Author's response to reviews

Title: Early Life Environment and Natural History of Inflammatory Bowel Diseases

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Author's response to reviews: see over
Editorial Board Comments:

Comments: This is a well-worth area to be studied, given that there are limited data on effects or influence of environmental factors on IBD disease course.
Since their paper was submitted, a recent paper has shown the protective effect of breastfeeding with a duration dependent response in incident IBD in a population of disease emergence, it will be useful to include in reference (Ng SC et al. Environmental Risk Factors in IBD: a population based case-control study in Asia-Pacific, Gut 2014).
Please also address minor comments from reviewers

Response: We appreciate the careful and thorough review by the reviewers and the editorial board. We have addressed the minor comments of the reviewers and have added the citation of the important study by Ng et al.
Reviewer: Whitney Tang

Minor Essential Revisions

1. Method: There is no sample size justification. Will the non-significant association between exposures and surgery in UC due to under power of small sample size?

Response: Thank you for raising this concern. The survey was distributed to all patients recruited as part of our prospective registry and was not restricted to a specific target sample size. One possible reason for the lack of association in UC is that surgery is a less frequent occurrence in UC compared to Crohn’s disease, lowering the power to examine associations. As one will note from Table 3, none of the associations except for daycare exposure even bordered statistical significance suggested that there truly was a null effect. However, daycare exposure may be selected as a variable for examination in future studies with larger sample sizes.

*Lines 253-256*

Fourth, due to fewer events (surgery) in UC, the statistical power to detect associations may be lower. However, most associations except for daycare exposure did not demonstrate even a trend towards an association.

2. Results: There is a category of “unsure” in the questionnaire. Are they excluded from the analysis? What is the sample size included in the multivariate regression models and mutually adjusted model?

Response: Yes, for each variable, those who answered as ‘unsure’ were excluded from the analysis. We have now clarified this in the methods. The sample size for multivariate regression models in Crohn’s disease was 249 patients with non-missing data. Inclusion of those with missing information as a separate stratum did not change any of our estimates. We have clarified the above as a footnote to the tables listing the multivariate effect sizes.

*Lines 108-109*
Those who answered “Not sure” for any of the exposures were treated as missing for that variable.

*Lines 424-425*

Multivariate analysis included 249 patients in Crohn’s disease with non-missing data for all variables.

3. Results: Although studies showed conflicting result, exposure to antibiotics is also a factor of interest as an important environmental factors associated with IBD pathogenesis. There is question about antibiotics included in the questionnaire. Was the association between exposure to antibiotics and disease outcome analyzed?

**Response:** Yes, the question between antibiotic exposure and disease outcomes was analyzed and showed no effects. This is also in part due to the large number of missing responses in this category as early life antibiotic exposure isn’t easily recalled, particularly when anchored to specific time points of age like 1 year or 5 years.

4. Table 1: Characteristics of study population can be separated into UC and CD since they were analyzed separately, and only factors associated with CD showed significant result.

**Response:** We have modified table 1 to provide disease specific frequencies.

5. Typo in line 166: 95% CI

**Response:** Thank you. We have corrected this.
This study addresses an important question on the effect of early life exposure on the course of IBD. The study has a decent study design and methodology, and the statistical methods are valid. This paper is potentially publishable if the following questions are addressed.

Minor essential revisions as follows

1. The authors have included several factors like the age of diagnosis, disease location and perianal involvement as potential confounding factors. Have they included other known adverse factors, such as steroid use at first presentation, as a potential confounding factor?

Response: Thank you for raising this important question. We did not have information on steroid use at first presentation as ours is a referral centers and many patients have a length of care outside our institution prior to seeing us in consultation. We agree that steroid use has been proposed as a risk factor for severe Crohn’s disease, however this has usually been for a composite endpoint including need for immunosuppressive or biologic therapy and less frequently for an endpoint consisting only of surgery. Furthermore, for it to be a confounder, it would have to be differentially associated with both the outcome (surgery) and exposure, and we believe this unlikely.

Lines 256-257
We also did not have information on other factors that have been associated with aggressive disease course including steroid use at diagnosis.

2. The chance of surgery is affected by the duration of disease. This is very major confounding factor. While the authors have rightly put that in the regression model, can they provide more details on this factor? What are the the mean durations and the SD of the two groups (surgery vs no surgery), and are there significant differences between the groups?

Response: The mean disease durations for those who did not undergo surgery was 9.7 years (SD 9.3 years) vs. 16.6 years in those who underwent surgery (SD 11.9) (p < 0.05). Hence, our

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variables were all adjusted for duration of disease ensuring that associations between environmental exposures and outcomes were not confounded by this variable.

3. The first letter of the italicized bacteria names should be capitalized in lines 208-209.

Response: Thank you for pointing this out. We have made the suggested change.

Lines 210-211
who were exclusively breastfed had reduced colonization with Clostridium difficile, Bacteroides, and Lactobacilli and higher frequency of the protective

4. Please provide evidence or reference for the statement "early life exposure may have greater and more sustained effects on the gut microbiome than exposures later on in adult life" (line 226-227)

Response: Several studies have shown that breastfeeding and mode of delivery have significant exposure on the composition of adult microbiota and are indeed one of the strongest determinants of adult microbiota composition. Furthermore, much of the stability and composition of gut microbiota is obtained by age 3-5 years. We have now provided the supporting citations.

Lines 227-229
It has been consistently demonstrated that early life exposure may have greater and more sustained effects on the gut microbiome than exposures later on in adult life\textsuperscript{29, 32, 33, 44}. 