HHS Public Access

Author manuscript

Nat Rev Gastroenterol Hepatol. Author manuscript; available in PMC 2024 June 04.

Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2024 February ; 21(2): 86–100. doi:10.1038/s41575-023-00854-4.

Deciphering the different phases of preclinical inflammatory bowel disease

Jonas J. Rudbaek[1,2], **Manasi Agrawal**[1,4], **Joana Torres**[4,5,6,7], **Saurabh Mehandru**[4], **Jean-Frederic Colombel**[4], **Tine Jess**[†,1,3]

[1]Center for Molecular Prediction of Inflammatory Bowel Disease, Department of Clinical Medicine, Aalborg University, Copenhagen, Denmark

[2]Section for Biomarkers, Immunology and Antibodies, Department for Congenital Disorders, Statens Serum Institut, Copehangen, Denmark.

[3]Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

[4]Division of Gastroenterology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

[5]Department of Gastroenterology, Hospital Beatriz Ângelo, Loures, Portugal

[6]Division of Gastroenterology, Hospital da Luz, Lisbon, Portugal

[7]Faculdade de Medicina, Universidade de Lisboa, Portugal

Abstract

Inflammatory bowel diseases (IBD) are immune-mediated inflammatory diseases (IMIDs **[G]**) of the gastrointestinal tract and include two subtypes, Crohn's disease and ulcerative colitis. It is well-recognized that IBD is associated with a complex multifactorial etiology that includes genetic predisposition and environmental exposures, with downstream dysregulation of systemic immune function and host-microbial interactions in the local environment in the gut. Evidence to support the notion of a multistage development of IBD is growing, as has been observed in other IMIDs such as rheumatoid arthritis and systemic lupus erythematosus. With the rising worldwide incidence of IBD, it is increasingly important to understand the complex interplay of pathological

[†] jess@dcm.aau.dk .

Author contributions

All authors contributed substantially to discussion of the content and reviewed and/or edited the manuscript before submission. J.J.R., M.A., and T.J. researched data for the article. J.J.R, M.A and T.J. wrote the article.

Competing interests

S.M. has received research grants from Genentech and Takeda; payment for lectures from Genentech, Morphic and Taleda; and consulting fees from Arena Pharmaceuticals, Ferring, Morphic and Takeda. J.-F.C. reports has received research grants from AbbVie, Janssen Pharmaceuticals and Takeda; payment for lectures from AbbVie, Amgen, Allergan. Ferring Pharmaceuticals, Shire and Takeda; has received consulting fees from AbbVie, Amgen, Arena Pharmaceuticals, Boehringer Ingelheim, BMS, Celgene Corporation, Eli Lilly, Ferring Pharmaceuticals, Galmed Research, Genentech, Glaxo Smith Kline, Janssen Pharmaceuticals, Kaleido Biosciences, Imedex, Immunic, Iterative Scopes, Merck, Microbia, Novartis, PBM Capital, Pfizer, Protagonist Therapeutics, Sanofi, Takeda, TiGenix, Vifor; and holds stock options in Intestinal Biotech Development. J.T. has received research grants from AbbVie and Janssen Pharmaceuticals; has received ad board fees from AbbVie, Janssen Pharmaceuticals, Pfizer and BMS; has received speaker fees from AbbVie, Janssen Pharmaceuticals and Pfizer. J.J.R., M.A. and T.J. declare no competing interests.

Peer review information

Nature Reviews Gastroenterology & Hepatology thanks Luc Biedermann, Iago Rodriguez-Lago and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

events during the different stages of disease development to enable IBD prediction and prevention strategies. In this article, we review comprehensively the current evidence pertaining to the pre-clinical phase of IBD, including at risk, initiation and expansion phases. We also discuss the framework of preclinical IBD, expanding on underlying pathways in IBD development, future research directions and IBD development in the context of other IMIDs.

ToC Blurb

Inflammatory bowel disease (IBD) is an immune-mediated inflammatory disease (IMID). Here, the authors review evidence on the preclinical phase of IBD, outlining and describing the proposed at risk, initiation and expansion phases. Overlap with other IMIDs is discussed alongside the possible future directions for research into preclinical IBD.

Introduction

Inflammatory bowel diseases (IBD) are chronic immune-mediated diseases (IMIDs) of the gastrointestinal tract. IBD takes two major forms, ulcerative colitis and Crohn's disease, which differ in clinical presentation, disease location, and molecular pathology. Despite such differences, ulcerative colitis and Crohn's disease share a complex etiology covering a multifaceted interplay of genetic susceptibility, environmental factors, dysregulated immune responses, and altered gut microbiota, which, in a yet unknown combination, lead to disease development[1–4].

The increasing incidence of IBD worldwide[5] makes research into ways of achieving secondary or primary prevention of IBD of great importance. To attain the goal of earlier diagnosis and prevention in IBD it is imperative that we understand the complex interplay of molecules and pathways that drives disease development in the pre-clinical phase, including pathological changes occurring in the early life period. It has been proposed that the clinical onset of IBD is preceded by a crucial pre-clinical phase, as observed for several other IMIDs, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)[6–8]. One of the first studies to suggest the presence of a pre-clinical phase in IBD was a study published in 2005 by Israeli *et al.*[9] after the observation of an association between anti-*Saccharomyces cerevisiae* (ASCA) positivity, an anti-microbial antibody commonly associated with Crohn's disease, and later development of IBD in a cohort of 32 patients with Crohn's disease, 8 patients with ulcerative colitis and 95 healthy individuals as controls. Since that study, new studies have brought evidence for a pre-clinical phase of IBD and a series of exciting observations have been made that might help shape our overall understanding of the length and form of the pre-clinical phase of IBD and guide future research in the field.

In this Review, we seek to compile the available evidence pertaining to the pre-clinical phase of IBD to better characterize the individual stages of the pre-clinical disease **[G]** period, using the nomenclature suggested in a 2021 European Crohn's and Colitis Organization (ECCO) workshop[10], and the importance of understanding the combination of factors that drive disease development in the different pre-clinical phases. We also discuss the overlap

with other IMIDs and outline possible future directions of research within the field of pre-clinical IBD.

Risk factors for IBD

A major focus within IBD research has been attempting to elucidate factors that increase the future risk of IBD development. Thus far, factors such as inherited genetic risk loci and exposures, both during early life and later in life, have been shown to increase risk of disease development[11–14]. These factors could have a synergistic role in both priming the intestinal immune system against commensal **[G]** and self-antigens **[G]**, and making it more prone to dysregulation and therefore breaking down immune tolerance **[G]**, culminating in disease initiation.

Genetic predisposition

A genetic component in the development of IBD is well established. A concordance rate of 50% for Crohn's disease and around 19% for ulcerative colitis was found for monozygotic twins[15,16]. Furthermore, first-degree relatives (FDRs) have a significantly higher risk of developing disease (Crohn's disease, relative risk (RR), 10; 95 % CI, 2.73–25.60; ulcerative colitis, RR, 8; 95% CI, 5.86–10.67)[17]. These findings sparked an interest in identifying the genetic elements causing this higher familial prevalence of the disease. Since the introduction of genome-wide association studies (GWAS), the identification of genetic risk loci for IBD have been steadily increasing, and some of the identified loci include genes associated with immune function, including *NOD2*, *OSMR, SMAD3, IL23R* and the *HLA* locus[12,18,19]. So far as many as 240 IBD-associated risk loci have been identified [11,18]. This development speaks to the possibility that with increasingly high-resolution studies, more relevant risk loci are likely to be uncovered. Present findings indicate that genetic risk explains disease development only to a relatively minor extent. A large trans-ancestry association study utilizing a cohort of 86,640 individuals of European decent and 9,846 individuals of East Asian, Indian or Iranian decent found that the identified loci explain only 13.1% and 8.2% of the variance in disease liability, for Crohn's disease and ulcerative colitis, respectively[18], with substantial differences in genetic risk determinants across European and Asian populations, indicating that most of the variance is either due to undiscovered loci or other factors such as environmental exposures. Furthermore, notably few of the identified loci have presently been mechanistically linked to the disease[11,12,18]. Overall, genetics only explain a subset of the identifiable IBD risk, thereby highlighting the role of the environment or other non-genetically mediated effects in disease development[13]. Relative contribution of genetic risk versus environmental risk is likely inter-individual with the rarer monogenic forms of IBD having the largest contribution from genetic factors, whereas in other cases environmental factors could play a more prominent part.

Early life exposures

The early life period, considered conventionally to extend from prenatal life up to 5 years of age, represents a critical period towards immune maturation. Exposures during this period can modulate the risk of disease later in life[20]. The influence of early life exposures extends to IBD and other IMIDs[13,21,22]. In a systematic review and meta-analyses of 114

studies, restricted to exposures occurring during the first 5 years of age, Agrawal *et al.* have reported that infections, antibiotics, immigration from low to high incidence areas, passive smoking and breastfeeding modulate IBD risk [13]. This finding is reminiscent of murine studies in which a 'critical window of time' in early life determines the immunological tone of the host and changes occurring within this timeframe associate with increased susceptibility to inflammatory pathologies later in life [23]. Although breastfeeding seems to be protective against IBD [13], other exposures are detrimental. In subsequent epidemiological analysis, Agrawal, M. *et al.* have reported on increased ulcerative colitis risk with ≥3 courses of antibiotics during pregnancy and mebendazole, a broad-spectrum anthelminthic agent, exposure in early life[24,25]. Others have reported on the influence of greenspace and air pollutants on IBD risk, with increased greenspace conferring decreased risk and air pollutants conferring increased risk [26,27]. Parental Crohn's disease diagnosis at the time of birth, but not later in life, is associated with risk of Crohn's disease in the offspring[28]. Last, in a case–control pilot analysis of deciduous teeth of 28 individuals with (n = 12) and without IBD (n =16), higher levels of heavy metals were positively associated with IBD diagnosis[29].

Although these data provide important insights into the influence of the early life period on IBD risk, omics analysis of early life biological samples are critical to unravel downstream effects of these exposures. For example, in analyses of offspring of mothers with IBD with and without IBD, the former was reported to have intestinal dysbiosis **[G]**, observed as altered microbial composition, which was found to trigger changes in adaptive immune cell subsets in germ free mice inoculated with stool from these individuals, and elevated faecal calprotectin **[G]** levels, a marker of intestinal inflammation, compared to the latter[30,31]. Based on these data, we hypothesize that early life exposures can modulate gut microbiome **[G]**, mucosal immunological maturation, and thereby prime the immune system towards future health and disease already during the first years of life.

Exposures later in life

Whereas physiological changes and environmental exposures in the early life period have been suggested to function as priming events making the immune system vulnerable to disease associated pathological changes, exposures encountered later in life might act as triggering events in the disease development or might function to further shape the immunological landscape. To date, several exposures have been suggested to increase risk of IBD, including urban living[32], ultra-processed foods[33–35], infections[36], smoking[37–39] and other factors [14]. The usage of antibiotics has also been shown to increase risk of IBD. A study utilizing Danish register data identified exposure to antibiotics to be a risk factor for IBD within IBD families (two or more affected FDRs)[40]. Substantiating these findings, a population-based study published in 2023 linked antibiotics use to IBD risk across ages (10 to ≥60 years) [41]. Interestingly, the risk increased with subsequent antibiotic courses and the highest risk of disease was observed 1–2 years after antibiotic exposures, indicating that antibiotics could be a potential trigger in the development of IBD, possibly acting through effects on the intestinal microbiome. Furthermore, nonsteroidal anti-inflammatory drugs (NSAIDs) usage has also been associated with increased risk of IBD. A study utilizing the nurses health cohort (n = 76,795), with 123 incident cases of Crohn's disease and 117

cases of ulcerative colitis) showed that usage of NSAIDs at least 15 days per month was associated with increased risk of Crohn's disease[42].

