Environmental triggers in IBD: a review of progress and evidence

Ashwin N. Ananthakrishnan[1], Charles N. Bernstein[2], Dimitrios Iliopoulos[3], Andrew Macpherson[4], Markus F. Neurath[5], Raja A. Raja Ali[6], Stephan R. Vavricka[7] and Claudio Fiocchi[8]

Abstract | A number of environmental factors have been associated with the development of IBD. Alteration of the gut microbiota, or dysbiosis, is closely linked to initiation or progression of IBD, but whether dysbiosis is a primary or secondary event is unclear. Nevertheless, early-life events such as birth, breastfeeding and exposure to antibiotics, as well as later childhood events, are considered potential risk factors for IBD. Air pollution, a consequence of the progressive contamination of the environment by countless compounds, is another factor associated with IBD, as particulate matter or other components can alter the host's mucosal defences and trigger immune responses. Hypoxia associated with high altitude is also a factor under investigation as a potential new trigger of IBD flares. A key issue is how to translate environmental factors into mechanisms of IBD, and systems biology is increasingly recognized as a strategic tool to unravel the molecular alterations leading to IBD. Environmental factors add a substantial level of complexity to the understanding of IBD pathogenesis but also promote the fundamental notion that complex diseases such as IBD require complex therapies that go well beyond the current single-agent treatment approach. This Review describes the current conceptualization, evidence, progress and direction surrounding the association of environmental factors with IBD.

IBD
Two diseases (Crohn's disease and ulcerative colitis) affecting primarily the digestive tract, characterized by chronic inflammation.

Microbiota
Community of microorganisms comprising bacteria, viruses, fungi, Archaea and eukaryotic microorganisms.

Correspondence to A.N.A. Massachusetts General Hospital, Harvard Medical School, 165 Cambridge Street, Boston, Massachusetts 02114, USA.
aananthakrishnan@mgh.harvard.edu

doi:10.1038/nrgastro.2017.136
Published online 11 Oct 2017

The changing epidemiology of IBD (Crohn's disease and ulcerative colitis) across time and geography suggests that environmental factors have a major role in inducing or modifying disease expression[1–3]. Considering that IBD emerged in Western countries throughout the middle of the 20th century, the emergence of IBD in developing nations over the past 25 years suggests that this epidemiological evolution is related to westernization of lifestyle and industrialization[2,3]. Urbanization of societies is associated with changes in diet, antibiotic use, hygiene status, microbial exposures and pollution, which have been implicated as potential environmental risk factors for IBD (FIG. 1). Environmental risk factors for individual, familial, community-based, country-based and regionally based origin could all contribute to the pathogenesis of IBD[1,4–6]. Lending further support to the critical importance of environmental influences is recognition of the central role of the gut microbiota in the development and propagation of inflammation in IBD[7]. Although host genetics might partly determine gut microbial structure, external environmental exposures from the time of birth to adulthood continue to alter the composition, structure and function of the gut microbiome, thereby dynamically altering the risk and natural history of disease throughout life[8,9]. Discovering how environmental factors influence the onset of IBD and contribute to its pathogenesis could, ultimately, help to determine how individuals can reduce their risk of disease or have a milder clinical course. The search for pathogenic environmental factors is also important, as many unmet therapeutic needs and suboptimal outcomes in IBD remain. Mechanistic insights obtained from robustly defining environmental influences could also lead to identification of new therapeutic targets and treatment strategies. In this Review, we summarize the latest literature on various environmental influences in IBD and discuss how such factors could provide insights into IBD pathogenesis and potential therapeutic pathways.

The gut microbiota in IBD

Dysbiosis is defined as a change in the normal microbial ecology, considered mainly for the intestine in the context of IBD. Infection can be defined as the invasion and multiplication of microorganisms in body tissues by a causative agent or agents (identified either through classic culture or indirectly through their genomic signatures) and credible pathogenicity. Normally, the gut

Key points

- Gut microbiota composition is known to be important in maintaining health and mediating disease
- Dysbiosis, a change in the normal microbial ecology, occurs in the intestine in the context of IBD
- Gut inflammation in IBD is characterized by a reduced diversity of microbiota, which could render the host more susceptible to colonization with pathogens or pathobionts
- Environmental factors probably have a major role in IBD; antibiotic use, childbirth mode, breastfeeding, air pollution, NSAID use, hypoxia or high altitude, diet and urban environments have been studied
- Future studies should adopt a multi-omic big data approach, integrating several layers of data on clinical parameters, environmental exposures, genetics, epigenetics, immunological function and microbial structure

microbiota varies longitudinally and transversely in the gastrointestinal tract in a single individual, and the microbial consortia vary between different individuals, with evidence of clusters of distinct microbial communities in different individuals[10]. These well-balanced host–microbial symbiotic states probably respond differently to episodes of infection, dietary changes, drug intake and exposure to xenobiotics.

The intestinal microbiota is known to be important in both maintaining health and mediating disease[8,9], and its composition is influenced by both environmental and host factors[8,9,11]. Approximately one-third of faecal bacterial taxa are heritable[11]. In 1,098 individuals in the discovery cohort individuals, 58 single-nucleotide polymorphisms (SNPs) were identified as associated with the relative abundance of 33 taxa. Among these SNPs, four loci were replicated in a second cohort of 463 individuals: rs62171178 (nearest gene *UBR3*) associated with Rikenellaceae, rs1394174 (*CNTN6*) associated with *Faecalibacterium*, rs59846192 (*DMRTB1*) associated with *Lachnospira*, and rs28473221 (*SALL3*) associated with *Eubacterium*[11].

That the gut microbiota is altered during intestinal inflammation is well known. In animal models of intestinal infection (for example, *Salmonella* infection), resolution of the infection only occurs once the normal microbiota is re-established[12]. Multiple studies have now investigated the difference in gut microbial composition between patients with IBD and healthy individuals. Gut inflammation in IBD is characterized by a reduced diversity of microbiota, which could render the host more susceptible to colonization with pathogens or pathobionts[7,13]. The underlying uninflamed mucosa in IBD has a different microbiota than healthy mucosa[7,13]. Hence, gut dysbiosis can trigger further changes in the gut microbiota of individuals with IBD, or alternatively, the gut epithelium of individuals with IBD could be conducive to development of an aberrant microbiota[14]. An elegant study examined samples from multiple gastrointestinal locations collected before treatment in new-onset paediatric Crohn's disease[13]. An axis defined by an increased abundance in certain bacteria, which included Enterobacteriaceae, Pasteurellaceae, Veillonellaceae and Fusobacteriaceae, and decreased abundance in Erysipelotrichales, Bacteroidales, and Clostridiales (FIG. 2) correlated strongly with presence of disease. Microbiome comparison between patients with Crohn's disease with and without antibiotic exposure indicated that antibiotic use amplifies microbial dysbiosis associated with the condition[13]. In addition to alterations in the composition and function of the bacterial taxa, studies have also suggested a potential role for enteric viruses, fungi and Archaea[7,15]. In a mouse model, murine norovirus infection was necessary in addition to mutations in the autophagy gene *ATG16L1* to cause altered Paneth cell phenotypes that predisposed to colitis[16]. Neither the genetic variant nor enteric infections alone were sufficient to result in a pathogenic change. Alterations in the human gut microbiome that develop as early as infancy could contribute to the pathogenesis of IBD[17]. Some of the earliest exposures perturbing the infant gut microbiota are mode of birth, breastfeeding and exposure to antibiotics, all of which have been examined as factors predisposing to the development of IBD and are explored herein.

Early-life factors

Antibiotics. Healthy humans before, during and after a 5-day course of oral ciprofloxacin demonstrated decreased diversity, richness and evenness of the faecal microbiota[18]. The abundance of roughly one-third of the bacterial taxa changed during ciprofloxacin administration, and, although the microbiota largely returned to pretreatment community composition at 4 weeks after treatment, several bacterial taxa failed to recover within 6 months, suggesting a persistent effect of even a short course of oral antibiotics[19]. In another study[18,19], the distal gut microbiota of three individuals examined over 10 months and spanning two courses of ciprofloxacin demonstrated that the effect of the antibiotic on gut

Xenobiotics
Substances that are foreign to the body.

Pathobionts
Microorganisms associated with chronic inflammatory diseases.

Archaea
A kingdom of single-cell microorganisms without a nucleus or membrane-bound organelles.

