Peer Review File
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Reviewer A:

Overall, I found this to be a timely and useful review. It is unbalanced in some places, which I highlight in this review, with suggestions for improvement. An emerging trend in IBD research is combining metabolomics and microbiome with epigenomic profiling, so this article should be of interest to many thinking about contributing to this field. The paper highlights many papers that
were novel for me, and maybe it is just a difference in perspective, but it also seems to miss many important studies.

Comment 1: Regarding the discussion of genetics and heritability (section starting on line 108), the description needs to be tightened-up. There are ~240 loci associated with IBD overall reported in the literature, and while line 131 highlights that 47 and 71 of these are UC- and CD-specific respectively, it is not clear what number the 60% shared refers to, or by what criteria this evaluation is made (GWAS significance, FDR, effect size?). Similarly, the phrase "commonly share" in relation to other inflammatory/auto-immune diseases would preferably be more quantitative. It would also be useful to estimate the proportion of IBD that is regarded as familial.

Reply 1: Thank you for pointing out this missing information. We have clarified the Section: Genome in Inflammatory Bowel Diseases, and written the missing information solicited. Please, see the changes in the manuscript highlighted in yellow.

Comment 2: On the same theme, it is great to list the contributions of multiple environmental exposures, from smoking to food choices, but again it is difficult for readers to have sense of the magnitude of the individual contributions. Might a table listing the studies and their estimates be
included in the review?

Reply 2: Thanks for the suggestion. We created a table (Table 2) with some environmental factors and added relevant references.

Comment 3: An important dietary component that the authors failed to include is omega-3 and omega-6 fatty acids, both shown by Mendelian Randomization to be causally linked to protection: PMIDs 34780722 and 36430859.

Reply 3: We appreciate the comment and added a paragraph with the mentioned content. Section: Diet in Inflammatory Bowel Diseases: "Through genetic colocalization and Mendelian randomization techniques, researchers have identified the causal relationship between n-6 PUFAs, and the derived metabolites as a causality factor for CD (47,48). Another study has shown the protective effects of n-3 PUFAs on IBD, commonly found in foods such as fish oil, flaxseed, and other sources (47). The recommended dietary intake ratio of omega-3:omega-6 depends on the disease under consideration (49). According to Cholewski et al. 2018 (49), the ideal consumption would be to follow the proportion of the Japanese population from 1:2 to 1:4. However, some researchers suggest consumption from 1:5 to 1:10 of n-3:n-6 would already be

adequate for the general population (49)."

Comment 4: Very minor point: line 229, what does "a few organisms were colonized by ..." refer to?

Reply 4: We have clarified the paragraph. Section: Gut Microbiome: "The microorganisms: Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, Ruminococcus bromii, Roseburia spp., Lactobacillus spp., and Bifidobacterium spp., are responsible for the most significant fraction of butyrate production (66-68)."

Comment 5: The discussion of methylation is very welcome, but also misses some critical papers describing large-scale methylome profiling in IBD, viz: Ventham et al 2016 (PMID: 27886173), McDermott et al 2016 (PMID: 26419460), Somineni et al 2019 (PMID: 30779925), to name just a few. A comprehensive review of this literature is beyond scope, but I think it is important that the authors review the comparison of blood-based and gut-based signatures (the former are largely inflammatory and inception-focused). Lloyd-Price et al 2019 (PMID: 31142855) reviewed multi- omics of the gut microbiome in IBD for Nature.

Reply 5: We added more information about methylation, as suggested. Section: DNA Modifications: DNA methylation and histone modifications: "Ventham et al. (119) found 439 differentially methylated positions and five differentially methylated regions, mainly VMP1 (vacuole-membrane protein 1). The major differentially methylated position was RPS6KA2, a ribosomal kinase in the serine/threonine kinase family that regulates cell growth and proliferation (119). In peripheral blood mononuclear cells, the promoter region of TRIM39-RPP2 was also significantly hypomethylated in colonic mucosa from pediatric UC patients (128). On the other hand, TRAF6 hypermethylation was observed. Methylation patterns observed in blood samples from the CD change the patterns during disease development/progression or in acute inflammation (129). In addition, the increased risk of colorectal cancer in IBD patients was associated with global hypermethylation, probably driven by inflammation (130).

The importance of correlating blood methylation findings with microbiome assessment results is worth mentioning due to the dysbiosis in patients with IBD. A large multi-omic study evaluated the microbiome of patients with CD and UC for one year and found several species of bacteria not yet cataloged, in addition to a reduction in enzymes, specific fatty acids, and some vitamins such as B5 and B3 (131). More studies like this are needed better understand the role of epigenome associated with the patient's microbiome.".