An experimental study showed that propyzamide, a commonly utilized herbicide, promotes intestinal inflammation in IBD animal models[43]. Similarly, a study investigating two common emulsifiers showed induction of low-grade inflammation in wild-type mice and robust colitis in engineered mouse strains (*Il10*$^{-/-}$ and *Tlr5*$^{-/-}$ mice)[44]. Studies like these might help elucidate the mechanisms by which certain exposures increases IBD risk. Increased focus has been placed on perfluoroalkyl substances (PFAS). These compounds have so far been associated with ulcerative colitis in two studies, of respectively, 3,713 individuals[45] and 32,254 individuals[46]. . However, evidence is still conflicting, underlined in a nested case–control study finding PFAS exposure to have no association with ulcerative colitis risk and an indication of an inverse association with Crohn's disease risk[47]. Further research into these exposures in the pre-clinical phase and the mechanism by which they affect disease risk might aid in elucidating possible disease triggering events for IBD. Importantly, similar to the inter-individual differences in the contribution of environment versus genetics, it is likely that the influence of the various exposures could vary at each individual at risk, depending on timing, dose, synergistic effect or individual gene–environment interactions. Thus, it is plausible that a specific exposure might increase future risk of disease or act as a trigger initiating disease associate pathological changes in the individual patient.

Preclinical phase: disease initiation

Studies utilizing samples from individuals who later develop IBD have increased rapidly in number in the past few years, with more studies on the horizon. The two main strategies utilized in these studies are retrospective studies utilizing serum and/or plasma repositories, exemplified by the Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects (PREDICTs) study from the USA, which utilizes a retrospective military cohort with longitudinal samples collected during active military service of the included individuals[48], and prospective studies following patients over time, exemplified by the Crohn's and Colitis Canada Genetic Environmental Microbial (CCC GEM) project. This development has given rise to a wealth of new evidence aiding in the characterization of the pre-clinical phase of IBD and has shown that changes associated with disease development can be observed as far back as 10 years before clinical onset of disease.

Intestinal permeability

IBD is characterized by a chronic inflammatory state in the intestine of the affected individual and a hallmark of the disease is observed changes in the intestinal environment, including dysbiosis[49–51], changes in epithelial barrier properties[52–55], and a dysregulated intestinal mucosal immune response[56–58]. The CCC GEM project have specifically set out to investigate if changes in intestinal environment and function that are described post-disease diagnosis might also be detectable in the pre-clinical phase of the disease [59,60]. The GEM cohort is composed of samples from close to 5,000 healthy FDRs from patients with Crohn's disease who were followed over 10 years, of when around 100 so far have developed IBD.

The first study published from this cohort measured fraction excretion ratio of lactulose to mannitol (LMR) in urine as a surrogate for intestinal barrier permeability in pre-clinical patients with Crohn's disease[59]. Higher LMR was found to be significantly associated with development of Crohn's disease (HR, 3.03; 95% CI, 1.64-5-63; $P = 3.97 \times 10^{-4}$) 3 years prior to diagnosis. Importantly, this association remained statistically significant when only including individuals with measurements from >3 years before diagnosis[59]. Presently, the etiology behind the increased permeability is not precisely defined. One view is that it is thought to represent a dysfunctional host–microbial interface. However, several environmental exposures, including smoking[61], NSAID usage[62] and alcohol consumption[63], have been shown to affect intestinal permeability in humans. A previous study from the same group found that the genetic background in FDRs of patients with Crohn's disease only had a limited effect on intestinal permeability[64], which might suggest that the largest contribution to the increased permeability is due to other factors such as changes in the local intestinal environment. Thus, it is not clear from the present data if the observed increase in intestinal permeability is caused by changes in the microbiome or if it is a consequence of separate factors, such as environmental exposures. Furthermore, a GEM study published in 2021 found that matrix metalloproteinase 12 (MMP12), a matrix metalloproteinase previously associated with barrier dysfunction in a IBD mouse model[65], and the chemokine Chemokine (C-X-C motif) ligand 9 (CXCL9) was simultaneously associated with Crohn's disease development and increased intestinal permeability[66], thereby linking proteolytic and inflammatory changes with increased permeability.

Dysbiosis

In line with the findings described in the previous section, another study from CCC GEM utilized fecal samples from 7 patients with ulcerative colitis, 13 patients with pre-ulcerative colitis, and 48 healthy individuals and found an increase in the fecal proteolytic activity in the pre-ulcerative colitis group compared with healthy controls, which was associated with microbiota changes, including increases in *Bacteriodes vulgatus* and decreases in *Akkemansia* and *Adlercreutzia* in these patients[60]. Furthermore, colonization of germ-free mice with the pre-ulcerative colitis microbiome gave rise to similar microbiota changes and increased the colonic cell counts of polymorphonuclear leukocytes, suggesting that the microbial changes and increased proteolytic activity possibly result in intestinal inflammation. Changes in the intestinal proteolytic environment have previously been reported for patients with IBD with active disease[67–69], and from the findings described earlier it seems that compositional and functional changes in the microbiota, which might augment intestinal inflammation, are present prior to clinical onset of ulcerative colitis in some patients. Several studies have previously shown that healthy siblings to patients with IBD have altered gut microbiota with lower microbial diversity than healthy individuals[70–72]. In 2023, utilizing data from the GEM cohort, the first study showing that gut microbiome changes associated with later Crohn's disease onset was published. In this study, the authors utilized a machine learning approach to produce a microbiome risk score (MRS) and showed that this MRS was associated with Crohn's disease onset (HR, 2.24, 95% CI, 1.03–4.84, $P = 0.04$) and could to some degree predict disease onset in up to 5 years before disease onset (AUC = 0.67)[73]. Interestingly, in the GEM cohort, healthy siblings shared aspects of the dysbiosis observed in patients with Crohn's disease, including lower concentrations

of *Faecalibacterium prausnitzii,* Clostridia cluster IV and *Roseburia* spp., additionally some of these changes in the siblings correlated with intestinal permeability. This finding might indicate a link between the observed changes in intestinal permeability in patients with pre-Crohn's disease and dysbiosis[70]. Besides the indicated link between dysbiosis and intestinal permeability, changes in the composition of the microbiome are likely also associated with changes in the metabolome **[G]**. One study showed that *P. gingivalis* induced dysbiosis in a mouse model directly correlated with metabolic changes, including metabolites involved in lipid metabolism and amino acids metabolism, [74].In another study, microbiome alterations in newborn mice induced behavioral impairment in the mice, which was mediated by changes in circulating metabolites, including 4-methylphenol [75]. Changes in metabolic processes have been found in patients with IBD and many of these changes are suggested to be associated with host–microbiota interactions[76]. A study utilizing two different pre-clinical cohorts showed for the first time that perturbances in metabolic profiles were present years before diagnosis of disease in both patients with Crohn's disease and ulcerative colitis[77]. Changes that could be hypothesized to be linked to compositional changes in the microbiome.

Autoimmune and antimicrobial humoral responses

Substantiating the observed changes in the intestinal environment, elevated levels of different antimicrobial antibodies, as well as some autoantibodies **[G]**, have also been associated with disease development, some as far back as 10 years before diagnosis[78–80]. Hence, dysregulated humoral responses seem to be activated years before diagnosis.

Hitherto, most studies have focused on antimicrobial antibodies. Israeli *et al.* were the first to cross-link records of patients with IBD with available serum from the Israeli Army's serum repository, thereby identifying 38 pre-diagnosis serum samples from 32 patients with Crohn's disease and 10 pre-diagnosis serum samples from 8 patients with ulcerative colitis, which were tested for ASCA **[G]** (anti-*Saccharomyces cerevisiae* antibody) IgA, ASCA IgG and ANCA (antineutrophil cytoplasmic antibodies). Overall, 31% of patients with Crohn's disease and 0% of healthy individuals as controls tested positive for ASCA. The mean titters of ASCA increased towards diagnosis, as did the proportion of patients with ASCA positivity. After ASCA detection, the mean interval to Crohn's disease diagnosis was 3.2 years (range 1.7–6.4 years). In ulcerative colitis, 25% of patients versus 0% of the matched controls tested positive for ANCA[9]. These results were later expanded using serum samples from the European Prospective Investigation into Cancer and Nutrition study that reported on 77 incident cases of Crohn's disease (mean time before diagnosis 4.5 years, SD 3.2) and 167 incident cases of ulcerative colitis (mean time before diagnosis 4.4 years, SD 3.1), matched to two healthy controls. Pre-diagnosis samples were tested for ASCA IgG, ASCA IgA, perinuclear ANCA (pANCA), anti-OmpC and anti-CBir1[81]. The predictive accuracy of combining markers was higher than the accuracy of each marker alone. Subsequently, the PREDICTs study showed that antimicrobial antibodies are present at least 5 years before diagnosis[82,83]. In 200 individuals who developed Crohn's disease compared with healthy individuals, the most predictive anti-microbial markers were ASCA-IgA, anti-FlaX and ASCA-IgG. Their predictive performance based on univariate models was 0.69 (95 % CI,0.61–0.76), 0.61 (95 % CI, 0.53–0.69), and 0.69 (95 % CI,

0.62–0.77), respectively, 5 years before diagnosis[82]. In this large study, pANCA was not predictive of ulcerative colitis development[82]. From the same study, investigators showed that antimicrobial antibodies, such as ASCA-IgA, were associated with an increased risk of developing Crohn's disease stricturing **[G]** and/or penetrating **[G]** complications at diagnosis (HR: 1.33; 95% CI 1.13–1.55 at 2–4 years before diagnosis; HR: 1.30; 95% CI 1.10–1.51 at 6 years before diagnosis)[84]. In the CCC GEM cohort, a baseline positivity for at least 2 antimicrobial antibodies (which was observed in 43% of FDRs developing Crohn's disease versus 11% of healthy FDRs as controls) was associated with an adjusted odds ratio of Crohn's disease of 6.5 (95% CI, 3.4–12.7; P <0.001), an observation that was independent of intestinal permeability, fecal calprotectin level, C-reactive protein level, and CD-polygenic risk score[78]. These data provocatively suggest that immune dysregulation might be an independent risk factor in the subsequent development of IBD.

