Peer Review File
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Reviewer A:
Comment 1:
I am assuming that this manuscript is a narrative review and as such it takes the reader too long to get to the point which is epigenetic mechanisms in inflammatory bowel disease. Too many sections proceed the narrative and the sections are overly long. Perhaps sections 1.1. and 1.2 could be combined as one introductory section with clear specific examples.
Reply to comment 1 from Reviewer A:
We have now shortened this section and combined sections 1.1 and 1.2 into one, as suggested.

Comment 2:
Sections 2.1 to 2.7 in this reviewer's view could be better rearranged in a more flowing manner that is more akin to the narrative of the manuscript.
Reply to comment 2 from Reviewer A:
We addressed this comment by fusing subsections and tried to connect the section better to improve the logical flow.

Comment 3a:
Sections 2.5 and 2.6 could be annexed together given that the narrative of these sections is built into the science of epigenetic changes.
Reply to comment 3 from Reviewer A:
As we have extended section 2.5, responding in part to Reviewer B, we would like to keep these subsections distinct for clarity.

Comment 3b: Also, when providing information re epigenetic changes the authors should define what is meant by this to further clarify the scientific messages being provided.
Reply to comment 3b from Reviewer A:
We define now epigenetic mechanisms in the context of this review right from the start in the abstract, writing:

“Epigenetic mechanisms maintain gene expression states within a cell and through cellular generations and involve DNA methylation and chromatin changes, such as histone modifications. These mechanisms play roles in inflammatory processes. Here we review recent advances about what we know about their impact in inflammatory bowel disease.”

On page 7, lines 241-246 we further define ‘epigenetic’:

“However, a more narrow, contextual understanding of the term ‘epigenetic’ is widely

accepted, whereby epigenetic mechanisms are regulatory functions involving DNA methylation, histone modifications, chromatin changes or noncoding RNA and this is how we interpret this concept here (Figure 1)."

Comment 4: The manuscript would gain immensely from an overall scientific language check.

Reply to comment 4 from Reviewer A:

We have edited the manuscript throughout for clarity. As this led to a large number of changes in almost every section (by breaking up of sentences, use of synonyms etc) which do not change the content of the text, we have not indicated these in the 'track changes' as this would make the tracked manuscript totally un-readable.

Reviewer B:

Comment 1:

I do not think the statement "The function of these latter in gut homeostasis is still being researched, and there have been no in-depth studies relating ultra-processed foods to IBD" is entirely true. Check out these and there are more:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8517080/

https://pubmed.ncbi.nlm.nih.gov/34261638/

https://www.bmj.com/content/374/bmj.n1554#:~:text=Ultra%2Dprocessed%20food%20intake%20and%20risk%20of%20IBD,-Table%203%20shows&text=risk%20of%20IBD.-,Higher%20intake%20of%20ultra%2Dprocessed%20food%20was%20associated%20with%20a,P%3D0.006%20for%20trend).

Reply to comment 1 from Reviewer B:

We have removed this contentious statement (and shortened the whole section), as this is not pertinent to the focus of this review.

Comment 2:

Section 2.3 and 2.5 is too superficial and needs more unique information compared to other reviews.

Reply to comment 2 from Reviewer B:

Addressing comment 1 of Reviewer A, we have fused now sections 2.1-2.3 into one section "Environmental, microbial and genetic factors affect IBD".

As this reviewer subsequently recognizes, the unique information is provided in a following section on SP140 and other genes, but we feel that we need to provide some context regarding the value of the genome-wide association studies. Furthermore, we extended the content on DNA methylation and IBD, by discussing relatively recent studies on methyl-CpG-binding protein Mbd2 and methylation processivity factor

Uhrf1.

Comment 3:
Reference to these other publications need to be done and to assess if anything new is stated in this review.
Reply to comment 3 from Reviewer B:
See reply to comment 2 and 4, below.

Comment 4:
The section on SP140 is good and more like that should be stated. There has been some evidence of epigenetic loss of a tumor suppressor, RASSF1A, in colorectal cancer and recently in IBD. Can the authors find non-immune epigenetically loss genes to focus on to stimulate the discussion for new therapeutics?
Reply to comment 4 from Reviewer B:
We now include the example of epigenetic loss of tumor suppressor RASSF1A, which is, indeed, a nice example illustrating how epigenetic changes (in this case DNA methylation) are linked to IBD. We write (page 8, lines 245-248):

"For instance, the epigenetic silencing of the tumor suppressor protein RASSF1A through DNA methylation changes has been linked not only to colorectal cancer but also to inflammatory bowel diseases(44,45)"

Furthermore we now include a new section describing recent studies on the histone methyltransferase SETD2 in colitis, page 15, lines 386-407:

"Numerous studies using tissue specific gene deletion in the mouse of have identified potential roles of epigenetic factors, histone modifying enzymes or chromatin remodelling enzymes in the colitis response and these studies also illustrate the roles of the various cell types involved in the pathology (reviewed in(68)). A recent example in this respect is the analysis of the role of SETD2 in the intestinal epithelium in colitis(69). SETD2 is the only known histone H3 lysine 36 trimethyl-transferase, mediating H3K36me3, a modification usually found over actively transcribed regions and thought to promote transcription. SETD2 mutations have been implicated in colorectal cancer(70), Deletion of Setd2 specifically in the intestinal epithelial epithelium worsened the pathological response in experimental colitis in the mouse(69). This exacerbation was found to be associated with dysregulated microRNAs (miRNAs) and genes involved in the response to oxidative stress, with these changes in gene expression linked to the loss of H3K36me3 over the affected genes(69,71). Pathology could be alleviated by treating the mice with anti-oxidant N-acetyl-L-cysteine. This is noteworthy, as oxidative stress contributes to the colitis pathology. SETD2 expression was down in IBD patients and mice subjected to experimental colitis(69) and SETD2 was found to be mutated in samples from ulcerative colitis patients with a high risk of developing colorectal carcinoma(72). Regulatory T (T reg) cell or group 3 innate lymphoid cells (ILC3s) specific deletion of Setd2 showed that this factor also regulates

the inflammatory response through these immune cells(73,74), Thus, these tissue-specific deletion studies allow dissection of the roles of epigenetic regulators in the various cell types that contribute to the inflammatory process."

See also reply to comment 2."