Comment 6: Conversely, the depth of discussion of the role of miRNAs in IBD is out of proportion with the rest of the article, for an area of research that in my opinion is quite a small niche.

Reply 6: We appreciate your feedback and have shortened the miRNAs section.

Comment 7: In the section on diet, I suggest adding discussion of the role of gut barrier integrity in regulating inflammation, and how it is modified by the microbiome

signaling to immune cells.

Reply 7: We have added the following information. Section Diet Can Modulate Microbiota: “Depletion of commensal microbes and consequent dysbiosis can impair mucosal healing and enhanced recruitment of proinflammatory leukocytes to the lamina propria and induce chronic inflammation and colitis in genetically predisposed individuals (62). High-fat Diet (HFD) modulates negatively the gut microbiome, which in turn can impact the mucosal integrity and immune system, altering the humoral response, mediated by B lymphocytes and antibodies produced by them, and cellular response, mediated by T lymphocytes and cytokines (75). In this case, the passage of luminal materials may be related to an exaggerated immune activation and the release of cytokines, such as IL-4, IL-6, IL-12, IL-13, TNF, IL-1B, IFN-y, and inhibition of cytokines, such as IL-10, IL-17, and IL-22. As a result, increased tight junction permeability may lead to the additional passage of macromolecules from the intestinal lumen and increased immune activation, exacerbating the symptoms of IBD (75).”

Comment 8: Penetrating Crohn’s Disease (Montreal B3) should be distinguished from stricturing/fibrotic (B2) as this is a critical distinction regarding disease progression. This is alluded to on line 215, but I would recommend a more pathway-oriented discussion, perhaps leaning on reviews by Xavier and colleagues, eg PMID 31249397 and 32103191 (ref 14 in the MS).

Reply 8: We appreciate your feedback and have added a new part to the introduction about Montreal classification. Section: Introduction: “The Montreal Classification for Crohn's disease is a worldwide system used to characterize CD patients (8). According to this classification, CD is classified into three main categories: age at diagnosis, location of disease, and disease behavior. The age-at-diagnosis category divides patients into pediatric (<17 years old) and adult (≥17 years old) groups (8). The location of the disease is classified according to the inflamed area in the gastrointestinal (GI) tract and includes three subcategories: L1 (terminal ileum), L2 (colon), L3 (ileocolon), and L4 (upper GI). Disease behavior is classified into three subcategories: B1 (nonpenetrating and nonstricturing disease), B2 (stricturing), and B3 (penetrating). Furthermore, the Montreal Classification also includes additional categories to classify disease behavior based on the presence or absence of perianal disease, which would be indicated by a ‘p’ appended to behavior categories (B1p, B2p, and B3p). This classification system is useful in predicting disease outcomes and guiding treatment decisions in patients with CD (8).”

Comment 9: Additionally, can the authors provide insight into the nutritional or other aspects of enteral and other IBD-specific diets that are thought to be curative?

Reply 9: We have provided information about the nutritional aspects of exclusive enteral nutrition: - Section Diet in Inflammatory Bowel Diseases: “According to the ESPEN guideline (53), an EEN is recommended as the first-line therapy to induce remission in children and adolescents with mild active CD. This nutrition therapy is based on administering a liquid nutrition formula (polymeric diet with moderate fat content) via a feeding tube, which completely excludes food intake for several weeks

(50).” In addition, other IBD-specific diets used to treat and reduce symptoms in IBD that are directly related to changes in the intestinal microbiota are described in the section: Diet Can Modulate Microbiota.

Reviewer B:
In general, the structure and content of the paper is good. The paper builds up from the exposome, via the microbiome, to the epigenome. However, some sentences are quite strange and unclear. This might be due to an incorrect use of words or grammar. I also noticed that references were not always correct. Some sentences missed references, e.g. when the own review is mentioned, and other times references were misplaced, e.g. sentence of 240 loci has references for NOD2.

Comment 10: references were misplaced, e.g. sentence of 240 loci has references for NOD2.
Reply 10: We double-checked all references. Thanks for the review and comments. All reference changes are highlighted in yellow in the manuscript for this Section - Genome in Inflammatory Bowel Disease.
I will go over each part one by one:

Comment 11: - Abstract: The background does not give a clear overview and already gives a conclusion, e.g. the exposome-diet-epigenome axis needs to be thoroughly investigated. Genetics is also not mentioned in the background but only later in the abstract.
Reply 11: We appreciate the reviewer’s suggestion. We rewrote the background of the abstract, and we added the following phrase to the end of the abstract’s conclusion: “This review highlights the need for a comprehensive investigation of the Exposome-Diet-Epigenome axis to improve our understanding of IBD and its outcomes.” All changes are highlighted in yellow in the text.