Using the PREDICTs study samples, a separate study looked into Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) autoantibodies (aGMAb), reporting that both IgG and IgA aGMAb were found 6 years prior to Crohn's disease diagnosis in 21% and 7% of samples, respectively, but not in ulcerative colitis or healthy individuals, with additional patients seroconverting and mean titer increasing from 1/190 to 1/320 towards the time of diagnosis.[79] By the time of diagnosis, the seroprevalence for IgA and IgG aGMAb in individuals that developed Crohn's disease was 12% and 25%, respectively, suggesting that increasing titers of the pro-inflammatory IgG antibodies[57] correlated with clinical disease. Additionally, these autoantibodies were associated with ileal and/or ileocolonic involvement and a 2.8 risk (HR) of developing penetrating and/or stricturing disease or undergoing surgery following Crohn's disease diagnosis[79]. IgA aGMAb was a predictor of Crohn's disease development with >97% specificity and with sensitivity increasing from 15% to 21% as time to diagnosis decreased (AUC 0.6)[79]. In a 2023 study, the presence of anti-integrin αvβ6 IgG autoantibodies (anti-αvβ6) were tested in the preclinical stages of ulcerative colitis. For this analysis, serum from 82 individuals who later developed ulcerative colitis who were matched to 82 individuals that remained healthy (healthy controls) was studied in the PREDICTs study, and validated in the GEM cohort. The authors found that anti-αvβ6 levels were significantly higher in sera from patients who developed ulcerative colitis than the controls at all timepoints tested (earliest sample obtained at 10 years before diagnosis). Seropositivity increased from 20.7% at the earliest timepoint to 52.4% at diagnosis, and the predictive performance of anti-αvβ6 increased from 0.79 at 10 years to 0.89 at two years before diagnosis[80].

Altogether, these findings suggest that evidence of dysregulated immune responses precede clinical disease and imply possibly breaking of immune tolerance similar to what is seen in other IMIDs such as RA and SLE[85,86]. The observation of such changes up to 10 years prior to diagnosis supports the notion of a long initiation phase in IBD.

Changes in protein markers

An earlier study from Swedish nationwide registries reported that elevated erythrocyte sedimentation rate (≥15 mm) among male individuals in late adolescence was associated with a diagnosis of Crohn's disease later in life (HR 5.95, 95% CI 4.47–7.92)[87].

Thereafter, the Nurses' Health Study investigated markers of inflammation in 83 cases of incident Crohn's disease and 90 cases of ulcerative colitis (one serum sample available per individual, median time interval between blood collection and diagnosis of Crohn's disease or ulcerative colitis of 6.6 and 6.8 years, respectively). Serum levels of IL-6 and C-reactive protein (CRP) of the pre-IBD groups were significantly higher than healthy individuals controls, with a positive correlation between levels of these systemic markers and risk of IBD[88]. The large PREDICTs study assessed the predictive value of a panel of 1,129 proteins (SomaLogic®, Colorado, USA)[82]. Overall, 51 proteins were predictive of Crohn's disease with a 76% accuracy up to 5 years prior to diagnosis, and 87% accuracy at 1-year pre-diagnosis. Proteomic markers preceding disease diagnosis were markers of inflammation (levels of CRP, serum amyloid P, trypsin 2), markers involved in cytokine signaling, innate immunity and response to bacteria (complement factors, TNF-receptor subunits, lipopolysaccharide binding protein, proteinase-3 and several interleukins)[82]. Several dysregulated pathways in preclinical Crohn's disease were identified, such as the lysosome pathway, pathways involved in glycosaminoglycan metabolism and the complement pathway[82]. The GEM cohort proteomics in serum were studied using the Olink® (Olink Holding, Uppsala, Sweden) Proximity Extension Assay platform (446 circulating proteins) and their association with levels of anti-microbial antibodies and fecal calprotectin, as well as measurement of intestinal permeability, was assessed. Overall, 71 healthy FDRs who developed Crohn's disease were matched to 284 FDRs that remained healthy. The authors reported that 25 proteins associated with risk of developing Crohn's disease (bearing biological plausibility) the most consistent being Chemokine (C-X-C motif) ligand 9 that presented the highest OR with future risk of Crohn's disease (OR=2.07 per SD, 95% CI 1.58–2.73, q=7.9e-5)[66]. Importantly, CXCL9 was also significantly associated with all other Crohn's disease-risk biomarkers with consistent direction of effect. Notably, in the GEM cohort, the timeframe before diagnosis is quite narrow in some patients (interquartile range (IQR) 1.0–3.5 years before diagnosis). Even if different panels were used to study proteomics it is interesting to note that no statistically significant overlap was identified with the set of proteins described in the PREDICTs study[66]. Finally, using the plasma samples biobanked from individuals who developed ulcerative colitis later in life (n = 72) and matched healthy controls (n = 140), Swedish investigators measured the abundance of 92 protein (Olink® panel). The median period from when a prediagnostic sample was obtained to the diagnosis of ulcerative colitis was 4.8 years (IQR 2.2–7.2 years). They described a set of six proteins (MMP10, CXCL9, CCL11, SLAMF1, CXCL11 and CCL2) to be upregulated in the serum of those with preclinical ulcerative colitis as compared with controls. However, the predictive accuracy of each marker alone was under 65%, and of the 6 markers combined was 0.71 (0.63–0.78)[89]. Thus, there are signs that disease-associated systemic inflammation is present years prior to diagnosis in IBD patients, indicating a long disease initiation phase.

Changes in the glycome

The human glycome, defined as the multitude of complex carbohydrates produced by our cells, covering surfaces of both cells and proteins, are increasingly recognized as having an important role in both homeostatic and pathological responses[90]. Dysregulation of the glycome has been shown in IBD[91] and have been linked to autoimmune diseases through changes in antibody glycosylation patterns[92,93] and reaction to host-derived glycans[94].

Presently, no studies have been conducted specifically to investigate glycome changes in pre-clinical IBD. However, in addition to the discovery of the aGMAb antibody in the previously mentioned PREDICTs study, it was also found that these antibodies targeted a changed glycosylation of the GM-CSF protein. These antibodies were further associated with altered glycosyltransferase expression in ILC3 (type 3 innate lymphoid cells) and T cells[79]. Furthermore, in another PREDICTs study, pathway analysis on serum protein markers identified glycosaminoglycan metabolism as a dysregulated pathway in pre-clinical Crohn's disease[82]. Thus, it is likely that changes in the glycome precede onset of IBD and might play and important part in disease development.

Pre-clinical phase : disease expansion

From the initiation phase data, we can infer increased intestinal permeability and dysbiosis leads to dysregulated innate and adaptive immune responses, and thereby the observed increases in inflammatory and serological markers. The latest evidence, especially within epidemiology, indicates the presence of an escalation of pathophysiological processes a few years prior to diagnosis, characterized by subclinical inflammation **[G]** and increased use of healthcare services.

Subclinical inflammation

Studies reporting on fecal calprotectin levels, a biomarker of intestinal inflammation, in asymptomatic individuals are key towards characterizing both the initiation and expansion phase. Several studies focusing on healthy relatives of patients with IBD have found that a subset of those relatives display elevated faecal calprotectin levels[78,95–97]. In a study from the GEM cohort including 1,420 FDRs of whom 50 developed Crohn's disease during follow-up (median follow-up time 2.95 years) faecal calprotectin level >100 μg/g was associated with an increased risk of developing Crohn's disease (HR, 7.76; 95% CI 3.99–15.11). Furthermore, faecal calprotectin level was weakly correlated with intestinal permeability at recruitment (R=0.069, P=0.012, Spearman correlation)[59]. A study that recruited 480 healthy FDRs (siblings, offspring or parents), calculated a risk score for disease development based on polygenic risk scores (PRS) and smoking history. Those individuals falling into the highest or lowest risk score quartiles were asked to undergo a video capsule endoscopy[98,99]. From these groups, 35% had elevated faecal calprotectin level ≥50 μg/g, and 21%, mostly from the high quartile risk score (n=22), presented a Lewis score ≥135 (abnormal inflammation); notably, in 11 participants, again from the high-risk group, Lewis score was ≥790 (moderate-to-severe small bowel inflammation), and one individual was diagnosed with Crohn's disease during the 3 year follow-up[98,99]. These data suggest that subclinical intestinal inflammation is likely to be present a few years prior to symptomatic disease onset. Notably, the pre-clinical samples utilized in the previously described GEM study investigating association between proteomic markers and serological markers, permeability and intestinal subclinical inflammation, corresponded to ~2 years prior to diagnosis and the disease-associated changes observed could therefore be hypothesized to be linked to subclinical inflammation during a phase of disease expansion[66]. This idea is underlined by their finding that several of the disease-associated proteomic markers were also associated with increased faecal calprotectin levels.

Epidemiological data

Use of medical records from population-based registers to investigate healthcare and laboratory parameters prior to IBD onset has helped understand better the pre-clinical phase, whilst also highlighting that the transition point from the initiation phase to the expansion phase seems to be fluid. Published in 2022, Cohen *et al.* reviewed medical records from an Israeli health register (n = 5,643) to investigate changes in laboratory parameters, healthcare services and medication use 5 years before diagnosis of IBD[100]. The authors reported a significant increase in the use of health care services, such as visits to general practitioner and ER visits, prior to diagnosis for both Crohn's disease and ulcerative colitis, with a marked increase 2 years prior to diagnosis (P <0.001). A finding in line with a Danish population-based cohort study including 9,019 patients with Crohn's disease and 20,913 patients with ulcerative colitis showing increased pre-clinical social costs compared with controls representing the general population (10 years before diagnosis: 1.4 times higher for Crohn's disease and 1.5 times higher for ulcerative colitis)[101]. A difference between ulcerative colitis and Crohn's disease was observed in that several parameters changed for Crohn's disease, but not ulcerative colitis, within the 5 years prior to diagnosis. These parameters included inflammatory biomarkers such as CRP levels, white blood cell count and platelet count, as well as a greater number of *Helicobacter pylori* tests taken and elevated use of medications, including antibiotics, proton pump inhibitors and NSAIDs, especially in the 2 years prior to IBD diagnosis. A limitation of the Israeli study is the lack of a matched healthy control population, hindering the comparability to the general population. Rodríguez-Lago *et al.* reported that of 31,005 individuals who underwent screening colonoscopy, an incidental diagnosis of IBD was made in 0.35% of the cohort. Of these individuals, some showed endoscopic signs indicative of subclinical inflammation and 36% of individuals developed symptoms after a follow-up period of 25 months (IQR = 10.5–42)[102,103]. Lastly, in a retrospective study that investigated healthcare and medication use preceding diagnosis in the same cohort, a higher use of primary and specialized care, as well as increased steroid use, was observed in the 3–5 years prior to diagnosis[104]. Another population-based case–control study published in 2021 investigated gastrointestinal symptoms before diagnosis of IBD in 19,554 cases and 78,114 healthy individuals as controls observed increases in reported gastrointestinal symptoms during a 10-year period prior to diagnosis (9.6% and 10.4% of Crohn's disease and ulcerative colitis cases, respectively, compared with 5.8% for controls)[105]. In line with these findings, a Danish population-based study investigating medication use of patients with IBD in the pre-clinical phase between years 2005 and 2018, found that medication use across all organ systems was increased in the 10 years prior to IBD diagnosis, compared with matched controls (1.1 to 1.8-fold higher), with marked increase in the 2 years prior to diagnosis[106]. The described findings suggest that not only do symptoms, apparently unrelated to disease, arise many years before diagnosis, but the general increased use of medication across all organ systems indicate that the disease might have a more far-reaching effect than previously expected.

