controls signal transduction during T-cell activation [53]. Selenium deficiency decreases antibody production, while selenium supplementation enhances T-cell responses and increases antibody synthesis. It is also known to also to exert antioxidative effects and to protect from deteriorating effects of reactive oxygen species [54]. Iron deficiency does lead to defective Tcell proliferative response and impaired cytokine production by lymphocytes. It should be noted that iron supports pathogen development and iron supplementation can also result in increased susceptibility to infections [55]. Of important note, dietary iron was also shown to enhance IBD and carcinogenesis by augmenting oxidative and nitrosative stress. Thus in an experimental study, a twofold iron-

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enriched diet significantly increased colorectal tumor incidence (14/16, 88%) as compared with animals fed the control diet (3/16, 19%; $P < 0.001$) [56]. Despite theoretically micronutrient deficiency may impact the immune system and influence onset and development of IBD, more research is needed to understand optimal levels and therapeutic implications [28]. Beyond micronutrients, specific food compound such as the green tea [57-59] or Echinacea [60-62] have been also suggested to reduce or enhance immune stimulation and potentially to be implicated in IBD prevention.

Dietary patterns in IBD management and prevention

The role of diet in preventing the onset of IBD is not well understood and the same refers to using diet as a mode for disease control [63,64] [65]. Overall no concerted effort has been made so far to provide nutritional guidelines for IBD patients and the existing guidelines largely follow the principle 'If it hurts, don't do it". Potential dietary suggestions include nutritional deficiency screening, advise patients to self-monitor and avoid foods that may worsen symptoms, eating smaller meals at more frequent intervals, drinking adequate fluids, avoiding caffeine and alcohol, taking vitamin/mineral supplementation, eliminating dairy if lactose intolerant, limiting excess fat, reducing carbohydrates and reducing high-fiber foods during flares. Mixed advice exists regarding pre/probiotics. Recommendations are largely different across regions/countries. For example, enteral nutrition is recommended for Crohn's disease patients in Japan, which differs from practices in the USA [65]. A potential reason for the lack of solid dietary recommendations is the scarcity of studies evaluating the impact of diet in the disease course [66] . So far, we could identify only one study that has assessed nutritional factors and their influence on disease outcome in newly diagnosed IBD [67]. In this inception cohort study, high intake of caffeine was associated to an increased risk of surgery, severe disease course and higher treatment step in CD patients; in UC patients, daily fast food intake was associated to an increased risk of surgery and high intake of caffeine was associated to higher risk of extra-intestinal manifestations and lower treatment step.

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In an attempt to fill this gap, over the recent years more effort has been done to evaluate specific diets in the management of IBD such as the Specific Carbohydrate Diet (SCD) and the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyol (FODMAP) diet. Exclusive enteral nutrition is recommended as first-line therapy to induce remission in children with active luminal CD [68]. In adults, long-term diet interventions such as total parenteral nutrition or elemental diet [69] have also shown promise, however their administration is more complicated to allow normal life of patients. The SCD is a dietary regime aimed to induce and maintain drug-free remission in patients with IBD initially developed by gastroenterologist Sidney Haas in 1951 and later popularized by biochemist Elaine Gottschall in the book Breaking the Vicious Cycle: Intestinal Health Through Diet [70]. The SCD diet is focused on avoiding complex carbohydrates that may lead to bacterial overgrowth and bowel injury with increased intestinal permeability. The diet allows carbohydrate foods consisting of monosaccharides only and excludes disaccharides and most polysaccharides and is supplemented by yogurt free of lactose. Recommended cultures include Lactobacillus bulgaricus, Lactobacillus acidophilus, and Streptococcus thermophilus. The SCD allows almost all fruits, vegetables containing more amylose (a linear-chain polysaccharide) than amylopectin (a branch-chained polysaccharide), nuts, nut-derived flours, cheese, meats, eggs, butters, and oils. It excludes sucrose, maltose, isomaltose, lactose, grain-derived flours and all true and pseudograins, potatoes, okra, corn, fluid milk, soy, cheeses containing high amounts of lactose, as well as most food additives and preservatives [70]. So far, several case-series studies have suggested an important potential of the SCD diet in the control and remission maintenance in IBD [48,71-73]. The low FODMAP diet gained much attention in research as means for IBD treatment. A recent meta-analysis including two randomized control trials and four before-after studies with a total of 319 patients (96% in remission) reported overall improvement in gastrointestinal symptoms such as diarrhea response abdominal bloating fatigue and nausea [74]. Recently, plant-based dietary patterns

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were suggested as valid means for long-term inflammation control [75]. In particular, the Mediterranean diet was suggested to exert strong immunomodulatory effects and even showing a potential to modulate epigenetic mechanisms. Recent data from the Predimed study, a randomised, controlled, parallel trial in high cardiovascular risk volunteers, revealed that over 5 years of intervention the Mediterranean diet is associated with the methylation of genes related to inflammation and exerts high regulatory effects [76]. Further intervention trials utilizing transcriptomics analyses revealed potential of Mediterranean to modulate gene expression and to normalise microbiota in IBD patients [77]. Similarly, semi-vegetarian diet was shown to exert preventive effects against IBD relapse in patients who have achieved remission in a prospective, single center, 2-year clinical trial [78].

On-going research activities

The current evidence on specific diets in the IBD course remains to be confirmed and updated by further well-designed and long-term follow-up studies, where the complex interactions between nutrients and microbiota should be taken into account.

In this context, several research projects have been recently launched or are being launched which aim to assess diet and microbiome related to IBD. These include the Food and Resulting Microbial Metabolites (FARMM) study by the Crohn's and Colitis Foundation of America (CCFA)[79], the Study on the Genetic, Environmental and Microbial Interactions that Cause IBD (GEM Project) by the Crohn's and Colitis Canada[80], the Prognostic effect of Environmental factors in Crohn's and Colitis (PREdiCCt) study [81]and the Diet, microbiome and inflammatory bowel disease course (microIBDiet) study. The FARMM study, part of the CCFA's Microbiome Initiative, is a controlled feeding experiment among healthy volunteers, with the objective of examining how different diets ("Western" diet, exclusive enteral nutrition and a vegan diet for 2 weeks) influence the gut microbiota and fecal metabolomics. The GEM Project is recruiting healthy first degree relatives of CD patients, and will assess genetic and environmental factors and gut microbiome; participants

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will be followed up to assess the risk of developing CD and the factors associated to the disease. The PREdiCCT study will recruit IBD patients in remission, and will assess diet, lifestyle, genetics and microbiome; participants will be followed-up for two years to study the factors associated to relapse. The microIBDiet study will recruit newly diagnosed IBD patients, and will assess diet and microbiome; patients will be followed up for five years to study the factors associated to an impaired outcome (personal communication from the principal investigator). These projects bring promise for shedding more light on the role of dietary and nutritional factors in IBD as a basis for further dietary intervention trials.

Conclusion

In summary, rapid technological bioscience development has opened new horizons for an improved understanding of the role of gene-environment interactions on the onset and development of IBD. Targeted nutrition taking into account individual genetic make-up, epigenetics and microbiota composition may represent novel platform for successful prevention and could offer successful strategy for disease control. Definitely, more work is required to evaluate the role of individual food compounds and complex nutritional interventions with potential to decrease inflammation, to modulate immune-modulatory epigenetic traits and maintain intestinal microbial balance as means for prevention and management of IBD.

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