PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	IMAGINE Network's Mind And Gut Interactions Cohort (MAGIC) Study: A protocol for a prospective observational multi-centre cohort study in Inflammatory Bowel Disease & Irritable Bowel Syndrome
AUTHORS	Moayyedi, Paul; MacQueen, Glenda; Bernstein, Charles; Vanner, Stephen; Bercik, Premysl; Madsen, Karen; Surette, Michael; Rioux, John; Dieleman, Levinus; Verdú, Elena; de Souza, Russell; Otley, Anthony; Targownik, Laura; Lavis, John; Cunningham, Jennifer; Marshall, Deborah; Zelinsky, Sandra; Fernandes, Aida

VERSION 1 - REVIEW

REVIEWER	Georgina Hold UNSW, Australia I lead a similar study - The Australian IBD Microbiome (AIM) Study whose protocol will be submitted to BMJ Open within the next few days.
REVIEW RETURNED	02-Jul-2020

GENERAL COMMENTS	This is an excellent study protocol for an excellent study! The study outcomes are clear and the novelty is in the intended study size and the richness of data and sample collection. The authors should indicate the recruitment period and whether the study has been registered prospectively or retrospectively.

REVIEWER	Igor Łoniewski Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, Poland.
REVIEW RETURNED	12-Jul-2020

GENERAL COMMENTS	I want to congratulate the awe-inspiring project and add a few minor comments to help realize the scientific aims. 1. Page 6, line 15: How do you plan to assess psychiatric co-morbidity (e.g., psychiatrist consultation)? Will, all patients with depression be enrolled in the study? Which criteria are planned to be used in psychiatric diagnosis? Antidepressants have antimicrobial activity (Macedo D, Filho AJMC, Soares de Sousa CN, et al. Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of

	the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. J Affect Disord. 2017;208:22-32.). Which antidepressants will be allowed to be used? Which data concerning depression medications will be collected (duration, dose, etc.)? How do you plan to warrant the good matching of healthy controls (age, sex, BMI, race, diet)? 2. Page 7, line 3: Please define the fecal calprotectin concentration unit. 3. Page 8, line 52: How will patients be recruited (advertisements, database)? In the case of patients with IBS, it can be an important source of bias (the particular psychological type of patients can look for the opportunity to take part in this study). How do you plan to optimize the recruitment of patients to avoid it? 4. Page 13, line 49: Do you plan to assess food supplements intake, especially pre-, and probiotics? 5. Page 15, line 33: What does it mean “significant portion of the stool samples”? How these samples will be selected (randomly, exceptional cases)? Line 52: How do you plan to treat the compositionality problem inherent to microbiome investigations (Nguyen, T.T.; Hathaway, H.; Kosciolek, T.; Knight, R.; Jeste, D.V. Gut microbiome in serious mental illnesses: A systematic review and critical evaluation. Schizophr. Res. 2019, doi:10.1016/j.schres.2019.08.026., Nguyen, T.T.; Kosciolek, T.; Eyler, L.T.; Knight, R.; Jeste, D.V. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. J. Psychiatr. Res. 2018, 99, 50–61.)? Do you plan to share your data using tools such as Qiita (qiita.ucsd.edu) (Gonzalez, A., Navas-Molina, J.A., Kosciolek, T. et al. Qiita: rapid, web-enabled microbiome meta-analysis. Nat. Methods 2018,15,796–798.)? 6. Page 16, line 17: Can you see sense and possibility to analyze: fecal SCFA, blood Tryptophan metabolites (Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2020;17(4):223-237), GABA, stress hormones? What is your opinion about immunological and gut integrity parameters analysis? I think that these issues should be mentioned in the paper. 7. Page 36: Table 1, please consider comments from point 1. Exclusion criteria should include pre- and probiotics, and other medications, which can strongly affect the gut microbiota.