The data discussed here suggest increased healthcare utilization up to at least 10 years prior to IBD diagnosis, especially in last 2–3 years preceding diagnosis, hence suggesting a transition from initiation phase to the expansion phase at this stage. Notably, there might

also be differences between preclinical Crohn's disease and ulcerative colitis. The described epidemiological findings could indicate a less pronounced pre-clinical phase in ulcerative colitis than in Crohn's disease or that clinically active disease happens more acutely in ulcerative colitis. However, the observations might be influenced by other factors as well, such as ulcerative colitis generally being a more mucosal disease[3], which could influence the measured laboratory markers.

Proposed integrated model

A critical step towards prediction and preventative strategies in IBD is to delineate the integrated pathways that lead to disease onset. With expansion of research in pre-clinical IBD, we now have insights into multilevel perturbations that lead to disease onset. Integrating these factors, we applied the putative model of preclinical IBD suggested by the European Crohn's and Colitis Organization (ECCO)[10] with grouping of the pre-clinical period into phases evolving from at-risk individuals through pre-clinical disease initiation, over a pre-clinical disease expansion phase of approximately 2 years, to actual diagnosis of disease (Figure 1). However, the sequence of events in the pre-clinical phase of IBD is ambiguous due to lack of longitudinal data and biological samples, and limited clarity on the timing of triggering events and disease initiation. Current data, including those from epidemiological studies, suggest increased healthcare utilization and subclinical inflammation years prior to diagnosis of clinical disease. From the described evidence, the pre-clinical phase in Crohn's disease seems to be characterized by a heightened inflammatory state that involves perturbations within host–microbial interactions, loss of epithelial integrity and dysregulation of mucosal immune pathways, some of which can also be detected within the systemic circulation. By contrast, although less is evident in the pre-clinical phase of ulcerative colitis, emerging data suggests that disruptions of the immune system lead to targeting of endogenous antigens. With ongoing research, more preclinical abnormalities are likely to emerge in both Crohn's disease and ulcerative colitis. The described findings seem to substantiate the previously hypothesized escalation of pathophysiological processes that culminate in overt clinical disease, therefore not only pointing to opportunities to presage the clinical diagnosis, but also to development of preventative or disease-modifying therapeutic interventions.

Future directions

Current evidence suggests a marked overlap across IMIDs, which could inform future directions in IBD research (Box 1). The preclinical phases of other IMIDs, such as RA and SLE, are relatively well-characterized; such frameworks are applicable towards IBD [6–8,85,86]. The disease with the longest studied pre-clinical phase is RA with studies looking at association between pre-clinical Rheumatoid Factor (RF) and disease development going as far back as 1988, in which patients diagnosed between 1967 and 1986 were investigated. This longitudinal population study found that higher titers of RF associated with increased incidence of RA (48.3 cases per 1,000 for RF titer >1:256, $P<0.001$)[107]. A later study utilizing serial measurements of IgM RF and anti-cyclic citrullinated peptide (anti-CCP) antibodies from 79 pre-clinical RA blood donor samples, showed seropositivity of either antibody in 49% of the patients before symptomatic onset of disease[108]. Additionally, a

study published in 2012 profiling autoantibodies in pre-clinical RA and their association with disease development showed that anti-CCP2 seropositive patients had increasing mean numbers of anti-citrullinated protein antibodies (ACPAs) and increased mean total number of elevated cytokines towards diagnosis[109], thereby linking production of autoantibodies with increases in inflammatory makers in pre-clinical RA. Similarly, a study using longitudinal serum samples from the Department of Defense Serum Repository (DoDSR) (84 SLE cases with ~3 samples per case) found that antinuclear autoantibodies and several soluble inflammatory markers, including IL-5, IL-6 and IFN-γ, were elevated more than 3 years prior to diagnosis in SLE. Furthermore, they observed elevation of several inflammatory mediators, including IFNγ and IL-4, IL-5 and IL-6, prior to development of seropositivity (HR range between 1.54 – 7.13)[110], indicating that an elevated inflammatory state might start prior to autoantibody production in SLE. These findings underline the strength of individual patient longitudinal samples in combination with measurement of several disease-associated parameters in attempting to elucidating temporal order of events within the pre-clinical phase.

From these described studies, common features across IMIDs seems to be the detection of autoantibodies in some individuals, as well as elevated levels of systemic inflammatory markers, many years prior to diagnosis of disease, implying breaking of immune tolerance and a heightened inflammatory state. Interestingly, a study investigated disease characteristic autoantibodies across IMIDs (RA, SLE and type 1 diabetes mellitus (T1DM)), utilizing 1,321 samples collected from patients with active disease and unaffected autoantibody positive and negative FDRs. They observed that alternative autoimmunity, defined as presence of at least one autoantibody associated with another IMID, occurred in all investigated groups (patients with SLE, 56%; SLE FDRs 57.4%; patients with RA, 32.6 %; RA FDRs, 34.8; patients with T1DM, 43%), therefore showing that autoantibody signatures can be shared across these IMIDs[111]. To our knowledge no such study design has been performed investigating pre-clinical parameters between IBD and other IMIDs. Such studies might be of interest as to elucidate possible common pathways between diseases. Notably, it is observed across IMIDs that only a subset of patients who develop disease are seropositive for a given autoantibody, indicating that the presence of these autoantibodies in themselves are not enough to cause disease. This observation might suggest that the development through the pre-clinical phase is driven by either one major pathway or an interplay between several personalized pathways, which has previously been suggested for RA[86]. As dysregulation of glycosylation patterns have been implicated in both active IBD and other diseases, and have shown to affect simultaneously dysbiosis, the mucosal immune system and epithelial barrier integrity, it is tempting to hypothesize that a pathological change in the glycome might be a common central pathway in IBD[90–92]. Interestingly, several studies have shown association between aberrant glycosylation of antibodies and disease development in RA[93,112]. One study utilizing longitudinal samples from 126 FDRs found extensive V-domain glycosylation in FDRs who later developed RA, and this glycosylation was strongly associated with future disease onset (HR = 6.07)[93]. Another study utilizing samples from several cohorts found that increased galactosylation of IgG autoantibodies was present prior to disease and correlated with disease activity in RA (Spearmans's $P = 0.37$, $P < 0.0001$)[112]. Thus, investigations of such aberrant antibody

glycosylation could be a relevant topic for future research in pre-clinical IBD, as well as in cross-IMID studies. Lastly, it has been suggested that changes in intestinal permeability and dysbiosis contributes to the pathogenesis in several IMIDs[113], therefore it can be hypothesized that such changes are a key event not only in IBD development but across IMIDs. Hence, investigations of pre-clinical changes in the microbiome and intestinal barrier properties across IMIDs could be of interest to test this hypothesis. Although many advances have been made over the past few years in finding biomarkers and highlighting pathogenic mechanisms in the pre-clinical stages of IBD, research in this area is still behind compared to what has been done in other IMIDs such as RA or T1DM, where disease-prevention trials have been started many years ago. A clue to what might be possible can be found in T1DM, in which a randomized controlled trial from 2019 with 76 high-risk patients showed that a 2-week course treatment with teplizumab, an anti-CD3 monoclonal antibody, could delay diagnosis of clinical T1DM in high-risk patients by a median of 2 years[114]. A finding that later in November 2022 resulted in a landmark approval of the drug to delay T1DM onset[115]. Similar observations of delay of disease onset by treatment of at-risk groups have also been noted for RA[116]. Thus, going forward it is crucial to work towards better stratification of risk-groups, and to elucidate underlying pathways that could serve as therapeutic targets for disease modulation and/or interception. This effort might in the future make it possible to propose different interventional strategies dependent on the specific pathways involved (personalized treatment), the level of disease risk and the specific stage of disease development (Figure 2).

From the described evidence, there is clearly substantial overlap in pre-clinical disease development across IMIDs. These commonalities and the evidence already present from other IMIDs can prove to be a valuable inspiration for future research in the field of pre-clinical IBD. To facilitate understanding of the complexities governing IBD development, the utilization of cohorts containing longitudinal pre-clinical samples within the same individual is likely crucial. Such studies will aid in the elucidation of temporal changes in disease relevant parameters before disease onset. Furthermore, combining several different data sources (such as proteomics, glycomics, metabolomics and serology), via state-of-the-art network analysis, seem essential for elucidating relationships between pathological events and discovering novel pathways. It is also crucial to investigate the mechanistic effects of identified exposures in the pre-clinical phase and to understand whether the influence of early life events is measurable at the molecular level already at birth, which could also enhance our understanding of priming of the intestinal immune system. Lastly, the overlap of pre-clinical features across IMIDs makes cross-IMID studies of great interest to identify common pathways and facilitate shared preventative strategies.

Conclusions

In summary, the vast expansion of studies investigating the pre-clinical phase of IBD within the last years have made it evident that events associated with disease development happens many years prior to its clinical onset, as observed for several other IMIDs[6,85,86]. Overlaps across preclinical disease across IMIDs suggest commonalities across underlying pathways pertaining to immune dysregulation. Recent data suggest that preclinical IBD occurs at least two years prior to IBD diagnosis and is characterized by systemic and intestinal subclinical

inflammation, as well as increases in non-specific symptoms and healthcare utilization. However, the precise sequential order of pathological events within the pre-clinical phase is still elusive with the current evidence, as is the exact timing of transition from disease initiation phase to disease expansion phase. To uncover the phases of disease development and discover how demonstrated pathological changes are associated with specific pathways, it will be crucial to utilize cohorts containing longitudinal pre-clinical samples within the same individual in combination with state-of-the-art bioinformatics methodologies, such as network analysis. Furthermore, studies investigating disease relevant parameters across IMIDs could prove crucial in understanding commonalities in development of immune mediated inflammatory disease.

Acknowledgements

The authors thank Jill Gregory, Certified Medical Illustrator, Icahn School of Medicine at Mount Sinai, for the original versions of the illustrations. M.A. is supported by the National Institute of Diabetes and Digestive and Kidney Diseases (K23DK129762-02). T.J. is funded by a center of excellence grant from the Danish National Research Foundation (DNRF148); the foundation had no role in the review.