Comment 12: - Introduction: All elements are present: IBD and its pathogenesis; why research for IBD is important; environmental factors work through epigenetics; and how diet influences epigenome. However, the introduction is quite difficult to follow.
A few sentences are very confusing: line 77-> 78 (new diseases discovered? Maybe elaborate on this) and line 80 -> 82 (no idea what you want to say here).

Reply 12: We rewrote the Section: Introduction. Please, see all changes highlighted in yellow.

- Methods: this is okay.

Comment 13: - Genome: This part needs revision! Some sentences are very unclear, e.g. hereditary susceptibility ... This part jumps from one thing to another which makes it hard to follow. Due to the jumping, some parts are easily interpreted wrong. This part is not the main focus of the review. However, if you want to include a part about

genetics, it should be described clear and correctly.
Reply 13: We appreciate the reviewer's suggestion. To clarify, we rewrote the Section: Genome in Inflammatory Bowel Diseases. All changes are highlighted in yellow.

Comment 14: - Exposome: Introduction seems to have some repetition. Confusing sentences: line 144 (where?) and line 167 -> 170 (translated -> influenced?). Besides these remarks, the part is well written.
Reply 14: We appreciate the reviewer's suggestion. To clarify, we reorganized some sentences. All changes are highlighted in yellow.

Comment 15: - Diet: Clear overview. Confusing sentences: line 200-> 201: does this refer to the previous sentence or to the entire paragraph.
Reply 15: We rewrote the sentence to make the information clearer. All changes are highlighted in yellow in the manuscript.

Comment 16: - Section Microbiome: In the second paragraph, it jumps from bacterial load to gut colonization. The connection between the two is not clear. An increase in the Proteobacteria phylum in IBD is specifically emphasized (line 258-> 261), however it is not mentioned why this matter.
Reply 16: We appreciate the reviewer's suggestion. We included the following phrase in the second paragraph: "This colonization by microorganisms in the gut starts during the intrauterine period and may play a role in IBD within a predisposing environment (55,57,58)." We included the following phrase in the antepenultimate paragraph of this section: "Proteobacteria are anaerobic bacteria whose expansion is facultative, usually associated with dysbiosis (74)." All changes are highlighted in yellow in the text.

Comment 17: The paragraphs about FODMAPs provide two opposing messages. FODMAPs play a role in IBD pathogenesis, however a low-FODMAP diet also increases the incidence of IBD. This contradiction should be adequately addressed.
Reply 17: We appreciate the suggestion. We have clarified the paragraph about FODMAPs by rewriting this paragraph. Section Diet Can Modulate Microbiota: "The high exclusion of fibers for a prolonged time can lead to harmful changes in the intestinal microbiota (71). Recent randomized controlled trials have demonstrated reduced levels of Bifidobacterium due to a low FODMAP diet (80). However, it is necessary to evaluate each patient individually. For example, reduced and controlled consumption of foods with FODMAPs for a determined period has been encouraged for IBD patients with a phenotype similar to IBS, with pain and abdominal distension (79,80)."

Comment 18: Confusing sentences: line 229 -> 232 (which organisms?)
Reply 18: We rewrote and clarified the sentence. Section Gut Microbiome: "The microorganisms: Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, Ruminococcus bromii, Roseburia spp., Lactobacillus spp., and Bifidobacterium spp., are responsible for the most significant fraction of butyrate production (66-68)."

Comment 19: line 235-> 237, 293 (irritable bowel syndrome?)
Reply 19: We appreciate the orientation. We included IBS term in the phrase. All changes are highlighted in yellow in the manuscript.

Comment 20: line 335 -> 337 (words missing?).
Reply 20: We rewrote the paragraph in the Section: Diet Can Modulate Microbiota: "Other diet protocols, like a gluten-free diet, have seemed used by IBD patients to treat symptoms (80,81). However, changes in the intestinal microbiota caused by the lack of grain-based foods, such as lower concentrations of Bifidobacterium spp. and Lactobacillus spp., can lead to a reduction in SCFA production, whose role is essential for several functions in the body, such as participation in our metabolism, production of anti-inflammatory mediators, and intestinal health (81)."

Comment 21: - Epigenome: The definition of epigenetics comes quite late. Epigenetics or epigenome has been mentioned already several times before. Chromatin is introduced in the first paragraph of DNA modifications, however it is discussed in the second paragraph.
Reply 21: We appreciate the reviewer's suggestion. We rearranged the ideas in the text, in the Section: Epigenome in Inflammatory Bowel Diseases: we changed the order and included at the beginning of the section the phrase: "Epigenetics can be mitotically heritable changes in gene function that cannot be explained by altered DNA sequences (12).", and in Section: DNA Modifications: DNA methylation and histone modifications: we included the discussion of chromatin in the first paragraph of this section. All changes are highlighted in yellow.