Author addresses

[1]Massachusetts General Hospital, Harvard Medical School, 165 Cambridge Street, Boston, Massachusetts 02114, USA.
[2]University of Manitoba IBD Clinical and Research Centre, 804-F-175 McDermot Avenue, Winnipeg Manitoba R3E 3P4, Canada.
[3]Center for Systems Biomedicine, Vatche & Tamar Manoukian Division of Digestive Diseases, Department of Medicine, UCLA, 650 Charles E. Young Drive South CHS 44–133, Los Angeles, California 90095–7278, USA.
[4]Gastroenterology/UVCM, Inselspital, Freiburgstrasse 8, 3010 Bern, Switzerland.
[5]I. Department of Medicine, University of Erlangen-Nürnberg, University Hospital, Ulmenweg 18, 91054 Erlangen, Germany.
[6]The National University of Malaysia, UKM Medical Centre, Jalan Yaacob Latif, 56000 Kuala Lumpur, Malaysia.
[7]Triemli Hospital, Department of Gastroenterology & Hepatology, Birmensdorferstrasse 497, 8063 Zurich, Switzerland.
[8]Department of Pathobiology, Lerner Research Institute, and Department of Gastroenterology & Hepatology, Digestive Diseases and Surgery Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA.

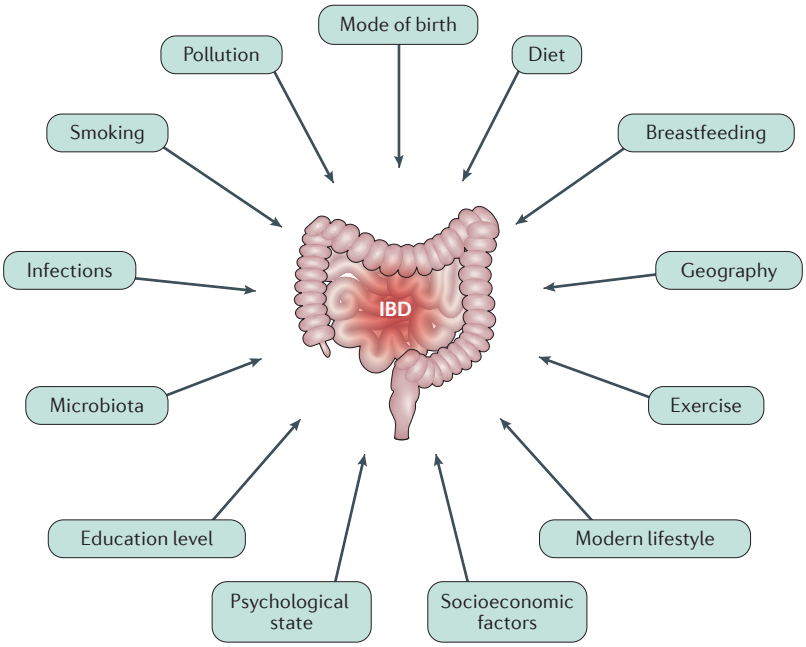


Figure 1 | **Environmental factors contributing to IBD pathogenesis.** Environmental factors including conception, maternal influences on the fetus, mode of birth, early childhood and diet and lifestyle exposures during adulthood modify the risk of development of Crohn's disease and ulcerative colitis.

microbiota was profound and rapid, with a loss of diversity and a shift in community composition occurring within 3–4 days of starting treatment. One week after the end of each course, communities began to return to their initial state, but the return was often incomplete. In all individuals, the composition of the gut microbiota stabilized by the end of the experiment, but was altered from its initial state[19]. As antibiotics have been widely used in both developing and developed nations, and are increasingly being used in developing nations, it is reasonable to consider that antibiotic use could be a key predisposing factor in IBD pathogenesis. Misuse and abuse of antibiotics as well as use in livestock might further compound this issue.

Several studies have examined whether antibiotic use early in life predisposes to IBD and have consistently demonstrated this association in Western populations[20]. A Canadian IBD epidemiological database study compared antibiotic use in the first year of life between 36 individuals with IBD and 360 healthy controls. Of children with IBD, 58% had one or more antibiotic dispensations in their first year of life compared with 39% of controls; moreover, those receiving one or more dispensations of antibiotics were nearly three times as likely to be diagnosed with IBD[21]. In a separate analysis using a much larger data set of 294 children with IBD matched to 2,377 healthy controls to examine whether a diagnosis of otitis media (serving as a proxy for antibiotic use) was associated with IBD, the investigators found that individuals with an otitis media diagnosis by age 5 years were nearly three times as likely to have IBD (95% CI 1.5–5.2; $P = 0.001$), providing further support for the association of early antibiotic use with ultimate development of IBD[22]. Another study from Manitoba, Canada, found that adults diagnosed with IBD were also more likely to have been prescribed antibiotics 2–5 years before their diagnosis[23]. Antibiotic dispensations were associated with both Crohn's disease and ulcerative colitis, with the association nominally stronger in Crohn's disease cases for ≥1 and ≥2 dispensations, whereas ≥3 dispensations was associated with 1.5 times the likelihood of being diagnosed with ulcerative colitis. A dose-dependent relationship between the number of antibiotic dispensations and the risk of IBD was observed, further implicating antibiotic use as a predisposing factor in IBD pathogenesis[23].

A meta-analysis investigating antibiotic exposure as a risk factor for developing IBD analysed 11 observational studies (eight case–control and three cohort) including 7,208 patients diagnosed with IBD. All antibiotics were associated with IBD, with the exception of penicillin. Exposure to metronidazole (OR 5.01, 95% CI 1.65–15.25) or fluoroquinolones (OR 1.79, 95% CI 1.03–3.12) was most strongly associated with new-onset IBD, particularly Crohn's disease, and this association was more marked in children[20].

Other early-life influences. Studies examining 16S ribosomal RNA (rRNA) gene sequencing demonstrated that, by the end of the first year of life, the idiosyncratic microbial ecosystems in each baby, although still distinct, had converged towards a profile characteristic of the adult gastrointestinal tract[24], emphasizing the potential importance of influences in this period on subsequent risk of IBD. Interestingly, babies born by caesarian section are deprived of contact with the maternal gut or vaginal microbiota, and their microbiotas are characterized by a lack of strict anaerobes and the presence of facultative anaerobes such as *Clostridium* species[25,26]. Infants born via caesarian section have a more slowly diversifying microbiota than those born vaginally. In a study using *in situ* hybridization of faecal samples from 7-year-old children, significantly higher numbers of clostridia were found in those delivered vaginally compared with caesarian-born children ($P = 0.0055$), indicating that an abnormal intestinal microbiota reported after caesarian section delivery could continue even beyond infancy[27]. However, despite data demonstrating that mode of birth exerts a strong influence on the intestinal microbiome, an association with IBD has not been demonstrated in epidemiological studies. A population-based analysis to determine whether mode of delivery (caesarian versus vaginal delivery) affects risk of IBD was carried out on data from 1,671 individuals with IBD and 10,488 healthy controls linked to mothers' obstetrical records. Overall, there was no difference in the percentage of individuals with IBD born by caesarian section (11.6%) versus controls (11.7%, $P = 0.93$). Furthermore, individuals with IBD were no more likely to have been born by caesarian section than were their siblings without IBD (1,740 siblings from 1,615 families; 11.6% versus 11.3%; $P = 0.79$)[28].

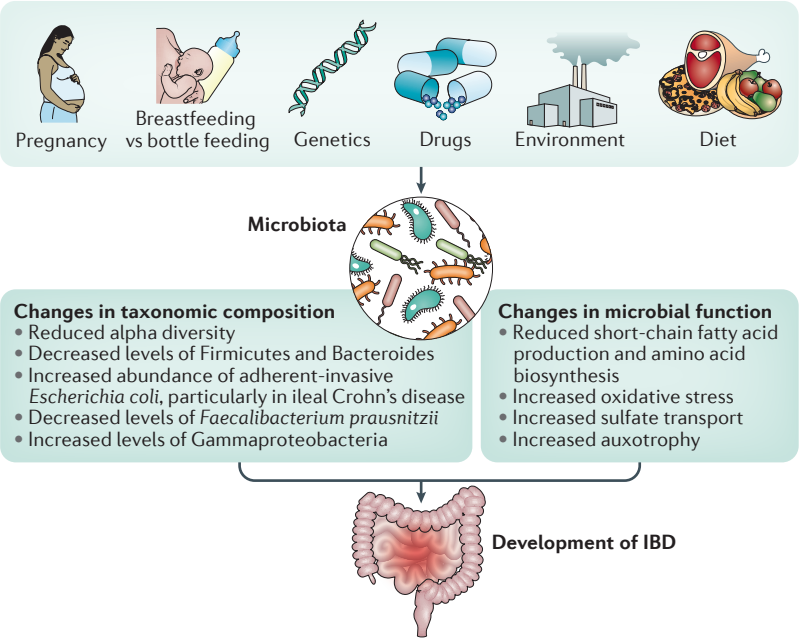


Figure 2 | **Lifelong influences on the gut microbiome from conception to adult life lead to dysbiosis and development of IBD.** The gut microbiome is susceptible to the influence of host genetics and environmental influences throughout childhood and adult life. The resultant alterations in taxonomic composition and function contribute to the development of intestinal inflammation in IBD.