Glossary

IMIDs
Immune-mediated inflammatory diseases comprise a diverse spectrum of diseases, which share commonalities in the inflammatory nature of the diseases and similar genetic, environmental and immunological factors

Dysbiosis
An imbalance in the composition of the microbial community that is associated with adverse health outcomes

Faecal calprotectin
An intracellular neutrophilic protein commonly measured in fecal samples as a way to measure degree of intestinal inflammation

Microbiome
The collection of genetic material from all the microorganisms present in which are part of a specific environment

Preclinical disease
The part of disease development prior to clinical onset of disease

Commensals
Microorganisms that derives benefits from the host without aiding or causing harm to said host

Self-antigen
An antigen produced by your own cells that elicits an immune response by your own immune cells leading to production of self-reactive antibodies known as autoantibodies, often as consequence of breaking of immune tolerance

Immune tolerance
The state of unresponsiveness of the immune system towards molecules produced by the host, as to prevent damage to healthy tissues

Autoantibodies
Antibodies targeting molecules produced by the hosts own cells, also known as self-antigens

Metabolome
The complete spectrum and number of metabolites present within an organism, tissue, or cell

ASCA
Antibodies targeting mannan molecules on the fungus Saccharomyces cerevisiae

Stricturing complications
The narrowing of a part of the intestine as a consequences of scar tissue in the intestinal wall, often as a result of chronic inflammation

Penetrating complications
Formation of fistulas or abscess in the intestinal wall as a result of chronic uncontrolled inflammation

Subclinical inflammation
An inflammatory condition that does not yet give rise to clinically apparent symptoms in the individual

References

1. Xavier RJ & Podolsky DK Unravelling the pathogenesis of inflammatory bowel disease. Nature 448, 427–434 (2007). [PubMed: 17653185]
2. Torres J et al. Crohn's disease. Lancet 389, 1741–1755 (2017). [PubMed: 27914655]
3. Ungaro RC et al. Ulcerative colitis. Lancet 289, 1756–1770 (2017).
4. Chang JT Pathophysiology of inflammatory bowel diseases. N. Engl. J. Med. 383, 2652–2664 (2020). [PubMed: 33382932]
5. Ng SC et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 390, 2769–2778 (2017). [PubMed: 29050646]
6. Frazzei G et al. Preclinical Autoimmune Disease: a Comparison of Rheumatoid Arthritis, Systemic Lupus Erythematosus, Multiple Sclerosis and Type 1 Diabetes. Front. Immunol. 13, 1–17 (2022).
7. Greenblatt KH et al. Preclinical Rheumatoid Arthritis and Rheumatoid Arthritis Prevention. Curr Opin Rheumatol 32, 289–296 (2020). [PubMed: 32205569]
8. Robertson JM & James JA Preclinical systemic lupus erythematosus. Rheum. Dis. Clin. North Am. 40, 621–635 (2014). [PubMed: 25437281]
9. Israeli E et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut 54, 1232–1236 (2005). [PubMed: 16099791]
10. Torres J et al. Results of the Seventh Scientific Workshop of ECCO: Precision Medicine in IBD-Prediction and Prevention of Inflammatory Bowel Disease. J. Crohn's Colitis 15, 1443–1454 (2021). [PubMed: 33730755]
11. De Lange KM et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. Nat. Genet. 49, 256–261 (2017). [PubMed: 28067908]

12. Graham DB & Xavier RJ Pathway Paradigms Revealed from the Genetics of Inflammatory Bowel Disease. Nature 578, 527–539 (2020). [PubMed: 32103191]
13. Agrawal M et al. Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. EClinicalMedicine 36, (2021). Comprehensive systemic review and meta-analysis collecting the available knowledge of early life risk factors and showing the importance of the early life period in the risk of later IBD development.
14. Piovani D et al. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. Gastroenterology 157, 647–659.e4 (2019). [PubMed: 31014995]
15. Halme L et al. Family and twin studies in inflammatory bowel disease. World J. Gastroenterol. 12, 3668–3672 (2006). [PubMed: 16773682]
16. Jess T et al. Disease concordance, zygosity, and NOD2/CARD15 status: Follow-up of a population-based cohort of Danish twins with inflammatory bowel disease. Am. J. Gastroenterol. 100, 2486–2492 (2005). [PubMed: 16279904]
17. Orholm M et al. Familial occurrence of inflammatory bowel disease. N. Engl. J. Med. 324, 84–8 (1991). [PubMed: 1984188]
18. Liu JZ et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat. Genet. 47, 979–986 (2015). [PubMed: 26192919] Large trans-ancestry study which not just discovers new genetic risk loci, but also show that some genetic heterogeneity is present between divergent populations in some of the risk loci.
19. Van Limbergen J et al. Advances in IBD genetics. Nat. Rev. Gastroenterol. Hepatol. 11, 372–385 (2014). [PubMed: 24614343]
20. Barker DJP The origins of the developmental origins theory. J. Intern. Med. 261, 412–417 (2007). [PubMed: 17444880]
21. Colebatch AN & Edwards CJ The influence of early life factors on the risk of developing rheumatoid arthritis. Clin. Exp. Immunol. 163, 11–16 (2011). [PubMed: 20964640]
22. Haupt-Jørgensen M et al. Maternal Antibiotic Use During Pregnancy and Type 1 Diabetes in Children-A National Prospective Cohort Study. Diabetes Care 41, e155–e157 (2018). [PubMed: 30327353]
23. Al Nabhani Z & Eberl G Imprinting of the immune system by the microbiota early in life. Mucosal Immunol. 13, 183–189 (2020). [PubMed: 31988466]
24. Agrawal M et al. Early-Life Mebendazole Exposure Increases the Risk of Adult-Onset Ulcerative Colitis: A Population-Based Cohort Study. Am. J. Gastroenterol. 117, 2025–2032 (2022). [PubMed: 36040420]
25. Agrawal M et al. Maternal antibiotic exposure during pregnancy and risk of IBD in offspring: a population-based cohort study. Gut 72, 804–805 (2023). [PubMed: 35676084]
26. Elten M et al. Ambient air pollution and the risk of pediatric-onset inflammatory bowel disease: A population-based cohort study. Environ. Int. 138, 105676 (2020). [PubMed: 32217428]
27. Elten M et al. Residential Greenspace in Childhood Reduces Risk of Pediatric Inflammatory Bowel Disease: A Population-Based Cohort Study. Am. J. Gastroenterol. 116, 347–353 (2021). [PubMed: 33038129]
28. Lee SH et al. Peri-natal exposure to parental Crohn ‘ s disease is associated with impaired gut barrier, microbiome composition differences and increased risk of Crohn’s disease. Science Abstract citation ID : jjac190. 40–41 at (2023).
29. Nair N et al. Association Between Early-life Exposures and Inflammatory Bowel Diseases, Based on Analyses of Deciduous Teeth. Gastroenterology 159, 383–385 (2020). [PubMed: 32224130] An interesting study showing the usefulness of an unconventional sample type (Deciduous teeth) to investigate environmental exposures and risk of IBD.
30. Torres J et al. Infants born to mothers with IBD present with altered gut microbiome that transfers abnormalities of the adaptive immune system to germ-free mice. Gut 69, 42–51 (2020). [PubMed: 31036757]
31. Kim ES et al. Longitudinal Changes in Fecal Calprotectin Levels Among Pregnant Women With and Without Inflammatory Bowel Disease and Their Babies. Gastroenterology 160, 1118–1130.e3 (2021). [PubMed: 33307026]

32. Soon IS et al. The Relationship Between Urban Environment and Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. Am. J. Gastroenterol. 105, S465 (2010).
33. Lo CH et al. Ultra-processed Foods and Risk of Crohn's Disease and Ulcerative Colitis: A Prospective Cohort Study. Clin. Gastroenterol. Hepatol. 20, e1323–e1337 (2022). [PubMed: 34461300]
34. Narula N et al. Association of ultra-processed food intake with risk of inflammatory bowel disease: Prospective cohort study. BMJ 374, (2021).
35. Chen J et al. Intake of ultra-processed foods is associated with an increased risk of Crohn's disease: a cross-sectional and prospective analysis of 187,154 participants in the UK Biobank. J. Crohn's Colitis 535–552 (2022) doi:10.1093/ecco-jcc/jjac167.
36. Axelrad JE et al. Systematic review: gastrointestinal infection and incident inflammatory bowel disease. Alimentary Pharmacology and Therapeutics vol. 51 1222–1232 at 10.1111/apt.15770 (2020). [PubMed: 32372471]
37. Cosnes J et al. Gender differences in the response of colitis to smoking. Clin. Gastroenterol. Hepatol. 2, 41–48 (2004). [PubMed: 15017631]
38. Karczewski J et al. The effect of cigarette smoking on the clinical course of inflammatory bowel disease. Prz. Gastroenterol. 9, 153–159 (2014). [PubMed: 25097712]
39. Mahid SS et al. Smoking and inflammatory bowel disease: A meta-analysis. Mayo Clin. Proc. 81, 1462–1471 (2006). [PubMed: 17120402]
40. Torres J et al. Risk Factors for Developing Inflammatory Bowel Disease Within and Across Families with a Family History of IBD. J. Crohn's Colitis 1–7 (2022) doi:10.1093/ecco-jcc/jjac111. [PubMed: 34417797]
41. Faye AS et al. Antibiotic use as a risk factor for inflammatory bowel disease across the ages: a population-based cohort study. Gut gutjnl-2022-327845 (2023) doi:10.1136/gutjnl-2022-327845.
42. Ananthakrishnan AN et al. Aspirin, Nonsteroidal Anti-inflammatory Drug Use, and Risk for Crohn Disease Ulcerative Colitis: A cohort study. Ann. Intern. Med. 156, 350–359 (2012). [PubMed: 22393130]
43. Sanmarco LM et al. Identification of environmental factors that promote intestinal inflammation. Nature 611, 801–809 (2022). [PubMed: 36266581]
44. Chassaing B et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 519, 92–96 (2015). [PubMed: 25731162]
45. Steenland K et al. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup. Environ. Med. 72, 373–380 (2015). [PubMed: 25601914]
46. Steenland K et al. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the Mid-Ohio Valley. Environ. Health Perspect. 121, 900–905 (2013). [PubMed: 23735465]
47. Lochhead P et al. Plasma concentrations of perfluoroalkyl substances and risk of inflammatory bowel diseases in women: A nested case control analysis in the Nurses' Health Study cohorts. Environ. Res. 207, 112222 (2022). [PubMed: 34662575]
48. Porter CK et al. Cohort profile of the PRoteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects (PREDICTS) study: Rationale, organization, design, and baseline characteristics. Contemp. Clin. Trials Commun. 14, 100345 (2019). [PubMed: 30989149]
49. Nishida A et al. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin. J. Gastroenterol. 11, 1–10 (2018). [PubMed: 29285689]
50. Manichanh C et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55, 205–211 (2006). [PubMed: 16188921]
51. Walker AW et al. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol. 11, (2011).
52. Johansson MEV et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. Gut 63, 281–291 (2014). [PubMed: 23426893]