Comment 22: Confusing sentences: line 370 (who is 'they'?),
Reply 22: Section: DNA Modifications: DNA methylation and histone modifications: We excluded this part of the first paragraph of this section: "They are, however, reversible ."

Comment 23: line 373 (just -> only?)
Reply 23: We removed the word "Just" at the beginning of the phrase. Section: DNA Modifications: DNA methylation and histone modifications: "Just DNA methylation is stably transferred through repetitive cell divisions, leading to the capacity to permanently transmit epigenetic information during an individual's lifetime (122,125,126)."

Comment 24: line 489 -> 491 (missing "and", and comma in wrong place)
Reply 24: - Section: ncRNAs and miRNAs: we corrected the phrase as suggested by the reviewer.

Comment 25: and line 495 -> 497 (not clear).
Reply 25: We removed the phrase. Section: ncRNAs and miRNAs: ~~"Many phytochemical compounds found in food and natural products that are capable of~~

~~affecting miRNAs, which are molecules involved in biological processes related to cancer, through epigenetic actions (103).”~~

Comment 26: - Conclusion: The beginning is very good, however the end is a bit more difficult to understand. Confusing sentences: line 529 (what is 'this'?).
Reply 26: We thank the reviewer for pointing this out. We rewrite the phrase to a better understanding: “These mathematical tools will make it possible to continuously record patient’s exposure to environmental factors and to better understand these complex immune-mediated diseases.”

Reviewer B:
Comment 27: This section combines too many points (that need a reference) and needs to be reworded for clarity and specificity: The cost of 72 mental and other health problem (too vague), such as extraintestinal manifestations (blend this in with your description below, too vague to stand here). Besides, several 73 patients become less productive due to sickness, which leads to premature retirement (1). (and disability) 71 medical care is high (in IBD, driven by....) to the public health care systems, and (numerous (proportion?) patients suffer from
Reply 27: We appreciate the reviewer’s suggestion. We rewrite the phrase to better conciseness and clarity. Section: Introduction: “Medical care costs are high to the public health care systems, and several patients become less productive due to sickness, which leads to disability and premature retirement (6,7).”

Comment 28: This statement is beyond the scope of this paper to make: Other organs 78 can be affected and other new diseases may be discovered.
Reply 28: We agree with the reviewer and excluded the phrase: “Other organs can be affected, and other new diseases may be discovered.” from the Introduction section.

Comment 29: This needs references, and extraintestinal manifestations is not needed (its explained above as often associated) Environmental factors are important in the genesis of IBD and extraintestinal 80 manifestations. Line 82: delete “these” as you have not specified. Sentence stands well without it.
Reply 29: Section: Introduction: we deleted the sentence about extraintestinal manifestations and the word “these” as suggested by the reviewer.

Comment 30: Lines 86-96: needs a lot more referencing for these statements, along with a better definition of “epigenome”.
Reply 30: We appreciate the reviewer’s suggestion. We rewrote the paragraph to inform a better definition of the epigenome, and moreover, we added more references to support the statements in Section: Introduction. All changes are highlighted in yellow in the text.

Comment 31: 111-114: This is redundant with your above writing. Please reword for novel thoughts.

Reply 31: We appreciate the suggestion. We rewrote the paragraph in Section: Genome in Inflammatory Bowel Diseases. All changes are highlighted in yellow in the text.

Comment 32: 137-139: references needed.
Reply 32: We added references as suggested by the reviewer at the end of Section: Genome in Inflammatory Bowel Diseases. All changes are highlighted in yellow in the text.

Comment 33: This doesn't make sense here: (144) Others chronic immune-mediated diseases such as IBD are emerging (11,22).
Reply 33: We appreciate the reviewer's suggestion. We deleted the word "Others" in the first sentence in Section: Environmental Factors "Exposome".

Comment 34: (155) with a large family (delete "or", replace with comma?) crowded rural homes (only rural?)
Reply 34: We thank the reviewer for pointing this out. We deleted the word "or" and replaced it with a comma in the sentence. All changes are highlighted in yellow in the text in Section: Environmental Factors "Exposome".