Studies have demonstrated a strong and durable effect of breastfeeding on composition of the infant gut microbiota. The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study collected faecal samples at 4 months of age and examined the association between gut microbiota composition (through high-throughput DNA sequencing) and perinatal factors including infant feeding[25]. Actinobacteria and Firmicutes were the most common phyla observed in both groups, with lower bacterial diversity observed in breastfed infants than formula-fed infants. Formula-fed infants had an increased abundance of *Peptostreptococcus* and notably *Clostridium difficile*, which has been associated with atopic response and allergic sensitization[25]. Exposure to human milk during the development of the infant immune system could also confer tolerance to dietary and microbial antigens. Intrapartum antibiotics in caesarian and vaginal delivery are associated with infant gut microbiota dysbiosis, and breastfeeding modifies some of these effects[26]. In animal studies, *Il10*$^{-/-}$ mice demonstrated reduced levels of systemic TNF and IFNγ and reduced severity of histological inflammation in the intestine when fed mouse breastmilk[29]. The possible association between breastfeeding and the incidence of ulcerative colitis in humans was established as long ago as 1961 (REF. 30). A meta-analysis of 17 relevant articles examined the association between breastfeeding and IBD and demonstrated a strong inverse association with both Crohn's disease (OR 0.45, 95% CI 0.26–0.79) and ulcerative colitis (OR 0.56, 95% CI 0.38–0.81)[31]. A systematic review focusing on paediatric IBD similarly demonstrated a strong inverse association with early-onset disease[32]. Other studies have demonstrated that longer duration of breastfeeding is associated with a stronger protective influence, with decreasing risk of IBD when infants were breastfed for durations of 3, 6, or 12 months[6]. Whether early-life influences such as breastfeeding continue to demonstrate an effect even in individuals with established disease is less well known. A single report from a referral centre of 333 patients with Crohn's disease and 270 patients with ulcerative colitis found that a history of being breastfed in infancy was associated with a decreased risk of Crohn's-disease-related surgery[33].

Together, the described data support the hypothesis that alterations in the gut microbiota both in the first year of life following delivery and subsequently could be critical risk factors for IBD, exerting their influences by altering the developing gut microbiota.

Urbanization, pollution and diet

Urbanization. Epidemiological studies have consistently shown that IBD is more common in urban centres[34]. In a Swiss cohort study, living in an urban zone was associated with both Crohn's disease and ulcerative colitis (relative risk (RR) 1.49, $P<0.001$; RR 1.63, $P<0.001$, respectively)[35]. From 1960 to 2005, a period with increasing urbanization, there have been increases of 2.4% (95% CI 2.1–2.8%; $P<0.001$) and 3.6% (95% CI 3.1–4.1%; $P<0.001$) per annum in the prevalence of ulcerative colitis and Crohn's disease, respectively, in industrialized countries[34]. The effect of urban environments on disease risk is particularly apparent in countries that have witnessed industrialization and westernization over the past few decades. Although the overall incidence and prevalence remains markedly lower than in the Western Hemisphere, when available, data on secular trends in disease burden suggest fairly rapid increases in incidence for both Crohn's disease and ulcerative colitis in the developed and developing countries in Asia. Elegant, comprehensive data from the Japan Ministry of Health demonstrated a substantial (more than tenfold) increase in the incidence of both Crohn's disease and ulcerative colitis since the 1970s, confirmed by similar data from South Korea, China and India[36]. The effect of urbanization on development of IBD might be mediated by a variety of changes that accompany such development, including changes in lifestyle and behaviour, exposure to environmental pollution, and alteration in diet, all of which are explored herein.

Environmental pollution. Various components of ambient air pollution have been associated with detrimental health conditions in urban environments. Short-term exposure of the gut to high levels of airborne particulate matter results in increased gut permeability and heightened innate immune response in the small intestine, while chronic exposure results in increased expression of pro-inflammatory cytokines and alterations in microbiota composition and function in the colon in mice[37]. In addition, long-term exposure exacerbated colitis in an *Il10*$^{-/-}$ mouse model[37]. However, epidemiological data examining the association between air pollution and IBD have yielded mixed results for particulate matter

exposure, indicating that when there is an association, other components of air pollution could have a role in disease pathogenesis. Kaplan *et al.*[38] studied whether ambient air pollution levels were associated with the incidence of IBD. Individuals who resided in regions with NO_2 concentrations in the highest quintile were more likely to be diagnosed with Crohn's disease before 23 years of age than individuals in regions in the lowest quintile. Individuals residing in areas of higher SO_2 concentrations were more likely to develop ulcerative colitis than those in areas with low SO_2 (REF. 38). In a European nested case–control study, exposure to fine particulate matter was inversely associated with risk of IBD but not individually for Crohn's disease or ulcerative colitis[39]. By contrast, proximity to heavy traffic load was associated with increased risk of disease, and other air pollutants such as nitrous oxides showed a trend towards positive associations with IBD[39]. An ecological analysis correlating emissions and number of hospitalizations for IBD by area code in Wisconsin, USA, identified a direct correlation between total density of air pollutant emissions and adult IBD hospitalizations[40]. Each 1-log increase in the density of total criteria pollutant emission was associated with a 40% increase in the rate of IBD (Crohn's disease or ulcerative colitis). Analysis of individual pollutants suggested statistically significant associations with CO, NO_2, SO_2 and fine particulate matter ($PM_{2.5}$)[40].

Diet. Evidence indicates that the composition of the gut microbiota can influence susceptibility to ulcerative colitis and Crohn's disease. Dietary changes are common in modern human society and cause alterations in the composition of the gut microbiota that, in turn, can lead to an aberrant intestinal immune response and, eventually, IBD[41–43]. Dysbiosis can induce colitis in mice[44], paralleling what is observed in patients with IBD who display reduced microbial diversity, with enrichment of bacteria of the family Enterobacteriaceae and depletion in bacteria from the phylum Bacteroidetes and certain bacteria from the phylum Firmicutes[7,13]. The mechanisms by which dysbiosis can trigger IBD are not fully understood, but the increase in invasive bacterial species coupled with a decrease in protective bacteria could disrupt local immune homeostasis, increase mucosal permeability and cause loss of immune tolerance[45].

The role of diet in the pathogenesis of Crohn's disease and ulcerative colitis was initially inferred through retrospective case–control studies, relying on recall for determination of pre-illness diet. A paediatric case–control study by Amre *et al.*[46] reported an inverse association between intake of fruits and vegetables and risk of Crohn's disease. Moreover, prospective cohort studies from Europe and North America have provided more robust estimates of the effects of dietary macronutrients and micronutrients on disease risk. In a large prospective cohort of 170,776 women followed for 26 years, women in the highest quintile of intake of dietary fibre were significantly less likely to develop incident Crohn's disease (OR 0.59, 95% CI 0.39–0.90) compared with those in the lowest quintile[47]. Furthermore, in addition to the total intake of fibre, the effect differed based on the source of fibre. The highest quintile of intake of dietary fibre from fruits, and to a lesser extent vegetables, was associated with a reduced risk of Crohn's disease; by contrast, fibre from whole grains, cereal or bran was not associated with a modified disease risk[47]. In two prospective cohort studies, dietary *n*-3 polyunsaturated fatty acid (PUFA) intake has been inversely associated with risk of ulcerative colitis, whereas dietary *n*-6 PUFA intake is associated with increased risk of incident ulcerative colitis[48–50]. In a study of spontaneous and NSAID-induced colitis in mice, dietary *n*-3 PUFAs reduced the clinical severity of colitis[51]. In addition, the production of TNF by splenic $CD4^+$ T cells was suppressed. As TNF plays a pivotal part in IBD pathogenesis, these findings are consistent with previous reports documenting the prophylactic effect of *n*-3 PUFAs on experimental colitis[51]. The association of incident IBD with intake of carbohydrates and protein has been more mixed, with some epidemiological cohorts, but not other studies, reporting a higher risk of incident IBD with a diet rich in animal protein[52,53]. The association between diet and risk of IBD seems to exist not just for recent diet before diagnosis of illness but also adolescent diet during high school, independent of diet during adulthood[54].