53. Zeissig S et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut 56, 61–72 (2007). [PubMed: 16822808]
54. Lassen KG et al. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. Proc. Natl. Acad. Sci. U. S. A. 111, 7741–7746 (2014). [PubMed: 24821797]
55. Breugelmans T et al. Aberrant Mucin Expression Profiles Associate With Pediatric Inflammatory Bowel Disease Presentation and Activity. Inflamm. Bowel Dis. 1–13 (2022) doi:10.1093/ibd/izac217. [PubMed: 33783507]
56. Uniken Venema WT et al. Single-Cell RNA Sequencing of Blood and Ileal T Cells From Patients With Crohn's Disease Reveals Tissue-Specific Characteristics and Drug Targets. Gastroenterology 156, 812–815.e22 (2019). [PubMed: 30391472]
57. Uzzan M et al. Ulcerative colitis is characterized by a plasmablast-skewed humoral response associated with disease activity. Nat. Med. 28, 766–779 (2022). [PubMed: 35190725]
58. van Unen V et al. Identification of a Disease-Associated Network of Intestinal Immune Cells in Treatment-Naive Inflammatory Bowel Disease. Front. Immunol. 13, 1–14 (2022).
59. Turpin W et al. Increased Intestinal Permeability Is Associated With Later Development of Crohn's Disease. Gastroenterology 159, 2092–2100.e5 (2020). [PubMed: 32791132] This prospective study, which is performed in pre-Crohn's disease first-degree relatives (FDRs) and healthy FDRs, is the first to show that abnormal intestinal permeability seems to be present prior to disease onset of Crohn's disease.
60. Galipeau HJ et al. Novel Fecal Biomarkers That Precede Clinical Diagnosis of Ulcerative Colitis. Gastroenterology 160, 1532–1545 (2021). [PubMed: 33310084] This study showed that functional changes in the intestinal environment, in the form of increased proteolytic activity, which associated with changes in the microbial composition, can be found prior several years prior to onset of ulcerative colitis in first-degree relatives.
61. Kevans D et al. Determinants of intestinal permeability in healthy first-degree relatives of individuals with Crohn's disease. Inflamm. Bowel Dis. 21, 879–887 (2015). [PubMed: 25734694]
62. Söderholm JD et al. Different intestinal permeability patterns in relatives and spouses of patients with Crohn's disease: An inherited defect in mucosal defence? Gut 44, 96–100 (1999). [PubMed: 9862833]
63. Leclercq S et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. Brain. Behav. Immun. 26, 911–918 (2012). [PubMed: 22521198]
64. Turpin W et al. Analysis of Genetic Association of Intestinal Permeability in Healthy First-degree Relatives of Patients with Crohn's Disease. Inflamm. Bowel Dis. 25, 1796–1804 (2019). [PubMed: 31251335]
65. Nighot M et al. Matrix Metalloproteinase MMP-12 Promotes Macrophage Transmigration across Intestinal Epithelial Tight Junctions and Increases Severity of Experimental Colitis. J. Crohn's Colitis 15, 1751–1765 (2021). [PubMed: 33836047]
66. Leibovitzh H et al. Immune response and barrier dysfunction-related proteomic signatures in preclinical phase of Crohn's disease highlight earliest events of pathogenesis. Gut 1–10 (2023) doi:10.1136/gutjnl-2022-328421. This study combines findings of disease-associated serum protein markers with markers of subclinical inflammation, humoral response to microbial antigens and intestinal barrier properties, therefore giving insight into the links between these different pathways.
67. Mills RH et al. Multi-omics analyses of the ulcerative colitis gut microbiome link Bacteroides vulgatus proteases with disease severity. Nat. Microbiol. 7, 262–276 (2022). [PubMed: 35087228]
68. Carroll IM & Maharshak N Enteric bacterial proteases in inflammatory bowel diseasepathophysiology and clinical implications. World J. Gastroenterol. 19, 7531–7543 (2013). [PubMed: 24431894]
69. Ruseler-van Embden JGH & Van Lieshout LMC Increased proteolysis and leucine aminopeptidase activity in faeces of patients with Crohn's disease. Digestion 40, 33–40 (1988). [PubMed: 3069527]

70. Hedin CR et al. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. Gut 63, 1578–1586 (2014). [PubMed: 24398881]
71. Hedin C et al. Siblings of patients with Crohn's disease exhibit a biologically relevant dysbiosis in mucosal microbial metacommunities. Gut 65, 944–953 (2016). [PubMed: 25856344]
72. Jacobs JP et al. A Disease-Associated Microbial and Metabolomics State in Relatives of Pediatric Inflammatory Bowel Disease Patients. Cell. Mol. Gastroenterol. Hepatol. 2, 750–766 (2016). [PubMed: 28174747]
73. Raygoza Garay JA et al. Gut Microbiome Composition Is Associated With Future Onset of Crohn's Disease in Healthy First-Degree Relatives. Gastroenterology 670–681 (2023) doi:10.1053/j.gastro.2023.05.032. [PubMed: 37263307] The first ever study showing association between preclinical changes in the microbial composition and later Crohn's disease onset.
74. Dong ZJ et al. Correlation Analysis of Gut Microbiota and Serum Metabolome With Porphyromonas gingivalis-Induced Metabolic Disorders. Front. Cell. Infect. Microbiol. 12, 1–13 (2022).
75. Liu G et al. Gut dysbiosis impairs hippocampal plasticity and behaviors by remodeling serum metabolome. Gut Microbes 14, (2022).
76. Gallagher K et al. Metabolomic Analysis in Inflammatory Bowel Disease: A Systematic Review. J. Crohn's Colitis 15, 813–826 (2021). [PubMed: 33175138]
77. Hua X et al. Inflammatory Bowel Disease Is Associated With Prediagnostic Perturbances in Metabolic Pathways. Gastroenterology 1–4 (2022) doi:10.1053/j.gastro.2022.09.007.
78. Lee SH et al. Anti-Microbial Antibody Response is Associated With Future Onset of Crohn's Disease Independent of Biomarkers of Altered Gut Barrier Function, Subclinical Inflammation, and Genetic Risk. Gastroenterology 161, 1540–1551 (2021). [PubMed: 34293299]
79. Mortha A et al. Neutralizing Anti-Granulocyte Macrophage-Colony Stimulating Factor Autoantibodies Recognize Post-Translational Glycosylations on Granulocyte Macrophage-Colony Stimulating Factor Years Before Diagnosis and Predict Complicated Crohn's Disease. Gastroenterology 163, 659–670 (2022). [PubMed: 35623454] In comprehensive study autoantibodies targeting GM-CSF were found to be elevated up to 6 years before Crohn's disease onset, and furthermore these autoantibodies were found to specifically target an altered post-translational glycosylation on GM-CSF, thereby suggesting that changes in glycosylations might be associated with disease development.
80. Livanos AE et al. Anti-integrin αvβ6 autoantibodies are a novel biomarker that antedate ulcerative colitis. Gastroenterology (2023) doi:10.1053/j.gastro.2022.12.042. In this study autoantibodies targeting integrin αvβ6 were for the first time found to be associated with later disease onset of ulcerative colitis in two independent cohorts, and presence of these antibodies could predict later disease onset.
81. Van Schaik FDM et al. Serological markers predict inflammatory bowel disease years before the diagnosis. Gut 62, 683–688 (2013). [PubMed: 22842615]
82. Torres J et al. Serum Biomarkers Identify Patients Who Will Develop Inflammatory Bowel Diseases Up to 5 Years Before Diagnosis. Gastroenterology 159, 96–104 (2020). [PubMed: 32165208] Large retrospective case-control study with longitudinal sampling that identified a panel of protein markers and serum antibodies that were predictive of Crohn's disease development within 5 years.
83. Choung RS et al. Serologic microbial associated markers can predict Crohn's disease behaviour years before disease diagnosis. Aliment. Pharmacol. Ther. 43, 1300–1310 (2016). [PubMed: 27117843]
84. Choung RS et al. Preclinical Serological Signatures are Associated with Complicated Crohn's Disease Phenotype at Diagnosis. Clin. Gastroenterol. Hepatol. (2023) doi:10.1016/j.cgh.2023.01.033.
85. Choi MY & Costenbader KH Understanding the Concept of Pre-Clinical Autoimmunity: Prediction and Prevention of Systemic Lupus Erythematosus: Identifying Risk Factors and Developing Strategies Against Disease Development. Front. Immunol. 13, 1–18 (2022).

86. Gravallese EM & Firestein GS Rheumatoid Arthritis - Common Origins, Divergent Mechanisms. N. Engl. J. Med. 388, 529–542 (2023). [PubMed: 36780677]

87. Melinder C et al. Physical Fitness in Adolescence and Subsequent Inflammatory Bowel Disease Risk. Clin. Transl. Gastroenterol. 6, e121–8 (2015). [PubMed: 26540026]

88. Lochhead P et al. Association Between Circulating Levels of C-reactive Protein and Interleukin-6 and Risk of Inflammatory Bowel Disease. Clin Gastroenterol Hepatol. (2017) doi:10.1016/j.cgh.2016.01.016.Association.

89. Bergemalm D et al. Systemic Inflammation in Preclinical Ulcerative Colitis. Gastroenterology 161, 1526–1539.e9 (2021). [PubMed: 34298022]

90. Reily C et al. Glycosylation in health and disease. Nat. Rev. Nephrol. 15, 346–366 (2019). [PubMed: 30858582]

91. Kudelka MR et al. Intestinal epithelial glycosylation in homeostasis and gut microbiota interactions in IBD. Nat. Rev. Gastroenterol. Hepatol. 17, 597–617 (2020). [PubMed: 32710014]

92. Zhou X et al. Antibody glycosylation in autoimmune diseases. Autoimmun. Rev. 20, 102804 (2021). [PubMed: 33727152]

93. Hafenscheid L et al. N- Linked Glycans in the Variable Domain of IgG Anti–Citrullinated Protein Antibodies Predict the Development of Rheumatoid Arthritis. Arthritis Rheumatol. 71, 1626–1633 (2019). [PubMed: 31067000]

94. Alves I et al. Host-derived mannose glycans trigger a pathogenic γδ T cell/IL-17a axis in autoimmunity. Sci. Transl. Med. 15, 1–16 (2023).

95. Pham M et al. Subclinical intestinal inflammation in siblings of children with crohn's disease. Dig. Dis. Sci. 55, 3502–3507 (2010). [PubMed: 20931283]

96. Thjodleifsson B et al. Subclinical intestinal inflammation: An inherited abnormality in Crohn's disease relatives? Gastroenterology 124, 1728–1737 (2003). [PubMed: 12806605]

97. Montalto M et al. Fecal Calprotectin in First-Degree Relatives of Patients with Ulcerative Colitis. Am. J. Gastroenterol. 102, 132–136 (2007). [PubMed: 17100982]

98. Taylor KM et al. Genetic and Inflammatory Biomarkers Classify Small Intestine Inflammation in Asymptomatic First-degree Relatives of Patients With Crohn's Disease. Clin. Gastroenterol. Hepatol. 18, 908–916.e13 (2020). [PubMed: 31202982]

99. Torres J Prediction of Inflammatory Bowel Disease: A Step Closer? Gastroenterology 158, 278–279 (2020). [PubMed: 31704299]

100. Cohen NA et al. Trends in Biochemical Parameters, Healthcare Resource and Medication Use in the 5 Years Preceding IBD Diagnosis: A Health Maintenance Organization Cohort Study. Dig. Dis. Sci. (2022) doi:10.1007/s10620-022-07714-2.

101. Vadstrup K et al. Cost burden of crohn s disease and ulcerative colitis in the 10-year period before diagnosis-A danish register-based study from 2003 2015. Inflamm. Bowel Dis. 26, 1377–1382 (2020). [PubMed: 31693731]

102. Rodríguez-Lago I et al. Characteristics and Progression of Preclinical Inflammatory Bowel Disease. Clin. Gastroenterol. Hepatol. 16, 1459–1466 (2018). [PubMed: 29133254]

103. Rodríguez-Lago I et al. Early microscopic findings in preclinical inflammatory bowel disease. Dig. Liver Dis. 52, 1467–1472 (2020). [PubMed: 32601034]

104. Rodríguez-Lago I et al. Subclinical bowel inflammation increases healthcare resources utilization and steroid use before diagnosis of inflammatory bowel disease. United Eur. Gastroenterol. J. 11, 9–18 (2023). Retrospective study case-control study showing that individuals who later develop IBD have elevated usage of steroids and increased health care utilization 3 and 5 years before diagnosis of disease.

105. Blackwell J et al. Prevalence and Duration of Gastrointestinal Symptoms before Diagnosis of Inflammatory Bowel Disease and Predictors of Timely Specialist Review: A Population-Based Study. J. Crohn's Colitis 15, 203–211 (2021).