Comment 35: (167). This needs more than one reference, with a possible understanding of why? (ie; nicotine or other ingredients in cigarettes, do we see this in vaping as well? Cigars? Chewing tobacco?)
Reply 35: We appreciate the reviewer's suggestion. Although the precise mechanism remains unknown, we added more information about this topic and related references in the section:
Environmental Factors "Exposome": "In this context, several potential active mediators in smoke may be responsible for these clinical effects, including nicotine and carbon monoxide, that could modulate the autoantibodies production, inflammatory profile, and leukocyte migration. However, the precise mechanism remains unknown (39,42)."

Comment 36: (200) see: https://ntforibd.org/research/research_table/
And, revise this statement (many studies demonstrating efficacy of therapeutic dietary strategies).
Reply 36: We agree with the reviewer and deleted the following phrase: "However, they do have not sufficient evidence to support their use in an evidence-based medicine approach."

Comment 37: (204) wrong word here: "accomplished"
Reply 37: We rewrote the sentence. Section: Gut Microbiome: "The microbiome plays a crucial role as an environmental factor that can modulate the exposome, leading to epigenetic modifications in the host."

Comment 38: (211) This is no longer the thinking, as babys microbiome is begun in utero. "A microorganism's gut colonization during the first hours of life" see: Peter I,

Maldonado-Contreras A, Eisele C, Frisard C, Simpson S, Nair N, Rendon A, Hawkins K, Cawley C, Debebe A, Tarassishin L, White S, Dubinsky M, Stone J, Clemente J, Sabino J, Torres J, Hu J, Colombel JF, Olendzki B. A Dietary Intervention to Improve the Microbiome Composition of Pregnant Women with Crohn's Disease and Their Offspring: the MELODY (Modulating Early Life Microbiome through Dietary Intervention in Pregnancy) Trial Design. Contemp Clin Trials Commun. 2020 May 4;18:100573. doi: 10.1016/j.conctc.2020.100573. eCollection 2020 Jun.PMID: 32617430 , among other publications.

Reply 38: We appreciate the reviewer's suggestion. We rewrote the sentence and added the reference for the MELODY trial. All changes are highlighted in yellow in the Section: Diet in Inflammatory Bowel Diseases.

Comment 39: (223) also found in abundance in flaxseed, chia seed, barley, oats, and other foods.

Reply 39: We appreciate the reviewer's suggestion. We rewrote the following phrase "Influenced by the microbiome and diet, SCFAs are gut microbiota-derived metabolites produced by anaerobic fermentation of indigestible fibers mainly found in fruits, vegetables and in abundance in flaxseed, chia seed, barley, oats, and other foods" in Section: Gut Microbiome.

Comment 40: (224) need references for these statements.

Reply 40: We added new references to support the statements presented in this paragraph. All changes are highlighted in yellow in the text.

Comment 41: 264: Here you just refute all that has come before? Evidence is pretty clear by now...please reword this statement (based on one study>)

Reply 41: We appreciate the reviewer's suggestion. We rewrote the last paragraph in Section: Gut Microbiome. All changes are highlighted in yellow in the text.

Comment 42: 292: need to reword the first part of this paragraph to blend better with your conclusions (prebiotics are good and needed), especially strong statement about FODMAPS responsible for pathogenesis....FODMAPS appear to be more about DIGESTION of these foods (enzymatic) than about disease process.

Reply 42: We appreciate the reviewer's suggestion. We rewrote and clarified the paragraph about FODMAPs in Section Diet Can Modulate Microbiota. All changes are highlighted in yellow in the text.

Comment 43: 314: see SCD diet and IBD-AID diet studies, as well as research on gluten intolerance and on GMOs in certain grains.

Reply 43: We rewrote it for better clarity of the information. All changes are highlighted in yellow in the text, in Section Diet Can Modulate Microbiota.

Comment 44: 355: this definition should come much earlier.

Reply 44: We appreciate the reviewer's suggestion. We allocate the phrase at the

beginning of the section: Epigenome in Inflammatory Bowel Diseases, for better clarity of the concept. All changes are highlighted in yellow in the text.

Comment 45: 365: need reference.
Reply 45: We added a reference to support the phrase: “Natural epigenetic modifications play a vital role in organism function, but if they occur incorrectly, they can cause serious health consequences (117).” in the section Epigenome in Inflammatory Bowel Diseases.

Comment 46: 525: need references to support.
Reply 46: We added references to support the information. All changes are highlighted in yellow in the text, in the Conclusion section.

Comment 47: Figure 1: needs revision to indicate perinatal influences, correct typo in “saturated”. Plus, not all polyunsaturated fats have the same effect (ie.; omega 6 vs omega 3). Might also want to include the non-nutritive additives to processed foods....
Reply 47: We appreciate the reviewer’s suggestion. We revised the figure, as pointed out by the reviewer, and made the corrections.