In addition to dietary macronutrients, micronutrients might also plausibly modify risk of IBD. Various micronutrients are recognized to have important roles as co-enzymes involved in maintaining the gut epithelial barrier, or directly influencing intestinal immune responses or gut microbial composition. For example, zinc is an important cofactor for various intestinal metalloproteinases and, in cell culture models, zinc deficiency is associated with reduced barrier integrity and increased permeability[55]. In a prospective cohort study, high intake of zinc was inversely associated with risk of Crohn's disease in women[56]. The reduction in risk was noted up to a daily zinc intake of 16 mg per day, twice the recommended daily intake. In established disease, low serum levels of zinc were associated with increased risk of hospitalizations, surgery and disease-related complications in both Crohn's disease and ulcerative colitis[57]. Furthermore, normalization of zinc levels was associated with improvement in outcomes. In a small interventional study, zinc supplementation was associated with reduction in intestinal permeability as measured using the lactulose:mannitol ratio[55]. Vitamin D was initially recognized for its role in bone health, but emerging epidemiological, experimental and interventional data support a plausible role in pathogenesis of Crohn's disease. In a prospective cohort study, women with high levels of predicted plasma vitamin D had a significantly reduced likelihood of developing incident Crohn's disease even after adjusting for relevant confounders (HR 0.38, 95% CI 0.15–0.97)[58]. In a study of 3,217 patients with established IBD, individuals with serum 25-hydroxy vitamin D levels <20 ng/ml had higher risk of IBD-related surgery and hospitalizations than those with levels >30 ng/ml (REF. 59). Importantly, those who were initially deficient but subsequently attained normal levels had a substantially lower likelihood of surgery than those who remained deficient in vitamin D.

Finally, the association between diet and risk of incident disease might not be through nutritive factors alone but also through other components added during food processing, such as emulsifiers. In elegant experimental studies, two emulsifiers, carboxymethyl cellulose (CMC) and polysorbate 80 (P80), were shown to alter the gut microbiota towards a pro-inflammatory state by increasing levels of bioactive flagellin within 1 day[60,61]. The pro-inflammatory microbiota was associated with alterations in gene expression and development of colitis. Transfer of stool from such mice to germ-free mice reproduced the changes in both microbial composition and intestinal expression of pro-inflammatory genes[60,61].

Other factors

NSAIDs. NSAIDs are among the most frequently consumed medications, and their association with gastric or duodenal ulcerations is well recognized. However, evidence has also linked them to the development of IBD[62]. Several explanations have been offered as the potential mechanism for the association between NSAIDs and IBD. First, non-selective inhibition of cyclooxygenase (COX) reduces synthesis of protective prostaglandins. *Il10*$^{-/-}$ mice administered NSAIDs had a 75% reduction in protective prostaglandin E_2 production not seen with selective COX2 inhibition[63]. Second, cytoskeletal disruption and increased mucosal permeability both involve activation by gut bacteria and uncoupling of mitochondrial oxidative phosphorylation[64,65]. A prospective cohort study assessed the association between aspirin and NSAID use and incidence of Crohn's disease and ulcerative colitis[62]. A higher risk of both conditions was observed with the highest frequency of NSAID use. Compared with non-users, women who used NSAIDs at least 15 days per month had an increased risk of both Crohn's disease (HR, 1.59, CI, 0.99–2.56) and ulcerative colitis (multivariate HR, 1.87; 95% CI 1.16–2.99). Similarly, increased risk of IBD was seen with prolonged duration of NSAID use (>6 years)[62]. Other studies have examined if NSAID use similarly has a deleterious effect in established IBD. Long *et al.*[66] examined outcomes in 791 individuals with IBD in remission with baseline and follow-up data available for analysis. Of these, 247 patients with Crohn's disease (43.2%) and 89 ulcerative colitis patients (40.6%) reported NSAID use. Patients with Crohn's disease with NSAID use ≥5 times per month had greater risk of active disease at follow-up (23% versus 15%, $P = 0.04$)[66]. No effect was observed in patients with ulcerative colitis (22% versus 21%, $P = 0.98$). In a prospective trial, patients with quiescent Crohn's disease and ulcerative colitis were randomly assigned to receive the non-NSAID analgesic paracetamol ($n = 26$) and the conventional NSAIDs naproxen ($n = 32$), diclofenac ($n = 29$), and indomethacin ($n = 22$) for 4 weeks. Non-selective NSAIDs, but not paracetamol, were associated with a 17–28% relapse rate within 9 days of ingestion[67]. Bonner *et al.*[68] showed high doses of NSAIDs to be associated with an increased numerical disease activity index score among patients with Crohn's disease with colonic involvement. The safety of selective COX2 inhibitors in 222 patients with ulcerative colitis in remission was examined in a study published in 2006 (REF. 69). Patients were randomly assigned to receive oral celecoxib (200 mg) or placebo twice daily for 14 days. In the celecoxib group, 3% experienced disease exacerbation through day 14, compared with 4% in the placebo group ($P = 0.719$), suggesting that selective inhibition of COX2 is not associated with disease relapse in IBD[69].

High altitude and hypoxia. Hypoxia is known to induce inflammatory responses in immune cells and endothelial cells: it is associated with an accumulation of inflammatory cells in multiple organs and elevated cytokines in experimental mouse models after short-term exposure to low oxygen concentrations[70–75]. In human studies, levels of circulating IL-6, IL-1ra and C-reactive protein are upregulated in response to hypobaric hypoxic conditions such as high altitude, and the systemic increases in these inflammatory markers could reflect local inflammation in the intestine[76]. Mammals have oxygen-sensing mechanisms that help them adapt quickly to hypoxia by increasing respiration, blood flow and survival responses[72]. If an inadequate supply of oxygen persists, additional mechanisms attempt to restore oxygenation or help the body adapt to hypoxia. Cellular adaptations to hypoxia rely on the transcription factor hypoxia-inducible factor (HIF), which is inactive when oxygen is abundant but is activated in hypoxic conditions[72].

Binding of HIF to the hypoxia response element of several HIF-regulated genes results in the increased transcription of several proteins involved in angiogenesis (such as vascular endothelial growth factor, VEGF), glycolysis (glucose transporter proteins), and erythropoiesis[72]. Serological studies have suggested that serum concentrations of VEGF, a potent angiogenic factor, are increased in patients with IBD[77]. Vermeulen *et al.*[78] reported that patients with ulcerative colitis or Crohn's disease have increased expression of HIF-1. Patients with IBD also have increased colonic mRNA expression of glycolytic enzymes, which is triggered by hypoxia through the transcription factor HIF-1 (REF. 78).

Effect of high altitude on healthy volunteers. In preliminary findings of a study of healthy volunteers (mountaineers after rapid ascent to high altitude), arterial oxygen saturation (sO_2) values were measured at three different altitudes (base at 490 m, 3,650 m and at 4,559 m). As expected, sO_2 values were significantly lower at both high-altitude locations than at the base location ($P < 0.001$). Peptic mucosal lesions on endoscopy were present in 28% of individuals on day 2 and in 61% on day 4, demonstrating that, even in healthy individuals, high altitude can induce ulcerations and inflammation in the gastrointestinal tract[79].

Hypoxia in IBD. Vavricka *et al.*[80] investigated whether flights and/or journeys to regions lying at an altitude of >2,000 m above sea level are associated with flare-ups in patients with IBD, within 4 weeks of the trip. Patients with IBD with at least one flare-up during a 12-month observation period were compared with a group of patients in remission. Patients with IBD experiencing flare-ups

Hypoxia
A state of reduced oxygenation in the tissues.