106. Bonfils L et al. DOP62 Medication use is increased in the decade prior to IBD diagnosis: A nationwide cohort study. Presented at ECCO2023, Copenhagen, Denmark https://www.ecco-ibd.eu/publications/congress-abstracts/item/dop62-medication-use-is-increased-in-the-decade-prior-to-ibd-diagnosis-a-nationwide-cohort-study.html (2023). Large

population-based study showing that usage of a broad spectrum medication types are increased in the 10 years leading up to disease onset in patients with IBD.

107. Puente A Del et al. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. Arthritis Rheum. 31, 1239–1244 (1988). [PubMed: 3178905]
108. Nielen MMJ et al. Specific Autoantibodies Precede the Symptoms of Rheumatoid Arthritis: A Study of Serial Measurements in Blood Donors. Arthritis Rheum. 50, 380–386 (2004). [PubMed: 14872479]
109. Sokolove J et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. PLoS One 7, 1–9 (2012).
110. Lu R et al. Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. J. Autoimmun. 74, 182–193 (2016). [PubMed: 27338520]
111. James JA et al. Latent autoimmunity across disease-specific boundaries in at-risk first-degree relatives of SLE and RA patients. EBioMedicine 42, 76–85 (2019). [PubMed: 30952617]
112. Ercan A et al. Aberrant IgG galactosylation precedes disease onset, correlates with disease activity, and is prevalent in autoantibodies in rheumatoid arthritis. Arthritis Rheum. 62, 2239–2248 (2010). [PubMed: 20506563]
113. Kinashi Y & Hase K Partners in Leaky Gut Syndrome: Intestinal Dysbiosis and Autoimmunity. Front. Immunol. 12, 1–9 (2021).
114. Herold KC et al. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N. Engl. J. Med. 381, 603–613 (2019). [PubMed: 31180194]
115. Keam SJ Teplizumab: First Approval. Drugs 83, 439–445 (2023). [PubMed: 36877454]
116. Frazzei G et al. Prevention of rheumatoid arthritis: A systematic literature review of preventive strategies in at-risk individuals. Autoimmun. Rev. 22, 103217 (2023). [PubMed: 36280095]

BOX 1: Prominent questions in pre-clinical IBD research.

- Are immunological changes associated with disease development already present at birth or shortly after birth and to what extent are these associated with early life events?
- What are the specific timings of transitions between the different phases of preclinical disease and which exposures or pathological changes are key events in these transitions?
- In which order do perturbations in disease associated pathways occur during disease development and how do the pathways interact with each other?
- To what extent do disease associated pathways overlap between different immune-mediated inflammatory disease?
- Would it be possible to utilize disease associated markers, such as genetic risk and biomarkers of altered pathways, to make a cumulative risk score for inflammatory bowel disease (IBD), which could be used to stratify patients towards personalized preventative interventions?
- Is it possible to find effective interventional strategies for at risk groups in IBD, similar to the delay of onset strategies developed for individuals at risk of rheumatoid arthritis and type 1 diabetes mellitus?

Key points

- Available evidence pertaining to the pre-clinical phase of inflammatory bowel disease (IBD) has expanded substantially, providing a foundation for understanding the development of IBD
- The development of IBD seems to evolve from at-risk individuals through several distinct subphases including, disease initiation and a disease expansion approximately 2 years prior to diagnosis.
- Observed changes within the pre-clinical phase of IBD include dysregulation of the adaptive and innate immune system of the intestine, as well as compositional changes in the gut microbiome, increased intestinal permeability, and changes in the glycome and clinical parameters.
- The specific temporal order of events within pre-clinical disease initiation are presently hard to decipher, owing to limited availability of longitudinal pre-diagnostic samples globally.
- Longitudinal studies linking data from several relevant data sources are likely needed to elucidate the pathways, molecular interplays and order of events in the development of IBD.
- Disease development in IBD seemingly share many general features with those of other immune-mediated inflammatory disease (such as rheumatoid arthritis) making studies focused on comparing pre-clinical development between diseases of great relevance.

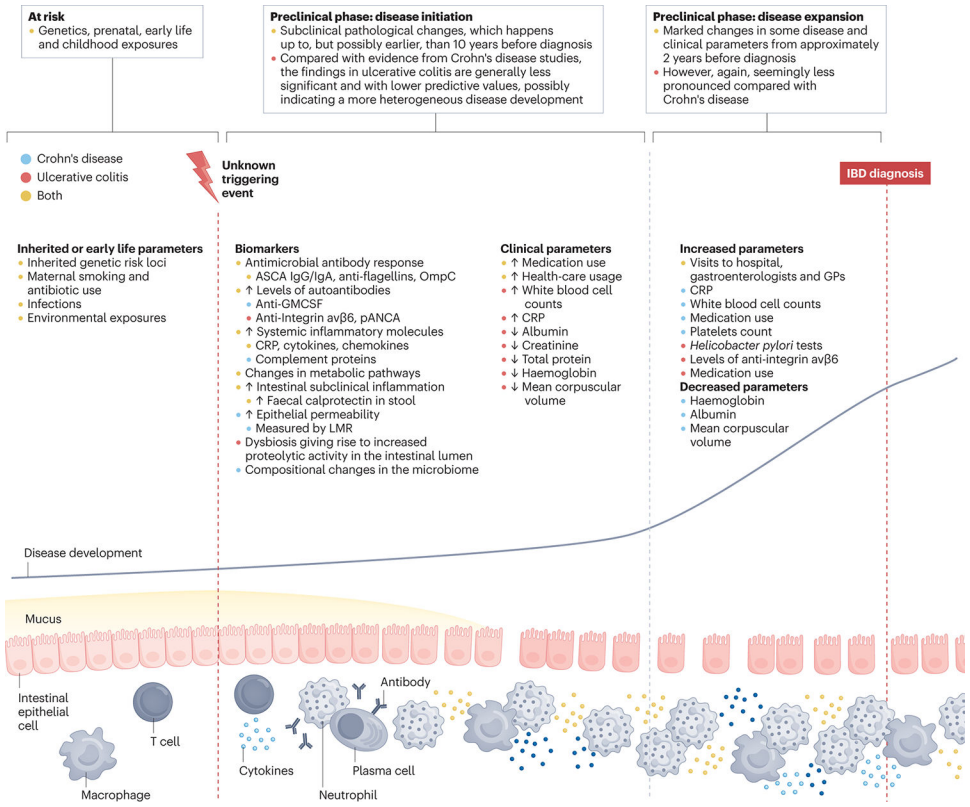

Figure 1 |. Proposed model of disease development in IBD based on the current evidence.

It is hypothesized that development of inflammatory bowel disease (IBD) is structured into several subphases, as initially suggested by the European Crohn's and Colitis Organization (ECCO)[10], evolving from at-risk individuals through pre-clinical disease initiation, over a disease expansion phase of approximately 2 years, to actual diagnosis of disease. The at-risk period is characterized by genetic and environmental risk factors, which could prime the intestinal immune system towards dysregulation and future disease. One or more unknown triggering events are suggested to push susceptible individuals towards disease initiation. This disease initiation phase is characterized by several pathological changes, including breaking of immune tolerance, as implied by autoantibody and antimicrobial antibody production, increased intestinal permeability, dysbiosis and changes in some clinical parameters, with so far elusive temporal order. It is proposed that from approximately 2 years prior to diagnosis escalation of pathophysiological processes that culminate in overt clinical disease takes place, characterized by marked changes in some parameter's indicative of possible subclinical inflammation and acceleration of not yet disease attributed symptoms. Evidence pertaining to the different disease subtypes has been indicated as follows (see key): blue dot, evidence pertaining pre-clinical Crohn's disease; pink dot, evidence pertaining to pre-clinical ulcerative colitis; yellow dot, evidence pertaining to both subtypes. ASCA; anti-Saccharomyces *cerevisiae antibodies*; CRP, C-reactive protein; GP, General practitioner; LMR, lactulose-to-mannitol ratio; OmpC, Outer-membrane porin C; pANCA, perinuclear anti-neutrophil cytoplasmic antibodies. Original illustration by Jill K. Gregory, adapted with permission of © Mount Sinai Health System

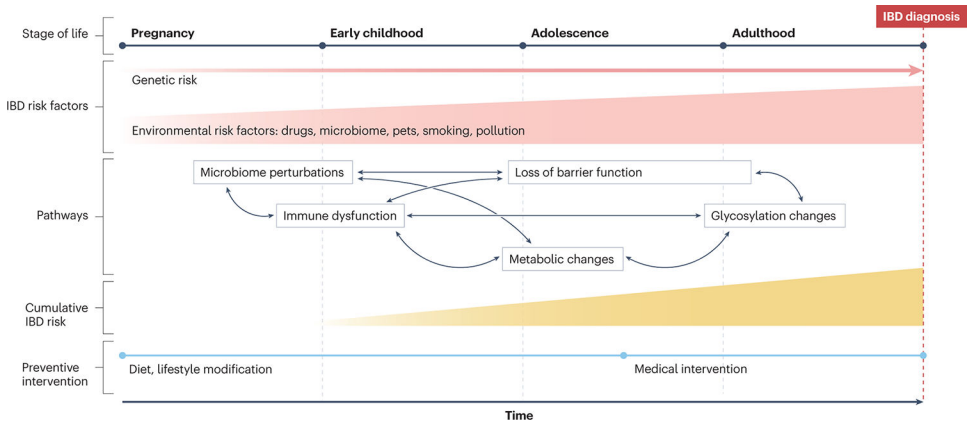

Figure 2 |. Proposed chronology of events starting from the prenatal period to IBD diagnosis and potential opportunities for preventive intervention.

In this figure, we hypothesize that the window of susceptibility starts during the prenatal period and continues until inflammatory bowel disease (IBD) diagnosis. In a background of genetic risk, progressive and cumulative exposure to relevant environmental risk factors (for example, infections, antibiotics, pets, smoking, pollution) might contribute to IBD risk over the course of an individual's life. These exposures lead to downstream events, including gut microbiome perturbations with loss of microbial diversity and alterations in specific taxa, altered innate and humoral immune function, loss of intestinal epithelial integrity, metabolic changes, and altered glycosylation of potentially pathogenic antibodies. The order in which these perturbations occur is not yet established, but crosstalk across pathways is likely. We propose that a cumulative risk score representing genetic risk and biomarkers of altered pathways can help estimate preclinical IBD risk and it can be stratified towards personalized preventive interventions. For example, diet and lifestyle modification might be a reasonable intervention for low-risk persons, whereas medical intervention might be appropriate for high-risk individuals. Notably, this timeline represents a broad overview of IBD. Considering substantial heterogeneity across IBD risk, phenotypes and outcomes, these events and timeline are likely to vary across different subsets of IBD. Original illustration by Jill K. Gregory, adapted with permission of © Mount Sinai Health System