REVIEWER	Bahtiyar Yilmaz University of Bern, Switzerland
REVIEW RETURNED	20-Jul-2020

GENERAL COMMENTS	This study will be one of the biggest cohort ever established for IBD and IBS related microbiome study. Therefore, their findings will be quite important for the field for better understanding the relationship between microbiota and these disease. There is not much to add \ comment for improving the study. It is already in a really good shape. The only thing that makes me sad to see is that the authors

	claim that the IBD microbiota studies published up to now are with the limited numbers which is not entirely true. It is a pity that the authors failed to give a proper credit to the papers published last years in Nature and Nature medicine (Human Microbiome Project 2 and Swiss IBD Cohort) on IBD studies with relatively quite informative numbers. Besides that, everything else seems ok and the study will deliver important messages for the IBD and IBS society. We are excited to see their informative and game-changer findings.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1.

We thank Georgina Hold for her kind comments and interested to hear of her AIM study.

She makes and excellent point that we had not clarified that the protocol was prospectively registered. We have added this to page 13 lines 4-6: “The study was prospectively registered on April 27th 2017 (ClinicalTrials.gov Identifier: NCT03131414) and this was last updated on March 7th 2019. Recruitment commenced in October 2017.”

Reviewer 2

We are grateful to Igor Łoniewski for his generous comments and detailed review of the manuscript.

1. How do you plan to assess psychiatric co-morbidity (e.g., psychiatrist consultation)?

We apologize for not making this clearer. We are not asking all 8000 to have a psychiatrist consultation but ask them to complete validated questionnaires that were outlined in table 3. This has been clarified on page 10 lines 6-9 “Questionnaires measuring anxiety, depression, generalized anxiety disorder, sleep disturbance, generalized anxiety disorder, perceived stress, adverse childhood experiences, resiliency, and pain catastrophizing in both adults and children are outlined in Table 3.”

2. Will all patients with depression be enrolled in the study?

No. Neither the IBD, IBS or healthy controls are recruited on the basis of depression. They are recruited on the basis of having either IBS and/or IBD or being healthy (as defined in the manuscript). Anxiety or depression is not an inclusion or exclusion criteria for any of these groups. We expect that 10-20% of our healthy controls will have anxiety and/or depression according to the validated questionnaires. We expect the corresponding proportion for those with IBD/IBS to be 30-40%.

3. Which criteria are planned to be used in psychiatric diagnosis?

The criteria are outlined in the validated questionnaires that we are using. Given the number of questionnaires we are using we have not provided scores and cut offs for every measure but if Dr Łoniewski is interested I would refer him to http://www.healthmeasures.net/images/PROMIS/manuals/PROMIS_Adult_Profile_Scoring_Manual.pdf as an example of a helpful resource in this regard.

Antidepressants have antimicrobial activity. Which antidepressants will be allowed to be used? Which data concerning depression medications will be collected (duration, dose, etc.)?

We do state on page 10 that drug history will be obtained but we are not prohibiting the use of any antidepressant. This is study will follow participants up for up to 4 years and it would be unethical to prohibit antidepressants for this length of time. We read the reference with interest and it appears that most commonly used antidepressants could have an antimicrobial effect but looking at the original papers (the citation provided was a review) the in vivo data are not compelling as any microbial changes could relate to the underlying disorder as opposed to the drug. Nevertheless, we are recording the drugs that participants are on at baseline and during follow up so could explore this further with our dataset. Ultimately, any hypothesis generated would need evaluating in a randomized controlled trial.

How do you plan to warrant the good matching of healthy controls?

We are not aiming for "good matching" as this is not a matched analysis. The controls come from the same population as the cases and any confounding will be adjusted for with the logistic regression analyses we are conducting. Dr Łoniewski makes an excellent point as it would be important to ensure that the sex proportion, BMI, and age of the controls are similar to the cases so that these factors can be adjusted for. We have monitored our control recruitment and they are similar to the cases in terms of age, sex and BMI.

Please define the fecal calprotectin concentration unit.

Thank you for pointing this omission out. The units are mcg/g and this has been added.

How will patients be recruited (advertisements, database)?