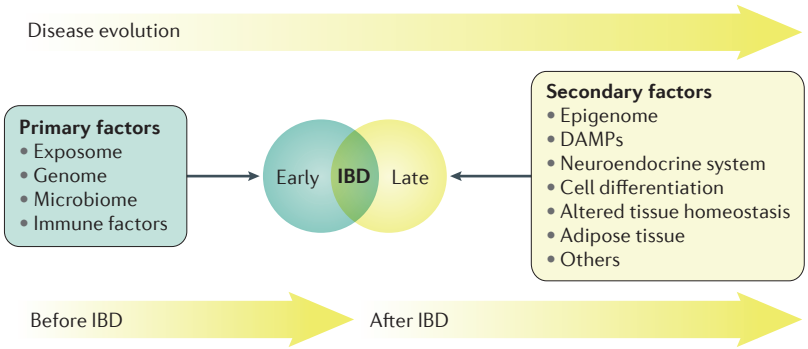


Figure 3 | **IBD — a continuously evolving biological process.** IBD is a continually evolving biological process, susceptible to various '-ome' influences that result in secondary biological alterations in the individual, altering the expression and progression of disease. DAMP, damage-associated molecular patterns.

had more frequently undertaken flights and/or journeys to regions >2,000 m above sea level within 4 weeks of the flare-up when compared with patients in remission (21 of 52 (40.4%) versus 8 of 51 (15.7%, $P = 0.005$)). These results suggest that journeys and/or flights to high-altitude regions are risk factors for IBD flare-ups occurring within 4 weeks of travel, and the role of hypoxia in IBD warrants further investigation.

Based on the hypothesis that hypoxia leads to intestinal inflammation, several studies have examined whether hyperbaric oxygen therapy can be used to treat Crohn's disease or ulcerative colitis. A systematic review of 17 studies (mostly small case studies) included 631 patients and demonstrated that hyperbaric oxygen therapy was associated with an 86% overall response rate with an 88% response for perineal Crohn's disease[81]. A small pilot proof-of-concept randomized trial that included 18 patients demonstrated hyperbaric oxygen therapy to be beneficial in moderate-to-severe ulcerative colitis[82].

Systems biology approach

The next step in unravelling the pathogenesis of such complex diseases such as IBD requires bridging of the gap between clinical and epidemiological observations regarding various environmental influences and mechanistic effects on intestinal inflammation, translating into new therapeutic targets. Owing to the large number and variety of environmental factors, the investigation into these factors and their targets requires mathematical modelling, enabling information to be organized into networks — a systems-biology-based approach that seeks to understand the complexity of disease pathogenesis. A systems approach in which biodata (that is, blood, stool and biopsy samples) are inputted and integrated via high-throughput technologies is ideal to study complex conditions such as ulcerative colitis and Crohn's disease. An example of this approach is the use of the iCluster algorithm for identification of IBD molecular subtypes by performing pattern discovery that integrates diverse data types: binary (somatic mutation), categorical (copy number gain, normal, loss), and continuous (gene expression) values[83]. Another example is the use of integrative Bayesian analysis (iBAG) for integration of IBD mechanistic data with clinical data[84]. The basic construction of the iBAG model consists of two components: a mechanistic model to capture information by partitioning the gene expression into components explained by different upstream platforms and a clinical model that subsequently incorporates these components to model the effects on a clinical outcome of interest. Combined, these approaches enable the identification of genes related to clinical outcome and generate insight into the biological mechanisms underlying these effects[85].

Although it cannot be described as examining the effect of an environmental factor, the identification of the microRNA miR-214 as a potential therapeutic target in ulcerative colitis network is a good example of how effective an IBD systems approach can be in identifying new therapeutic targets and providing mechanistic evidence in support of its efficacy. Specifically, it was the integration of transcriptomic and epigenomic data directly derived from colonic tissues from mouse models with colitis that identified miR-214 as a central regulator of an inflammatory molecular network[86]. The transcription factor signal transducer and activator of transcription 3 (STAT3) is upregulated in adult ulcerative colitis, which led to the subsequent investigation of the IL-6–STAT3 pathway in paediatric disease[87]. The levels of phosphorylated STAT3 and the genes it regulates were upregulated in tissue samples from patients with paediatric ulcerative colitis compared with controls. MiR-124 is a known epigenetic factor that regulates the expression of STAT3 (REF. 88). The reduced levels of miR-124 in colon tissues of children with ulcerative colitis result in increased expression and activity of STAT3, which promotes inflammation[88]. Consistent with this finding, a high-throughput functional screen of the full set of human microRNAs found that miR-214 regulated the activity of nuclear factor-κB. Integration of bioinformatic and genome-wide profile analyses showed that miR-214 activates an inflammatory response that is amplified through a feedback loop circuit mediated by phosphatase and tensin homologue (PTEN) and PDZ and LIM domain protein 2 (PDLIM2)[86]. Thus, based on these computational and molecular data, miR-214 is a promising drug target for ulcerative colitis. In fact, a miR-214 inhibitor blocked the inflammatory network and reduced the severity of colitis in mice induced by dextran sulfate sodium[86]. This example demonstrates the power of the systems biology approach to identify key molecules involved in IBD pathogenesis and develop novel therapies. Integration of clinical data and high-throughput molecular data, including epigenetic data, can therefore provide the most efficient and comprehensive approach to treating complex disorders such as IBD.

An integrated approach for new therapies

The currently accepted components of IBD pathogenesis include the exposome, the genome, epigenome, the gut microbiome and the immune system (FIG. 3). These components all interact with each another, and each represents a potential therapeutic target.

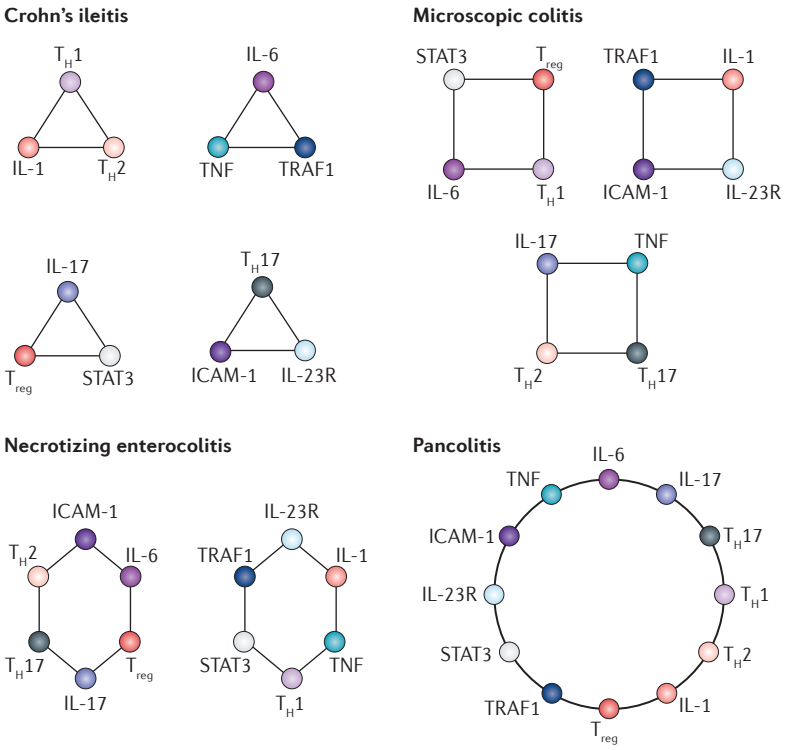
Exposome
The entirety of all environmental exposures of an individual in a lifetime.

Epigenome
DNA methylation and histone modifications that regulate expression of genes within a cell.

Genome. A number of exposome-derived epigenetic factors determine the expression of an IBD epigenotype or clinically evident IBD. The combination of IBD-associated gene variants, maternal factors, perinatal microbial exposures, antibiotics, diet and xenobiotics, among others, may determine the risk of developing IBD and the type and severity of the associated clinical manifestation. Deletion or replacement of gene variants leading to disease is technically feasible (in experimental animals) but might be associated with unknown risks, and effectiveness in humans is uncertain.

Box 1 | **Different network interactions can result in different diseases**

Different interactions between hypothetical biological networks that contain the same number and type of biological components can result in different diseases. The four hypothetical scenarios (networks) are each composed of the same number of biological components: four classes of T cells (T helper (Th)1, T_H2, T_H17 and regulatory (T_{reg}) cells), four cytokines (IL-1, IL-6, IL-17 and TNF), two receptors (IL-23R and ICAM-1) and two signalling molecules (TRAF1 and STAT3). Each biological component can only connect to two other components, but the connections vary drastically between the scenarios, even though the number of components (12) remains the same: in the upper left scenario, the 12 biological components connect in a way that results in four triangles; in the upper right scenario, the 12 components connect in a way that result in three squares; in the lower left scenario, the 12 biological components form two hexagons; and in the lower right scenario, they form one circle. Each of the four different arrangements (networks) has the same number of components but yields a different biological outcome that, if the outcome is a disease process, results in four different diseases. As a purely hypothetical example, the outcome of the arrangement composed of three triangles could yield Crohn's disease; the three triangles could yield microscopic colitis; the two hexagons could yield necrotizing enterocolitis; and the circle could yield pancolitis. Each of these distinct clinical entities would then require different forms of treatment, even though each entity derives from exactly the same number and type of biological components that only differ in the way they connect to each other.