Table 1 |

Selected studies investigating the preclinical phase of IBD

Reference	Disease subtypes investigated	Timepoint(s) before diagnosis[a]	Cohort details	Main findings
Singular timepoint in the pre-clinical phase				
Van Schaik et al. (2013)[81]	Crohn's disease and ulcerative colitis	~4.5 years	77 pre-Crohn's disease cases (mean age at diagnosis, 52.6 years) and 154 healthy individuals as controls (mean age at recruitment, 52.5 years); 167 pre-ulcerative colitis cases (mean age at diagnosis, 58 years) and 334 healthy individuals as controls (mean age at recruitment, 53.6 years)	A combination of serological markers (pANCA, ASCA, CBir1 and OmpC) could to some degree predict disease development (Crohn's disease: AUC = 0.679; ulcerative colitis: AUC = 0.657).
Choung, R. S. et al. (2016)[83]	Crohn's disease	~6 years	100 pre-Crohn's disease cases (median age at diagnosis, 30 years)	Showed that increased seropositivity towards antimicrobial serological markers is associated with complications at or shortly after diagnosis.
Lochhead, P. et al. (2017)[88]	Crohn's disease and ulcerative colitis	~6–7 years	83 pre-Crohn's disease cases (median age, 52.7 years), 90 pre-ulcerative colitis cases (median age, 50.4 years), 344 healthy individuals (median age, 51.7 years)	Elevated plasma levels of CRP and IL-6 was associated with disease development in both Crohn's disease and ulcerative colitis.
Turpin, W. et al. (2020)[59]	Crohn's disease	~3 years	50 pre-Crohn's disease FDRs (median age at recruitment, 16 years) and 1,370 healthy FDRs (median age at recruitment, 19 years)	Observed increased intestinal permeability (through LMR measurements) in FDRs that later develop Crohn's disease.
Lee, S. H. et al. (2021)[78]	Crohn's disease	~3 years	77 pre-Crohn's disease FDRs (median age at recruitment, 16 years) and 307 healthy FDRs (median age at recruitment, 16.1 years)	High seropositivity of a panel of antimicrobial antibodies was significantly associated with Crohn's disease development (OR 6.5). Association was independent of abnormal barrier function, subclinical inflammation and Crohn's disease-related genetic risks.
Bergemalm, D. et al. (2021)[89]	Ulcerative colitis	~5 years	Pre-clinical cohort: 72 pre-ulcerative colitis cases (median age at diagnosis, 54 years) and 140 healthy individuals as controls (median age at sampling, 50 years). Inception cohort: 101 treatment-naïve ulcerative colitis cases (median age at diagnosis, 37 years) and 50 healthy individuals as controls (median age at sampling, 26 years). Healthy twin cohort: 37 healthy twins (median age at sampling, 59 years) and 41 healthy individuals as controls (median age at sampling, 60 years)	Six inflammatory proteins were found to be elevated in pre-clinical ulcerative colitis cases compared with healthy individuals. These proteins could discriminate Treatment-naïve patients with ulcerative colitis from healthy individuals with an AUC of 0.92.
Galipeau, H. J. et al. (2021)[60]	Ulcerative colitis	~4.5 years	13 pre-ulcerative colitis FDRs (mean age at recruitment, 19.7 years) and 48 healthy individuals as controls (mean age at recruitment, 20.3 years)	Increased proteolytic activity was observed for patients with pre-ulcerative colitis and this changed activity was associated with functional changes in the gut microbiome.
Hua, X. and Ungaro R. C. et al. (2022)[77]	Crohn's disease and ulcerative colitis	Nurse cohort: ~10 years for ulcerative colitis and ~8 years for Crohn's disease. PREDICTs cohort: ~12 years	Nurse cohort: 55 pre-ulcerative colitis cases (mean age at diagnosis, 55.8 years), 49 pre-Crohn's disease cases (mean age at diagnosis, 55.7 years) and 208 healthy individuals as controls (mean age at blood sampling, 55.8 years).	Several metabolic pathways were significantly associated with disease development for both ulcerative colitis and Crohn's disease. First study to show that there are changes in metabolic pathways in the pre-clinical phase of IBD.

Reference	Disease subtypes investigated	Timepoint(s) before diagnosis[a]	Cohort details	Main findings
		for ulcerative colitis and ~6.5 years for Crohn's disease	PREDICTs cohort: 25 pre-ulcerative colitis cases (mean age at diagnosis, 34.1 years), 25 pre-Crohn's disease cases (mean age at diagnosis, 33.3 years) and 25 healthy individuals as controls.	
Leibovitzh, H. et al. (2023)[66]	Crohn's disease	~2 years	71 pre-Crohn's disease FDRs (median age at recruitment, 15 years) and 284 healthy FDRs (median age at recruitment, 16 years)	25 serum proteins were found to be significantly associated with Crohn's disease development. Several of these proteins were also associated with markers of subclinical inflammation, antimicrobial antibody responses and gut barrier function.
Garay, R. et al. (2023)[73]	Crohn's disease	~3 years	73 pre-Crohn's disease FDRs and 3,483 healthy FDRs Median age at recruitment was 17 years (range, 6–35 years)	Utilizing a machine learning approach on 16S ribosomal RNA sequencing data and the construction a microbiome risk score (MRS) the authors showed for the first time an association between changed microbial composition and later Crohn's disease onset (HR: 2.24). Furthermore, this MRS could to some degree predict patients with pre-Crohn's disease up to 5 years before onset (AUC: 0.67)
Longitudinal timepoints in the pre-clinical phase				
Israeli, E. et al. (2005)[9]	Crohn's disease and ulcerative colitis	>60 months, 37–60 months, 1–37 months	32 pre-Crohn's disease cases (mean age at diagnosis, 24.8 years) and 95 healthy individuals as control, 8 pre-ulcerative colitis cases (mean age at diagnosis, 23.5 years) and 36 healthy individuals as controls	31.3% of the patients with Crohn's disease in the cohort were ASCA positive prior to diagnosis and ACSA positivity was seen in 15.4% of the samples taken 60 months prior to diagnosis.
Torres, J. et al. (2020)[82]	Crohn's disease and ulcerative colitis	~5 years, ~4 years, ~2 years and at diagnosis (± 1 year)	Four longitudinal samples from 200 pre-Crohn's disease cases (mean age at diagnosis, 31.4 years), 199 pre-ulcerative colitis cases (mean age at diagnosis, 28.9 years) and 200 healthy individuals as controls (mean age at latest sample, 28 years)	A panel of serum antibodies and proteins found to be associated with disease development every year from 5 years before disease development was shown to predict Crohn's disease development with high accuracy (AUC range: 0.76 at −5 years and 0.85 at −1 year).
Mortha, A. et al. (2022)[79]	Crohn's disease and ulcerative diagnosis	~ 6 years, ~3.5 years and at diagnosis	Three to four longitudinal samples from 220 pre-Crohn's disease cases (mean age ~31 years), 200 pre-ulcerative colitis cases (mean age, ~ 31 years) and 200 healthy individuals as controls (mean age, ~32 years)	Anti-GM-CSF autoantibodies were found up to 6 years prior to disease development in patients with pre-Crohn's disease and these antibodies were associated with complications within 100 days of diagnosis (HR: 2.8). Titers of autoantibody increased towards diagnosis. The autoantibodies were found to be specific for an altered post-translational glycosylation on GM-CSF.
Livanos, A. E. et al. (2023)[80]	Ulcerative colitis	~10 years, ~4 years, ~2 years and at diagnosis	Main cohort: four longitudinal samples from 82 pre-ulcerative colitis cases (mean age at diagnosis, 32 years) and 82 healthy individuals as controls (mean age at recruitment, 32.6. years) Validation cohort: 12 pre-ulcerative colitis (mean age at recruitment, 17.7 years) and 49 healthy individuals as controls (mean age at recruitment, 17.3 years)	Anti-integrin-αvβ6 autoantibodies was significantly increased in patients with pre-ulcerative colitis up to 10 years prior to diagnosis and predicted ulcerative colitis development (AUC range: 0.79 at -10 years and 0.89 at -2 years). The autoantibodies were significantly associated with adverse disease outcomes (HR: 1.39).
Choung, R. S. et al. (2023)[84]	Crohn's disease	~6 years, ~4 to ~2 years (aggregated) and at diagnosis	Three to four longitudinal samples from 201 pre-Crohn's disease cases (mean age at diagnosis, ~31 years)	Levels of antimicrobial antibodies and protein markers (reflecting inflammatory, fibrosis and tissue protection markers) were associated

Reference	Disease subtypes investigated	Timepoint(s) before diagnosis[a]	Cohort details	Main findings
			and 201 healthy individuals as controls (mean age, 28.5 years)	with development of complicated Crohn's disease onset up to 6 years prior to diagnosis.
Register data to investigate the pre-clinical phase				
Vadstrup, K et al. (2020)[101]	Crohn's disease and ulcerative colitis	10 years before diagnosis to diagnosis	9,019 pre-Crohn's disease cases (mean age at diagnosis, 42.2 years) and 20,913 pre-ulcerative colitis cases (mean age at diagnosis, 47.5 years). Controls matched 1:1.	Societal costs and number of additional diagnosis were found to be substantially higher for patients with IBD than the general population in the 10 years prior to diagnosis with a spike from 2 years prior to diagnosis.
Blackwell, J. et al. (2021)[105]	Crohn's disease and ulcerative colitis	10 years before diagnosis to diagnosis	5,874 pre-CD cases, 13,681 pre-ulcerative colitis cases and 54,616 healthy individuals Age at diagnosis of cohort mostly >39 years.	One in four cases of IBD was found to have reported gastrointestinal symptoms > 6 months prior to diagnosis with 9.6% and 10.4% reported symptoms 5 years before Crohn's disease and ulcerative colitis diagnosis, respectively. Previous diagnosis of IBS and depression was associated with delays in specialist review.
Cohen, N. A. et al. (2022)[100]	Crohn's disease and ulcerative colitis	5 to 1 year before diagnosis	3,039 pre-Crohn's disease cases (mean age, 37.48 years), 2,322 pre-ulcerative colitis cases (Mean age, 41.2 years) and 282 pre-indeterminate colitis (Mean age, 44.21 years)	Showed changes in laboratory parameters, healthcare service and medication use occur in the 5 years preceding IBD diagnosis. A spike in the 2 years prior to diagnosis was noted in CD patients for some parameters, including levels of CRP, White blood cells counts, platelets and usage of medications, such as NSAIDs and antibiotics. Both disease subtypes showed similar spikes in health care utilization.
Rodríguez-Lago, I. et al. (2023)[104]	IBD	5 and 3 years prior to diagnosis	124 pre-IBD cases, 305 symptomatic-onset IBD and 372 healthy individuals as controls Median age of 56 years (Interquartile range [IQR], 53–62)	Increased visits to primary care and use of steroids in the periods of 3 and 5 years prior to diagnosis for patients with IBD.
Bonfils, L et al. (2023)[106]	IBD	10 years before diagnosis to diagnosis	29,219 pre-IBD cases and 292,190 non-IBD cases	Increased use of a broad range of medications was observed for patients with IBD up to 10 years prior to diagnosis with a steep increase in the 2 years prior to diagnosis.

A table showing information about publications on the preclinical phase of IBD divided into publications looking at a single pre-clinical timepoint, several pre-clinical timepoints (longitudinal studies) and publications utilizing data from registries. ASCA; anti-*Saccharomyces cerevisiae* antibodies, AUC; Area under the ROC Curve; CRP, C-reactive protein; FDR, First degree relative; HR, Hazard ratio; LMR, lactulose-to-mannitol ratio; OmpC, Outer-membrane porin C; OR, Odds ratio; pANCA, perinuclear anti-neutrophil cytoplasmic antibodies; PREDICTs, PRoteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects.

[a]The noted timepoint(s) for the non-register-based studies are reflecting mean or median time from sample acquisition to diagnosis in the cohort for the given studies.