Recruitment is an important source of bias although whichever approach you use will always only focus on a certain subset of IBS patients. Dr Łoniewski feels that we may preferentially recruit patients with psychological disturbance. This is not the case as research staff are instructed to invite anyone that meets Rome 4 criteria for IBS to participate and no attention is paid to any psychological issues before recruitment. Patients are recruited from gastroenterology clinics, so the diagnosis of IBS is more robust. This has been clarified in second paragraph of page 9 "IBS and IBD patients will primarily be recruited from gastroenterology clinics at participating centres."

Do you plan to assess food supplements intake, especially pre-, and probiotics?

We do capture the use of prebiotics and probiotics from the questionnaire completed by participants.

What does it mean "significant portion of the stool samples"? How these samples will be selected (randomly, exceptional cases)?

We are grateful to the reviewer for highlighting that we were not explicit about this. We have clarified this by adding the sentence: "We aim to reduce costs so it is possible to perform this on all samples but if this is not possible, we will perform shotgun metagenomics on at least 20% of randomly selected samples from each group".

How do you plan to treat the compositionality problem inherent to microbiome investigations?

Dr Łoniewski raises an important issue with regards to microbiome analysis and there has been a move to more methodology that accounts for the compositional nature of the microbiome. This issue does remain an active debate in the field and among the experts in statistical analysis in this field the

debate is not yet resolved, and we will continue to explore new statistical methods as they become available. We have updated the analysis section and added new references to reflect this.

Do you plan to share your data using tools such as Qiita (qiita.ucsd.edu)?

The microbiome data and all scripts used for analysis will be made publicly available. At this time a decision about whether to deposits processed data at a single site has not been made.

Can you see sense and possibility to analyze: fecal SCFA, blood Tryptophan metabolites, GABA, stress hormones? What is your opinion about immunological and gut integrity parameters analysis?

We absolutely agree with the reviewer that all of these are important parameters to be examined. In serum, we will be examining a panel of cytokines and chemokines to establish an immune phenotype as well as tryptophan metabolites, neurotransmitters, hsCRP, and stress hormones. LPS will be measured as a marker of gut integrity. We have added this information into the manuscript on page 10 lines 16-22.

Exclusion criteria should include pre- and probiotics, and other medications, which can strongly affect the gut microbiota.

We disagree with this suggestion. We are not aware of any evidence that probiotics “strongly” impact the gut microbiota. Stool metagenomics usually only show modest changes related to probiotics and probiotics also have minor impacts on clinical symptoms of IBD and IBS according to randomized controlled trial data. Furthermore, many patients take probiotics despite lack of evidence for efficacy and so excluding these would make the cohort less representative. It would be particularly problematic to prevent participants from taking these during follow up. We record whether patients are taking pre and probiotics and can do a subgroup analysis of those taking and not taking these interventions.

Reviewer 3.

We thank Bahtiyar Yilmaz for his kind and positive comments. We stand by tour comment that to date the case control studies comparing IBD and IBS with healthy controls have evaluated modest numbers of patients that do not allow for robust analysis due to multiple testing problems. This is based on systematic reviews of the literature (which we reference – references 18 and 19) and so we can be confident that at the time of publication this statement was true. What Dr Yilmaz is referring to is cohorts of IBD patients without healthy controls as a comparator. This will evaluate different research questions from the main analysis of the recruitment phase of our study but nevertheless we agree that this can provide very helpful information as he describes. Dr Yilmaz was the first author of the Swiss cohort study, and the senior author was Andrew Macpherson who used to work at the institution I am now working in and is a world-renowned gastroenterologist and immunologist. I have added this reference to the article as he suggests and added a sentence to the “strengths” section – “There is the possibility of pooling data with similar cohorts to provide more robust data on microbiome changes over time in these diseases (59).”

VERSION 2 – REVIEW

REVIEWER	Igor Łoniewski Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, Poland
REVIEW RETURNED	12-Sep-2020

GENERAL COMMENTS	Dear Authors, Congratulations and good luck in conducting so impressive study!