Networks concept adapted from REF. 103, Macmillan Publishers Limited.

Exposome. Exposome-derived epigenetic factors able to modulate the microbiome and immune system proteins are innumerable. Among them are changes in dietary micronutrients or macronutrients, salt intake, artificial sweeteners, emulsifiers, smoking and titanium dioxide nanoparticles, all of which can induce chronic inflammation. Studies in sheep have shown that maternal obesity induces intestinal inflammation in the offspring, which correlated with increased expression of transforming growth factor-β and IL-17, suggesting that maternal obesity predisposes to IBD[89]. Comparisons of the distal gut microbiota of genetically obese mice and their lean littermates, as well as those of obese and lean human volunteers, have revealed that obesity is associated with changes in the relative abundance of two dominant bacterial phyla, Bacteroidetes and Firmicutes[90]. Important obstacles exist when considering IBD therapeutic interventions directed towards environmental factors: targets are mostly unknown, interventions only have selective efficacy (for example, smoking cessation) and behavioural modifications must be lifelong, leading to compliance and effectiveness issues.

Gut microbiome. Various microbial strategies could be used to maintain gut microbial homeostasis: antibiotics have a role in the removal or suppression of undesirable microorganisms; probiotics can introduce missing desirable microorganisms, whereas prebiotics facilitate the proliferation of beneficial microorganisms; defensins might become available to replenish antimicrobial peptides to balance the gut microbiota. Clinical studies demonstrating a beneficial effect of antibiotics or probiotics on disease activity in IBD have been carried out only with metronidazole or ornidazole in preventing postoperative recurrence[91], rifaximin in mild ileal Crohn's disease[92], and the probiotic VSL#3 in preventing recurrent pouchitis[93] and in mild to moderate ulcerative colitis[94]. Faecal microbiota transplantation (FMT) is a new strategy involving the infusion of a faecal suspension of stools from a healthy donor into the gastrointestinal tract of a patient to cure a specific disease[95,96]. To date, in patients with IBD, FMT only offers selective, transient, donor-dependent and recipient-dependent modification of the gut microbiota with unpredictable clinical benefits[96,97]. Two randomized clinical trials of FMT in ulcerative colitis reached diverging conclusions regarding efficacy, with one demonstrating a benefit and the other showing no effect of FMT in ulcerative colitis[96,97]. However, more detailed analysis of one of the trials revealed a strong donor effect in determining the efficacy of FMT[98,99]. A third, multicentre trial adopted a more aggressive regimen of FMT via enema 5 days a week for 8 weeks in comparison with placebo[100]. At the end of the trial, which included 85 patients, steroid-free clinical remission was achieved by 27% of patients receiving FMT compared with 8% receiving placebo ($P = 0.021$)[100]. Thus, treatments targeting gut microbiota can occasionally be efficacious but are not target selective, can be used only for limited periods of time, and might have unpredictable adverse effects.

Metabolomics
Study of chemical fingerprints (metabolites) present within an organism.

Phenomics
Measurement of physical and biochemical traits of an organism.

Box 2 | **Questions on the environment and IBD**

- How do environmental factors modify the risk or natural history of Crohn's disease and ulcerative colitis?
- When, during one's lifetime, are environmental exposures important for development of Crohn's disease and ulcerative colitis?
- How do environmental exposures exert their effect on risk of IBD, and why do some have divergent effects on Crohn's disease compared to ulcerative colitis?
- Are some individuals more susceptible to specific environmental factors, and is this governed by genetics, ethnicity, geography, competing influences or other biological mechanisms?
- Can modification of environmental factors be effective in primary and secondary prevention of IBD?
- Can changing environmental factors be used as stand-alone or adjunct treatment for management of IBD?
- Will better definition of environmental factors improve our understanding of disease biology and identify new therapeutic pathways?

Immune system. Modulating the immune system is by far the most common approach to treating IBD, and it involves using drugs to target cytokines, receptors, cell adhesion molecules and signalling pathways. However, these targets might change with gut inflammation, which is an evolving biological process controlled by primary and secondary factors that differentially modulate the immune response in the early and late stages of IBD. To distinguish immune events in early-stage and late-stage Crohn's disease, mucosal T cell function was investigated in paediatric IBD. At disease onset, mucosal T cells can mount a typical type 1 T helper response that resembles an acute infectious process, but this response pattern is lost with progression to late disease. This finding suggests that immunoregulation changes with the course of human IBD, affecting the timing and efficacy of immunomodulatory agents[101]. The main considerations in targeting the immune factors in IBD are that current treatments are reasonably effective, there is a limited target selection, the response is dependent on immune status, and efficacy can be lost over time.

Complex diseases require –omics-based therapy. Biological malfunctions responsible for disease vary from patient to patient, even when the diseases display similar clinical manifestations. As illustrated in this Review, this aspect is due to the multiplicity and complexity of the underlying molecular networks that cannot be controlled by blocking individual molecular targets. A detailed network analysis must be done to identify specific biological differences, probable outcomes and therapeutic target selection[79]. BOX 1 further explains the hypothesis on how the effect of different biological networks between the same components could yield different clinical phenotypes.

In IBD, there are numerous –omics fields contributing to disease development, including genomics (~20,000 genes), metagenomics (~1×10^{14} microorganisms), transcriptomics (~1×10^{5} RNA transcripts), proteomics (~1×10^{6} proteins), metabolomics (~1×10^{4} metabolites) and phenomics or exposomics (~1×10^{8} compounds). Their interaction creates an almost infinite number of pathogenic outcomes that can only be understood by adopting a systems biology approach to identify the central regulators of the networks. This multi-omics integration of IBD networks should enable specific target identification and is potentially curative, forming the basis for personalized treatments for IBD.

Conclusions

The gut microbiome is central to the pathogenesis of IBD. Multiple environmental influences, from the perinatal period to adulthood, do, however, influence the risk and natural history of these complex, immunologically mediated diseases. Emerging evidence has provided epidemiological support and mechanistic plausibility for several of these associations. Further studies must adopt a multi-omic big data approach, integrating several layers of information on clinical data, environmental exposures, genetics, epigenetics, immunological function and microbial structure. Such an approach offers the potential to further our in-depth understanding of the pathogenic mechanisms behind these diseases, which, in turn, could lead to the development of new avenues for treatment and disease prevention (BOX 2).

1. Ananthakrishnan, A. N. Epidemiology and risk factors for IBD. *Nat. Rev. Gastroenterol. Hepatol.* **12**, 205–217 (2015).
2. Kaplan, G. G. & Ng, S. C. Globalisation of inflammatory bowel disease: perspectives from the evolution of inflammatory bowel disease in the UK and China. *Lancet Gastroenterol. Hepatol.* **1**, 307–316 (2016).
3. Kaplan, G. G. & Ng, S. C. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology* **152**, 313–321.e2 (2017).
4. Burke, K. E., Boumitri, C. & Ananthakrishnan, A. N. Modifiable environmental factors in inflammatory bowel disease. *Curr. Gastroenterol. Rep.* **19**, 21 (2017).
5. Ng, S. C. *et al.* Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut* **62**, 630–649 (2013).
6. Ng, S. C. *et al.* Environmental risk factors in inflammatory bowel disease: a population-based case-control study in Asia-Pacific. *Gut* **64**, 1063–1071 (2015).
7. Kostic, A. D., Xavier, R. J. & Gevers, D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* **146**, 1489–1499 (2014).
8. Kahrstrom, C. T., Pariente, N. & Weiss, U. Intestinal microbiota in health and disease. *Nature* **535**, 47 (2016).
9. Lynch, S. V. & Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.* **375**, 2369–2379 (2016).
10. Arumugam, M. *et al.* Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
11. Turpin, W. *et al.* Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat. Genet.* **48**, 1413–1417 (2016).
12. Endt, K. *et al.* The microbiota mediates pathogen clearance from the gut lumen after non-typhoidal *Salmonella* diarrhea. *PLoS Pathog.* **6**, e1001097 (2010).
13. Gevers, D. *et al.* The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15**, 382–392 (2014).
14. Forbes, J. D., Van Domselaar, G. & Bernstein, C. N. Microbiome survey of the inflamed and noninflamed gut at different compartments within the gastrointestinal tract of inflammatory bowel disease patients. *Inflamm. Bowel Dis.* **22**, 817–825 (2016).
15. Miyoshi, J. & Chang, E. B. The gut microbiota and inflammatory bowel diseases. *Transl Res.* **179**, 38–48 (2017).
16. Cadwell, K. *et al.* Virus-plus-susceptibility gene interaction determines Crohn's disease gene *Atg16L1* phenotypes in intestine. *Cell* **141**, 1135–1145 (2010).
17. Kronman, M. P., Zaoutis, T. E., Haynes, K., Feng, R. & Coffin, S. E. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics* **130**, e794–e803 (2012).
18. Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl Acad. Sci. USA* **108** (Suppl. 1), 4554–4561 (2011).
19. Dethlefsen, L., Huse, S., Sogin, M. L. & Relman, D. A. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* **6**, e280 (2008).

20. Ungaro, R. *et al.* Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. *Am. J. Gastroenterol.* **109**, 1728–1738 (2014).
21. Shaw, S. Y., Blanchard, J. F. & Bernstein, C. N. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am. J. Gastroenterol.* **105**, 2687–2692 (2010).
22. Shaw, S. Y., Blanchard, J. F. & Bernstein, C. N. Association between early childhood otitis media and pediatric inflammatory bowel disease: an exploratory population-based analysis. *J. Pediatr.* **162**, 510–514 (2013).
23. Shaw, S. Y., Blanchard, J. F. & Bernstein, C. N. Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. *Am. J. Gastroenterol.* **106**, 2133–2142 (2011).
24. Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A. & Brown, P. O. Development of the human infant intestinal microbiota. *PLoS Biol.* **5**, e177 (2007).
25. Azad, M. B. *et al.* Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* **185**, 385–394 (2013).
26. Azad, M. B. *et al.* Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG* **123**, 983–993 (2016).
27. Salminen, S., Gibson, G. R., McCartney, A. L. & Isolauri, E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* **53**, 1388–1389 (2004).
28. Bernstein, C. N. *et al.* Cesarean section delivery is not a risk factor for development of inflammatory bowel disease: a population-based analysis. *Clin. Gastroenterol. Hepatol.* **14**, 50–57 (2016).
29. Madsen, K. L., Fedorak, R. N., Tavernini, M. M. & Doyle, J. S. Normal breast milk limits the development of colitis in IL-10-deficient mice. *Inflamm. Bowel Dis.* **8**, 390–398 (2002).
30. Acheson, E. D. & Truelove, S. C. Early weaning in the aetiology of ulcerative colitis. A study of feeding in infancy in cases and controls. *Br. Med. J.* **2**, 929–933 (1961).
31. Klement, E., Cohen, R. V., Boxman, J., Joseph, A. & Reif, S. Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. *Am. J. Clin. Nutr.* **80**, 1342–1352 (2004).
32. Barclay, A. R. *et al.* Systematic review: the role of breastfeeding in the development of pediatric inflammatory bowel disease. *J. Pediatr.* **155**, 421–426 (2009).
33. Guo, A. Y. *et al.* Early life environment and natural history of inflammatory bowel diseases. *BMC Gastroenterol.* **14**, 216 (2014).
34. Benchimol, E. I. *et al.* Rural and urban residence during early life is associated with a lower risk of inflammatory bowel disease: a population-based inception and birth cohort study. *Am. J. Gastroenterol.* **112**, 1412–1422 (2017).
35. Juillerat, P. *et al.* Prevalence of inflammatory bowel disease in the Canton of Vaud (Switzerland): a population-based cohort study. *J. Crohns Colitis* **2**, 131–141 (2008).
36. Ng, S. C. *et al.* Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* **145**, 158–165.e2 (2013).
37. Kish, L. *et al.* Environmental particulate matter induces murine intestinal inflammatory responses and alters the gut microbiome. *PLoS ONE* **8**, e62220 (2013).
38. Kaplan, G. G. *et al.* The inflammatory bowel diseases and ambient air pollution: a novel association. *Am. J. Gastroenterol.* **105**, 2412–2419 (2010).
39. Opstelten, J. L. *et al.* Exposure to ambient air pollution and the risk of inflammatory bowel disease: a European nested case-control study. *Dig. Dis. Sci.* **61**, 2963–2971 (2016).
40. Ananthakrishnan, A. N., McGinley, E. L., Binion, D. G. & Saeian, K. Ambient air pollution correlates with hospitalizations for inflammatory bowel disease: an ecologic analysis. *Inflamm. Bowel Dis.* **17**, 1138–1145 (2011).
41. Devkota, S. *et al.* Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10*$^{-/-}$ mice. *Nature* **487**, 104–108 (2012).
42. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
43. Lewis, J. D. & Abreu, M. T. Diet as a trigger or therapy for inflammatory bowel diseases. *Gastroenterology* **152**, 398–414.e6 (2017).
44. Couturier-Maillard, A. *et al.* NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* **123**, 700–711 (2013).
45. Brown, K., DeCoffe, D., Molcan, E. & Gibson, D. L. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients* **4**, 1095–1119 (2012).
46. Amre, D. K. *et al.* Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. *Am. J. Gastroenterol.* **102**, 2016–2025 (2007).
47. Ananthakrishnan, A. N. *et al.* A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology* **145**, 970–977 (2013).
48. Ananthakrishnan, A. N. *et al.* Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. *Gut* **63**, 776–784 (2014).
49. Chan, S. S. *et al.* Association between high dietary intake of the *n*-3 polyunsaturated fatty acid docosahexaenoic acid and reduced risk of Crohn's disease. *Aliment. Pharmacol. Ther.* **39**, 834–842 (2014).
50. de Silva, P. S. *et al.* An association between dietary arachidonic acid, measured in adipose tissue, and ulcerative colitis. *Gastroenterology* **139**, 1912–1917 (2010).
51. Chapkin, R. S. *et al.* Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. *J. Nutr.* **137**, S200–S204 (2007).
52. Chan, S. S. *et al.* Carbohydrate intake in the etiology of Crohn's disease and ulcerative colitis. *Inflamm. Bowel Dis.* **20**, 2013–2021 (2014).
53. Jantchou, P., Morois, S., Clavel-Chapelon, F., Boutron-Ruault, M. C. & Carbonnel, F. Animal protein intake and risk of inflammatory bowel disease: the E3N prospective study. *Am. J. Gastroenterol.* **105**, 2195–2201 (2010).
54. Ananthakrishnan, A. N. *et al.* High school diet and risk of Crohn's disease and ulcerative colitis. *Inflamm. Bowel Dis.* **21**, 2311–2319 (2015).
55. Sturniolo, G. C., Di Leo, V., Ferronato, A., D'Odorico, A. & D'Inca, R. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm. Bowel Dis.* **7**, 94–98 (2001).
56. Ananthakrishnan, A. N. *et al.* Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study. *Int. J. Epidemiol.* **44**, 1995–2005 (2015).
57. Siva, S., Rubin, D. T., Gulotta, G., Wroblewski, K. & Pekow, J. Zinc deficiency is associated with poor clinical outcomes in patients with inflammatory bowel disease. *Inflamm. Bowel Dis.* **23**, 152–157 (2017).
58. Ananthakrishnan, A. N. *et al.* Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology* **142**, 482–489 (2012).
59. Ananthakrishnan, A. N. *et al.* Normalization of plasma 25-hydroxy vitamin D is associated with reduced risk of surgery in Crohn's disease. *Inflamm. Bowel Dis.* **19**, 1921–1927 (2013).
60. Chassaing, B., Van de Wiele, T., De Bodt, J., Marzorati, M. & Gewirtz, A. T. Dietary emulsifiers directly alter human microbiota composition and gene expression *ex vivo* potentiating intestinal inflammation. *Gut* **66**, 1414–1427 (2017).
61. Chassaing, B. *et al.* Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* **519**, 92–96 (2015).
62. Ananthakrishnan, A. N. *et al.* Aspirin, nonsteroidal anti-inflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. *Ann. Intern. Med.* **156**, 350–359 (2012).
63. Berg, D. J. *et al.* Rapid development of colitis in NSAID-treated IL-10-deficient mice. *Gastroenterology* **123**, 1527–1542 (2002).
64. Mahmud, T., Rafi, S. S., Scott, D. L., Wrigglesworth, J. M. & Bjarnason, I. Nonsteroidal antiinflammatory drugs and uncoupling of mitochondrial oxidative phosphorylation. *Arthritis Rheum.* **39**, 1998–2003 (1996).
65. Krause, M. M. *et al.* Nonsteroidal antiinflammatory drugs and a selective cyclooxygenase 2 inhibitor uncouple mitochondria in intact cells. *Arthritis Rheum.* **48**, 1438–1444 (2003).
66. Long, M. D. *et al.* Role of nonsteroidal anti-inflammatory drugs in exacerbations of inflammatory bowel disease. *J. Clin. Gastroenterol.* **50**, 152–156 (2016).
67. Takeuchi, K. *et al.* Prevalence and mechanism of nonsteroidal anti-inflammatory drug-induced clinical relapse in patients with inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **4**, 196–202 (2006).
68. Bonner, G. F., Fakhri, A. & Vennamaneni, S. R. A long-term cohort study of nonsteroidal anti-inflammatory drug use and disease activity in outpatients with inflammatory bowel disease. *Inflamm. Bowel Dis.* **10**, 751–757 (2004).
69. Sandborn, W. J. *et al.* Safety of celecoxib in patients with ulcerative colitis in remission: a randomized, placebo-controlled, pilot study. *Clin. Gastroenterol. Hepatol.* **4**, 203–211 (2006).
70. Eckle, T. *et al.* A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* **111**, 2024–2035 (2008).
71. Eltzschig, H. K. *et al.* HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J. Exp. Med.* **202**, 1493–1505 (2005).
72. Eltzschig, H. K. & Carmeliet, P. Hypoxia and inflammation. *N. Engl. J. Med.* **364**, 656–665 (2011).
73. Rosenberger, P. *et al.* Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. *Nat. Immunol.* **10**, 195–202 (2009).
74. Thompson, L. F. *et al.* Crucial role for ecto-5′-nucleotidase (CD73) in vascular leakage during hypoxia. *J. Exp. Med.* **200**, 1395–1405 (2004).
75. Eltzschig, H. K. *et al.* Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J. Exp. Med.* **198**, 783–796 (2003).
76. Hartmann, G. *et al.* High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine* **12**, 246–252 (2000).
77. Giatromanolaki, A. *et al.* Hypoxia inducible factor 1α and 2α overexpression in inflammatory bowel disease. *J. Clin. Pathol.* **56**, 209–213 (2003).
78. Vermeulen, N. *et al.* Seroreactivity against glycolytic enzymes in inflammatory bowel disease. *Inflamm. Bowel Dis.* **17**, 557–564 (2011).
79. Fruehauf, H. *et al.* Unsedated transnasal esophago-gastroduodenoscopy at 4559 M (14957 Ft) — endoscopic findings in healthy mountaineers after rapid ascent to high altitude. *Gastroenterology* **138**, S483 (2010).
80. Vavricka, S. R. *et al.* High altitude journeys and flights are associated with an increased risk of flares in inflammatory bowel disease patients. *J. Crohns Colitis* **8**, 191–199 (2014).
81. Dulai, P. S. *et al.* Systematic review: the safety and efficacy of hyperbaric oxygen therapy for inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **39**, 1266–1275 (2014).
82. Dulai, P. S. *et al.* Hyperbaric oxygen therapy is safe and effective for hospitalized ulcerative colitis patients suffering from moderate-severe flares: a multi-center, randomized, double-blind, sham-controlled trial. *Gastroenterology* **152**, S198 (2017).
83. Shen, R. *et al.* Integrative subtype discovery in glioblastoma using iCluster. *PLoS ONE* **7**, e35236 (2012).
84. Wang, W. *et al.* iBAG: integrative Bayesian analysis of high-dimensional multiplatform genomics data. *Bioinformatics* **29**, 149–159 (2013).
85. Weiser, M. *et al.* Molecular classification of Crohn's disease reveals two clinically relevant subtypes. *Gut* http://dx.doi.org/10.1136/gutjnl-2016-312518 (2016).
86. Polytarchou, C. *et al.* MicroRNA214 Is associated with progression of ulcerative colitis, and inhibition reduces development of colitis and colitis-associated cancer in mice. *Gastroenterology* **149**, 981–992.e11 (2015).
87. Yu, L. Z. *et al.* Expression of interleukin-22/STAT3 signaling pathway in ulcerative colitis and related carcinogenesis. *World J. Gastroenterol.* **19**, 2638–2649 (2013).
88. Koukos, G. *et al.* MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology* **145**, 842–852.e2 (2013).
89. Yan, X. *et al.* Maternal obesity induces sustained inflammation in both fetal and offspring large intestine of sheep. *Inflamm. Bowel Dis.* **17**, 1513–1522 (2011).
90. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).

91. D'Haens, G. R. *et al.* Therapy of metronidazole with azathioprine to prevent postoperative recurrence of Crohn's disease: a controlled randomized trial. *Gastroenterology* **135**, 1123–1129 (2008).
92. Prantera, C. *et al.* Rifaximin-extended intestinal release induces remission in patients with moderately active Crohn's disease. *Gastroenterology* **142**, 473–481.e4 (2012).
93. Shen, J., Zuo, Z. X. & Mao, A. P. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm. Bowel Dis.* **20**, 21–35 (2014).
94. Sood, A. *et al.* The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin. Gastroenterol. Hepatol.* **7**, 1202–1209.e1 (2009).
95. Preidis, G. A. & Versalovic, J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology* **136**, 2015–2031 (2009).
96. Aroniadis, O. C. & Brandt, L. J. Intestinal microbiota and the efficacy of fecal microbiota transplantation in gastrointestinal disease. *Gastroenterol. Hepatol. (N. Y.)* **10**, 230–237 (2014).
97. Costello, S. P. *et al.* Systematic review with meta-analysis: faecal microbiota transplantation for the induction of remission for active ulcerative colitis. *Aliment. Pharmacol. Ther.* **46**, 213–224 (2017).
98. Moayyedi, P. *et al.* Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* **149**, 102–109.e6 (2015).
99. Rossen, N. G. *et al.* Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology* **149**, 110–118.e4 (2015).
100. Paramsothy, S. *et al.* Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* **389**, 1218–1228 (2017).
101. Kugathasan, S. *et al.* Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. *Gut* **56**, 1696–1705 (2007).
102. Gearry, R. B. IBD and environment: are there differences between east and west. *Dig. Dis.* **34**, 84–89 (2016).
103. Sarajilic, A., Malod-Dognin, N., Yaveroglu, O. N. & Przulj, N. Graphlet-based characterized of directed networks. *Sci. Rep.* **6**, 35098 (2016).

Author contributions

A.N.A. and C.F. drafted the manuscript. All authors made critical revisions of the manuscript for content and figures, and edited the manuscript.

Competing interests statement

A.N.A. has served on advisory boards for AbbVie, Merck and Takeda. C.N.B. is supported in part by the Bingham Chair in Gastroenterology. He has served on advisory Boards for AbbVie Canada, Ferring Canada, Janssen Canada, Napo Pharmaceuticals, Pfizer Canada, Shire Canada and Takeda Canada, and he has acted as a consultant to Mylan Pharmaceuticals. He has received educational grants from AbbVie Canada, Janssen Canada, Shire Canada and Takeda Canada. He has been on the speaker's panel for AbbVie Canada, Ferring Canada and Shire Canada. The other authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Review criteria

A symposium entitled '*New Treatment Targets in Gut and Liver Diseases*' was held in Lucerne, Switzerland, in October 2016, with one session focused on environmental factors associated with IBD. While the list of potential environmental factors is extensive and discussed in detail in many recent publications[1,4,102], the aim of this article is to reflect the content of that session in a comprehensive review of current conceptualization, evidence, progress and direction surrounding the factors discussed. For this Review, PubMed/Medline was searched for relevant articles between 1968 and July 2017 using the following MeSH terms: "inflammatory bowel disease", "Crohn's disease", "ulcerative colitis" in combination with "environment", "diet", "pollution", "antibiotics", "breastfeeding", "early life", "anti-inflammatory agents, non-steroidal", "hypoxia", "elevation", and "microbiome". Citation lists of the retrieved articles and reviews on the topic were reviewed to identify additional articles of interest.

FURTHER INFORMATION

iCluster: https://cran.r-project.org/web/packages/iCluster/index.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF