BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (http://bmjopen.bmj.com).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-041733
Article Type:	Protocol
Date Submitted by the Author:	18-Jun-2020
Complete List of Authors:	Moayyedi, Paul; McMaster University Faculty of Health Sciences, Medicine MacQueen, Glenda; University of Calgary Bernstein, Charles; University of Manitoba, Gastroenterology Vanner, Stephen; Queen's University Bercik, Premysl; McMaster University, Medicine Surette, Michael; McMaster University, Madsen, Karen; University of Alberta Rioux, John; Universite de Montreal Dieleman, Levinus; University of Alberta, Edmonton, Alberta Verdú, Elena; McMaster University, Division of Gastroenterology de Souza, Russell; McMaster University, Department of Clinical Epidemiology & Biostatistics Otley, Anthony; Dalhousie University Targownik, Laura; Mount Sinai Hospital Lavis, John; McMaster University Marshall, Deborah; University of Calgary, Department of Community Health Sciences Zelinsky, Sandra; University of Calgary, PaCER Innovates Fernandes, Aida; McMaster University, Medicine
Keywords:	Inflammatory bowel disease < GASTROENTEROLOGY, Functional bowel disorders < GASTROENTEROLOGY, Depression & mood disorders < PSYCHIATRY, Microbiology < NATURAL SCIENCE DISCIPLINES, Nutritional support < GASTROENTEROLOGY

SCHOLARONE™
Manuscripts

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

For peer review only

IMAGINE Network's Mind And Gut Interactions Cohort (MAGIC) Study:
A protocol for a prospective observational multicentre cohort study in Inflammatory Bowel Disease & Irritable Bowel Syndrome

Authors:
Paul Moayyedi[1], Glenda MacQueen (post-humous)[2], Charles N Bernstein[3], Stephen Vanner[4], Premysl Bercik[1], Karen L Madsen[5], Michael G Surette[1], John D Rioux[6,7], Levinus A. Dieleman[5], Elena F Verdu1[1], Russel De Souza[1], Antony Otley[8], Laura Targownik[9], John Lavis[10] Deborah Marshall[2], Sandra Zelinsky[2], Aida Fernandes[1,2], on behalf of the IMAGINE Network

1. Farncombe Family Digestive Health Institute, McMaster University, Hamilton, ON, Canada
2. University of Calgary, AB, Canada
3. University of Manitoba, Winnipeg, MB, Canada
4. Queen's University, Kingston, ON, Canada
5. University of Alberta, Edmonton, AB, Canada (bercikp@mcmaster.ca)
6. Université de Montréal, Montréal, QC, Canada
7. Montreal Heart Institute, Montreal, QC, Canada
8. Dalhousie University, Halifax, NS, Canada
9. University of Toronto, ON, Canada
10. McMaster Health Forum, McMaster University, Hamilton, ON, Canada

Key Words:
Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, prospective observational cohort, microbiome, diet, genetic, microbiome, metabolome

ClinicalTrials.gov Identifier: NCT03131414

For peer review only

ABSTRACT (reduce to 250 words)

Introduction: Gut microbiome and diet may be important in irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and comorbid psychiatric conditions but the mechanisms are unclear. We will create a large cohort of patients with IBS, IBD and healthy controls and follow them over time, collecting dietary and mental health information and biological samples, to assess their gastrointestinal (GI) and psychological symptoms in association with their diet, gut microbiome and metabolome.

Methods and Analysis: This five-year observational prospective cohort study is recruiting 8000 participants from 15 Canadian centers. Persons with IBS who are 13 years of age and older or IBD ≥ 5 years will be recruited. Healthy controls will be recruited from the general public and from friends or relatives of those with IBD or IBS who do not have GI symptoms. Participants answer surveys and provide blood, urine, and stool samples annually. Surveys assess disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet. The main outcomes evaluated will be the association between the diet, inflammatory, genetic, microbiome and metabolomic profiles in those with IBD and IBS compared with healthy controls using multivariate logistic regression. We will also compare these profiles in those with active versus quiescent disease and those with and without psychological comorbidity.

Ethics and Dissemination: Approval has been obtained from the institutional review boards of all centres taking part in the study. We will develop evidence-based knowledge translation initiatives for patients, clinicians and policy-makers to disseminate results to relevant stakeholders.

For Peer Review Only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Strengths and Limitations

- This is the largest observational study evaluating the microbiome in inflammatory bowel disease and irritable bowel syndrome.
- The patients' disease type and activity are well characterized with detailed information on diet and mental health.
- The degree of patient engagement is another strength of the study.
- The microbiome and diet assessment are conducted once per year and may not correlate with disease flare-ups.
- As with all observational studies, any association may not be causal and will need evaluation in randomized controlled trials.

INTRODUCTION

Two thirds of the population experience significant gastrointestinal (GI) symptoms at some point in their life (1). One of the most common GI disorders is irritable bowel syndrome (IBS), affecting up to 10% of persons worldwide, depending on the definition (2). Another GI disorder that is associated with significant health care resources is inflammatory bowel disease (IBD) which affects approximately 0.3% of the world's industrialized population (3).

The cardinal features of IBS include chronic abdominal pain over 3 months per year related to a change in bowel habit and the disease can present at any age (2,4). IBD is a term encompassing two distinct but related diseases: ulcerative colitis (UC) and Crohn's disease (CD). The cardinal symptom of UC is bloody diarrhea whilst in CD abdominal pain is a more prominent symptom, but

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

diarrhea is a feature as well (5). IBD has the highest incidence in second and third decades of life and are lifelong relapsing and remitting diseases. Growth impairment can be an issue in children with CD.

Both IBS and IBD significantly impact quality of life (6, 7) and often surgery is needed in patients with IBD (8). IBS and IBD also account for significant health care spending in the developed world with many countries spending billions of dollars per annum (9). Persons with IBS and IBD have higher rates of anxiety and depression compared to the general population (10, 11) and those with other chronic diseases (12). The corollary is also true; persons with anxiety and depression have more GI symptoms compared to healthy controls (13). Therapy for IBS has traditionally focused on drugs that alter motility or visceral sensitivity of the GI tract and although various interventions are superior to placebo, the overall impact on symptoms is only modest (14). Therapy for IBD has traditionally focused on drugs that inhibit the exaggerated pro-inflammatory immune response, however only 50% of the patients achieve clinical remission, whereas clinical relapses are common.

There is evidence that the gut microbiome and diet are important in IBS (15), IBD (16), and comorbid psychiatric conditions (17). There is however a need for more longitudinal prospective data on this interaction in patients with IBD and IBS compared with healthy controls. We have conducted systematic reviews in both IBS (18) and IBD (19); although there are numerous case control studies exploring the gut microbiome in these conditions, the median sample size is around 20 per arm and in all cases the sample size was insufficient to deal with the multiple testing issues that relate to microbiome research in humans. In addition, inter-individual differences of the gut microbiome are large. Large sample sizes and longitudinal sampling within the same individuals

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

over time are therefore needed to evaluate the interaction between diet, the microbiome, IBS, IBD and associated mental health issues.

The Inflammation, Microbiome, and Alimentation: Gastro-Intestinal and Neuropsychiatric Effects (IMAGINE) (20) (see appendix for members) Strategy for Patient Oriented Research (SPOR) Network is conducting a five-year multicenter prospective observational cohort study, Mind And Gut Interactions Cohort (MAGIC). It will explore the interaction between the diet, microbiome and the host associated with IBS and IBD in order to better target treatment of IBD and IBS and the psychiatric disorders associated and affecting these diseases. The overarching hypothesis of this study is that IBS and IBD are driven by a perturbation of the gut microbiome and the associated host immune response. Alterations in the gut microbiome may also drive anxiety and depression associated with these GI disorders and these psychological factors may in turn influence gut symptoms and its microbiome. These mechanisms may also have a genetic predisposition.

Aims

The main aim of the MAGIC study conducted through the IMAGINE SPOR Network is to create a large cohort of patients with IBS, IBD and healthy controls and follow these individuals over time, assessing disease activity, diet, mental health and demographic information using validated questionnaires and collecting annual stool, urine and blood samples, to correlate GI and psychological symptoms with an individual's genetic variants, diet, and gut microbiome, as well host and microbiome metabolic products in stool, urine and serum.

Primary aims for baseline data

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

1. We will compare the gut microbiome and metabolomic profile between CD, UC, IBS and healthy controls. The main analyses will be between a specific disorder and healthy controls.

2. We will compare the gut microbiome and metabolomics profile of participants with active versus quiescent disease within CD, UC and IBS.

3. We will compare the gut microbiome and metabolomic profile of participants with and without psychiatric co-morbidity for each of CD, UC, IBS and healthy controls.

Primary aims for longitudinal data

1. Microbiome, metabolomic, genetic, inflammatory markers, dietary, disease phenotype, psychiatric comorbidity and demographic predictors of failure of therapy for each of UC, CD, and IBS.

2. Comparison of microbiome, metabolomic, genetic, dietary and demographic factors in IBD of participants who remain in clinical remission over 2 years versus those with recurrent active disease (UC and CD analyzed separately).

3. Comparison of gut microbiome, metabolomic, genetic, dietary and demographic factors in participants with IBS with mild/inactive disease (based on IBS-Symptom Severity Score (SSS)) compared with those with ongoing active disease. IBS will be evaluated overall and also within subgroups independently.

Secondary aims for baseline data

1. To compare dietary patterns between patients with CD, UC, IBS and healthy controls.

2. To compare genetic risk factors between CD, UC, IBS and healthy controls.

3. To compare gut microbiome, metabolomic, genetic, dietary and demographic factors in participants with IBD with quiescent inflammatory disease with and without concomitant IBS

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

(defined by fecal calprotectin <50 and subthreshold IBD symptom activity score but who have active IBS symptoms on IBS-SSS).

4. To compare the prevalence of mood and anxiety disorders in participants with each disorder against rates in healthy controls.

5. To compare the dietary, gut microbiome and metabolomics profile between participants with or without anxiety (CD, UC, IBS, healthy controls analyzed separately).

6. To compare the dietary, gut microbiome and metabolomics profiles between participants with and without depression (CD, UC, IBS, healthy controls analyzed separately).

7. To determine whether high rates of early adverse experiences are associated with history of more severe disease or treatment resistance in participants with CD, UC or IBS.

8. To examine the association between symptom severity and multiple domains of function in participants with CD, UC or IBS.

9. To develop models describing how factors such as microbiome, metabolome, diet, genes and psychiatric symptoms interact in CD, UC, and IBS.

Secondary aims for longitudinal data

1. To compare health care resource use between CD, UC, IBS and healthy controls.

2. To compare work productivity between CD, UC, IBS and healthy controls.

3. To compare baseline dietary and gut microbiome and urinary metabolome profiles and inflammatory markers in participants that develop anxiety during follow up versus those who did not have anxiety at any time (CD, UC, IBS and healthy controls analyzed separately).

4. To compare baseline dietary and gut microbiome and urinary metabolome profiles in participants that develop depression during follow up versus those that did not have depression at any time (CD,

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

UC, IBS and healthy controls analyzed separately and if appropriate combined).

7. To compare gut microbiome, urinary metabolome, genetic, dietary and demographic factors in IBS at baseline in those that change their IBS subtype during follow up and those that continue with the same IBS subgroup.

8. To compare which dietary, mental health, gut microbiome and metabolomics profiles precede a clinical relapse in patients with IBD and how these parameters are different in patients with active disease versus those who remain in clinical remission.

METHODS

Design

The IMAGINE MAGIC study is a prospective observational cohort study that is recruiting 2000 participants with each of CD, UC, and IBS and also 2000 healthy participants in 15 centers across Canada. Assessment includes psychological status, dietary intake, gut microbiome, urinary metabolomic profile, inflammatory markers, genotype, health-related quality of life, and health care resource use and associated costs. The cohort and healthy controls will be followed annually for up to 4 years after the baseline study enrollment.

At each visit, the participant provides blood, urine, and stool samples as well as complete questionnaires assessing disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet. Table 1 summarizes participant information collected at each visit.

Participants

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

A total of 8000 participants will be recruited, 2000 for each for healthy volunteers, IBS, UC and CD. Inclusion and exclusion criteria are outlined in Table 1.

Healthy participants over the age of 4 will be recruited from the relatives, spouses and friends of IBS and IBD cases taking part and also through advertisement for healthy volunteers.

IBS: Persons who meet Rome IV criteria and are 13 years of age or older are enrolled (Table 1). Persons with IBS are categorized into diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), IBS with mixed bowel habits (IBS-M), or unclassified with IBS (IBS-U) (21). Patients with IBS that also have celiac disease will be eligible provided they continue to have symptoms after six months of a gluten free diet and their tissue transglutaminase antibody has returned to normal.

IBD: Persons with either CD or UC over the age of 4 years are enrolled regardless of whether the disease is active or in remission (Table 1). Persons with unclassified IBD (IBD-U) are included. The Montreal Classification is used for adult CD and UC patients (22), and the Paris classification (23) for pediatric IBD. The research coordinator conducts a chart review to confirm the date of diagnosis and maximal phenotype at time of enrolment using the Montreal Classification.

Data Collection

All participants attend a baseline, 12, 24, 36 and 48-month visit. Sources of information for each patient are blood, urine and stool samples, questionnaires, and chart review (Table 2).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Participants complete a questionnaire to obtain age, sex, gender identity, education level attained, ethnic heritage, smoking/alcohol/drug history, comorbidities, medication and therapies, menstrual status at baseline. The study research coordinator records height, weight, BMI and disease-related information (24). Participants answer a series of questionnaires to assess disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet at each study visit. (Table 3). Healthcare resource use data and associated costs regarding physician visits, clinical procedures, imaging procedures, hospitalizations, emergency room visits, and medication use are collected by linking the participants to provincial administrative data from the Canadian Gastro-Intestinal Epidemiology Consortium (CanGIEC) (https://cangiec.ca).

Biosamples

A stool sample is collected for fecal microbiome and inflammatory markers, a urine sample is collected for metabolomics, and blood samples are taken for DNA isolation as well as for serum for inflammatory markers and metabolomics at each study visit. All biosamples are shipped to Population Health Research Institute (PHRI) for storing at -80 degrees Celsius.

Data Management

Questionnaires are completed electronically using tablets during the clinic with the option to complete the any questionnaires remaining after clinic visit at home on a computer using an e-mail link. The user questionnaires are available in the REDCap platform stored at a central database collection center, PHRI, at McMaster University. Study staff will review surveys within two weeks of receipt and highlight any missing answers that suggests a problem in completing the survey. These issues will be discussed with the PI, site lead and study team. The staff contact participants up

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

to three times by phone e-mail or in person at a regular study visit to remind them to complete questionnaires and to acquire answers to missing items.

Patient and Public Involvement

The research proposed in IMAGINE was informed by patients. Patient perspectives were sought in identifying priorities for strategic research funding opportunities. Diet, researching the gut microbiome, and mental health were initially identified as key priorities through this process. Through the Crohn's and Colitis Canada (CCC) "Gutsy Learning Series" these priorities were further defined with 289 lay participants in-person and on-line with IBD who gave feedback on what they felt were the most important research topics, identifying fecal transplants, diet and mental health as key priorities. Furthermore, through a workshop organized by the Canadian Digestive Health Foundation (CDHF) prior to this IMAGINE SPOR application, a key message was that IBS patients are concerned about using current pharmacological therapies to relieve their symptoms and prefer approaches that correct the imbalances they perceive to be the root cause of their disorder rather than taking drugs. Patients with IBS are concerned with the long-term use of powerful and sometimes expensive agents and would like more exploration of the factors that are driving the disease. One of the major areas this patient group wanted more research on was the role of diet and also the role that gut bacteria (and probiotics) play in driving IBS. There is remarkable congruence in the research priorities of the IBD and IBS patient communities. The IMAGINE SPOR proposal was informed by these priorities and seeks to address them by exploring the diet-gut microbiome-relationship and how this influences GI and mental symptoms of IBS and IBD.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

IMAGINE Patient Research Partners were involved in the development of the MAGIC demographic questionnaire. They also served to pilot test the online questionnaires and provide feedback on user experience and feasibility.

IMAGINE Patient Partners have been directly involved with study design and recruitment of subjects in IMAGINE and will support capacity development for patient engagement more broadly. In person interviews are being conducted by our Patient Partners to identify strategies to improve recruitment and retention rates. Also, this network of patient-engaged researchers is communicating knowledge derived from the study to healthcare professionals, policy makers, and other patients.

A key component of the IMAGINE research program is developing capacity for patient engagement, patient preferences for informing treatment choices and working with our Patient Partners to improve our recruitment rates and long-term retention of IMAGINE participants. We have a unique opportunity to integrate patient engagement as recommended by SPOR by leveraging our innovative Patient and Community Engagement Research (PaCER) program based at the University of Calgary (25, 26). PaCER is designed to promote new roles for patients and family members in health care and health culture through engagement in research. PaCER provides opportunities for patients to be involved in the development and conduct of research designed to affect the lives of patients living with IBD and IBS. Involving patients and families in research is an opportunity to increase the capacity to anticipate problems, manage their condition as a partner in their health care team, and to support other patients and families.

DATA MONITORING AND ETHICS

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

This is an observational study with no intervention mandated by the protocol and so there is no external data safety and monitoring board. Research ethics approval has been obtained for all 15 sites involved in the study. The protocol was approved on May 31, 2017 with an approval number 2017-3000-GRA with the last amendment to date being on June 25, 2019. The study was first registered on April 27th 2017 (ClinicalTrials.gov Identifier: NCT03131414) and this was last updated on March 7th 2019.

SAMPLE SIZE AND ANALYSES

Sample size

UC, CD and IBS cases will be analyzed. A sample size of 2000 cases in each disease group and 2000 healthy controls will have 90% power to detect a probability of 0.547 that an observed abundance in the disease group is more than the observed abundance in the control group using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.001 two-sided significance level (to adjust for multiple testing). This sample size also assumes 20% data dropout. The probability of 0.547 was derived from 75 IBD cases that we have obtained from pilot studies (27).

Analyses

Primary and secondary aims will be evaluated through multivariate logistic regression with further details regarding how diet, microbiome, metabolomics and genetics will be analysed below.

Diet Analyses

We will use principal component analysis to derive dietary patterns from self-reported semi-quantitative food-frequency questionnaires, and understand the differences in dietary patterns across

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

participants with UC, CD, IBS, and healthy controls; and for active versus non-active disease within each disease group, as outlined in the primary and secondary aims. Briefly, the purpose of PCA and/or machine learning is to reduce large and complex high-dimensional data into fewer dimensions — in this case comprehensive FFQ data (containing up to 150 items or more) is reduced to 2 or 3 dietary patterns (i.e., foods commonly consumed together) that explain the greatest amount of dietary variability within the reported eating habits of the cohort (28). The number of dietary patterns to be retained for subsequent analysis will be based on visual inspection of Scree plots in conjunction with eigenvalues, and principal component interpretability (29). To account for differences in total energy intake between participants, dietary pattern scores will be adjusted to the mean total population caloric intake using the residual method (28). Associations between a participant's adherence to a specific PCA-derived dietary pattern (e.g., Western or plant-based) and UC, CD, IBS and its disease activity will be quantified using logistic regression (case vs control) with appropriate adjustment for covariates (e.g., BMI, age, sex, etc.). For machine learning, dietary patterns will be derived using unsupervised methods that require little to no input or direction by the researcher. The use of both PCA and unsupervised machine learning methods will allow for validation of the derived diet patterns. Our experience suggests that the PCA and machine learning derided patterns will be largely similar (30).

In an exploratory analysis, of particular interest for the IBS group, we will collect data using a supplementary fermentable oligo-di-monosacharides and polyols (FODMAP) Questionnaire to capture foods rich in: a) oligosaccharides, including fructans and galacto-oligosaccharides; b) disaccharides, including lactose; c) monosaccharides, including fructose; d) polyols. These data will be used to better understand "trigger foods" or dietary components that are likely to produce

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

symptoms in participants with IBS.

Microbiome Processing and Analyses

All stool samples will be processed in one lab for consistency. Frozen samples sent from each site will be thawed on ice in an anaerobic environment, mixed thoroughly with a sterile spatula. Two aliquots of 0.3g will be transferred to DNA extraction buffer for molecular analysis. Three aliquots of 1.8 mL will be biobanked at -80°C. DNA will be extracted using established methods (31). Total bacterial load will be measured by quantitative PCR of the 16S rRNA gene. Microbial community profiling will be carried out by amplification and paired-end Illumina sequencing of the v3-v4 region of the 16S rRNA gene for bacteria (31, 32) and the Internal Transcribed Region of the ribosomal genes (ITS) for fungi (33). Microbiome profiles will be processed through in house bioinformatic pipelines (34) incorporating dada2 (35) to generate amplicon sequence variants (ASVs). As sequencing and library construction costs decrease, it will be feasible to carry out shotgun metagenomics on a significant portion of the stool samples. Metagenomic sequencing libraries will be constructed using NEBNext Ultra DNA Library kits with modifications to reduce reaction volume. Libraries will be sequenced to ~15,000,000 reads per sample (150nt paired-end) on an Illumina NovaSeq. After filtering and trimming for sequence quality and primer removal, DeconSeq (36) will be performed on the remaining reads in order to remove reads of human DNA. Genes and functional predictions and comparisons across sample groups will be computed using HUMAnN2 (37) and MetaPHlAn (38) for functional microbiome analysis.

Microbiome analysis will include α-diversity metrics for each sample and β-diversity measures (weighted and unweighted unifrac, Bray-Curtis, nonmetric multidimensional scaling) and other

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

statistical analysis using PhyloSeq and R (39). Using generalized linear mixed models (GLMM), we will identify microbial taxa and/or genes associated with disease phenotypes/progression, response to treatment, genotype, diet and other measured parameters. The large number of samples will also allow for application of machine learning methods such random forest and support vector machine methods (40, 41).

Metabolomics Processing and Analyses

Urinary metabolomic profiles will be determined and analyzed together with microbial and dietary profiles to identify relationships and associations with disease status and clinical phenotypes/response to therapies. Urinary metabolic profiles will be analyzed by ^{1}H-NMR on a 4-channel Varian INOVA 600 MHz NMR spectrometer. Assignment of endogenous urinary metabolites will be done using Chenomx NMR Suite 8.5 (Chenomx Inc, Edmonton, Canada) and online databases (HMDB) (42). Metabolite concentrations will be $\log_{10}$ transformed to normalize data prior to statistical analyses. Metabolome association study analyses will be done using multiple linear regression models in the R Project for Statistical Computing (R program). Projection-based principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), orthogonal partial-least squares (OPLS) analysis will be performed using R program.

Genetic Analyses

In terms of genetic analyses, genomic DNA samples will be tested using two different approaches: 1) genome-wide genotyping to capture common genetic variation and enable genome-wide association studies (GWAS) and 2) whole exome sequencing (WES) will primarily be used to capture rare genetic variation and identify non-synonymous coding variants as potential causal

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

variants. Both datasets will be used to identify genetic risk variants associated with disease status or clinical phenotypes/outcomes detailed above (e.g. response to therapy); both as previously described (43, 44, 45, 46). These data will also be used to impute the genetic variation at the highly polymorphic Human Leukocyte Antigens (HLA) and Killer cell Immunoglobulin-like Receptor (KIR) genes (47, 48), as these are key determinants of the host's immune response and genetic risk factors for many inflammatory diseases.

For the statistical analyses, following rigorous quality control of the genotype/sequence data, whole genome imputation of the dataset using a relevant public reference panel (e.g. 1000 Genomes, Haplotype Reference Panel, TOPMed, etc.) (49, 50) will be performed followed by principal components analysis (PCA). Principal components will be tested for phenotype association (using logistic regression with study indicator variables included as covariates) and evaluated for their impact on the genome-wide test statistics using λ (the genomic control inflation factor based on the median $\chi 2$) after genome-wide association of the specified principal component. Association testing as well as binary and linear genotype– phenotype analyses will be done with PLINK and multinomial and ordinal regression analyses with a custom program, Trinculo. Survival analysis and risk prediction will be done with R using the packages "survival" and "Mangrove", respectively. For integrated biomarker discovery (51), this genetic data will also be integrated with other biomarker data generated from the various IMAGINE platforms, in order to select those that estimate a large association with clinical outcomes (e.g. response to therapy), in order to create the best subset of predictors. Variable selection will be based on mathematical criteria for model selection, i.e., the Bayesian Information Criteria (BIC), and expert a priori (e.g., clinical knowledge, preliminary evidence). The selection of the model will be in the context of logistic regression, using the

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

candidate biomarkers as covariates and drug response (positive or negative) as the outcome. The BIC has been proven to lead to less over-fitting of the model to data compared to other less conservative approaches (52). This will reduce type-I errors and lead to increased robustness of the results.

Subgroup Analyses

There are a number of subgroup analyses planned. In particular, we will analyze the primary and secondary outcomes by sex. We will strive to ensure representative enrollment of men and women with a wide range of life experience and at different life stages. When women are surveyed or otherwise evaluated, we will take note of past, anticipated and ongoing pregnancies, obtain a menstrual history, and use instruments that are sensitive to the influence of gender on outcomes.

The pediatric population (IBD cases aged 4-18 years of age) is also an important group to study. All the primary and secondary outcomes described above will also be evaluated specifically in the pediatric population. This includes predictors of success and failure of therapy for IBD. Children developing IBD are predominantly treated from the time of first presentation at academic centres rather than in community practice. Hence the pediatric collaborators in this proposal offer access to the broad spectrum of IBD, including prior to alteration of the microbiome by any therapy. We will evaluate predictors of success and failure of these therapies for IBD over time in the pediatric population.

We will evaluate subtypes of IBS; constipation predominant, diarrhea predominant and mixed patterns. We will assess the microbiome, metabolomics, genetic, demographic and dietary predictors of response to therapies for IBS. Predictors of response to a low FODMAPs diet will help inform a

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

RCT of low FODMAPs diet in IBS, and in particular we will evaluate whether responders are more likely to initially be taking a diet that is very rich in FODMAPs and how this response relates to their microbiome compositional and functional changes, affected by diets. A subgroup analysis will be performed of those between the ages of 13 to 17 compared to adult patients. We will also evaluate those with stable celiac disease and compare results to those without this disorder.

DISSEMINATION

Our approach to dissemination involves developing evidence-based knowledge translation initiatives for research that is ready for prime time – for each of category of our three research partners (patient, clinicians and policymakers). For patients, we plan to create a 'white label' version of the McMaster Optimal Aging Portal (https://www.mcmasteroptimalaging.org) that focuses specifically on supporting self-management and more generally informed decision-making for GI disease. This involves 1) identifying existing Evidence Summaries and preparing new ones to provide patients with the key messages from scientific research (typically high-quality systematic reviews) that's ready to be acted on; 2) identifying existing Web Resource Ratings and preparing new ones to help patients identify the free health resources on the internet that are based on scientific research; 3) identifying existing Blog Posts and preparing new ones that provide commentaries for patients about on what the scientific research on a topic actually means and on why good science matters; and 4) identifying existing patient decision aids to help patients (and clinicians working with patients) to engage in shared decision-making.

We will work with network partners (e.g. CDHF and CCC) to determine the best online platform for patient-focused products, to develop inclusion criteria, and to ensure that their online resources

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

focused on GI disease meet the high standard necessary to be captured and ranked highly in the Web Resource Ratings. We plan to prepare 52 new Evidence Summaries, 24 Blog Posts, and 400 Web Resources Ratings for the 'white label' portal. We will also be working with network partners to ensure high rates of use of the white-label, GI disease-focused content site for patients.

The patients, family members and caregivers who live with the chronic conditions covered by the IMAGINE Network will be able to use the portal to find information that aids them in managing their conditions and making related health decisions. The content produced by the McMaster Optimal Aging Portal has been shown to be effective in informing health consumers of quality heath information related to aging but not other domains. We plan to conduct a randomized controlled trial (which will be detailed in a separate protocol) to assess how the online resources for patients, provided through the white label website, changes patient behaviour in regards to using information and making evidence-informed health decisions.

For clinicians, we will follow an approach that emerged from a comprehensive review of the literature on scaling up effective clinical interventions (summarized in an evidence brief) (53) and a stakeholder dialogue involving the key policymakers, stakeholders and researchers focused on supporting scale up of effective clinical practices in Ontario (summarized in a dialogue summary) (54): 1) supporting dynamic efforts to identify GI disease-related clinical practices to be optimized and the causes of underlying problems, using both empirical approaches like systematic reviews and theoretical approaches like the Behaviour Change Wheel and the Theoretical Domains Framework; 2) using rigorous processes to select and implement approaches to optimizing clinical practices that address the underlying causes of problems (e.g., audit and feedback, financial incentives; and 3)

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

monitoring, evaluating and reviewing the approaches selected to optimize clinical practices. We plan to support two approaches: 1) prioritizing clinical interventions to be scaled up by engaging network members using an explicit process; and 2) scaling up effective clinical interventions by developing and executing a scale-up plan, both of which will build on what has been learned from related work at the McMaster Health Forum, and will rely on the frequent engagement of key members of the IMAGINE SPOR Network in doing so.

For policymakers, we will use our tried and tested approach to supporting evidence-informed policymaking, which means: 1) preparing an evidence brief on a pressing need for scale-up and the factors hindering that achievement (e.g., nurses' scope of practice, existing financial incentives, lack of multidisciplinary teams), options for scaling up, and key implementation considerations (which includes an equity lens brought to bear on particularly vulnerable groups); 2) convening a stakeholder dialogue that brings together key policymakers, patient and clinical leaders and researchers who can consider the research evidence alongside the other factors that drive decision-making; and 3) preparing a dialogue summary, disseminating the evidence brief and dialogue summary, conducting personalized briefings to senior leaders in the system, and supporting their efforts to act on what they learned.

We will enhance this approach by convening citizen panels to capture the insights and values of a diverse group of citizens, with different types of lived experience with the issue at hand, in a panel summary, the key messages of which would be included in the evidence brief informing the stakeholder dialogue. We plan to address two topics using this approach, one focused on reducing emergency-department usage in people with IBD, and one focused on framing the work led by

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

IMAGINE in the context of rapid learning health systems for specific conditions (including those addressed by the IMAGINE Network). For each topic we will conduct consultations with a steering committee and key informants to define the terms of reference for evidence briefs, identify stakeholders and potential dialogue invitees, and review the outputs (briefs, summaries, and evaluation reports).

ARTICLE SUMMARY

Strengths

Studies that have evaluated the microbiome in IBS (18) and IBD (19) have been small and underpowered. These studies usually did not evaluate disease phenotype in detail and have not assessed diet (18, 19), which can be an important effect modifier (55). This will be the largest observational study published to date that is sufficiently powered to evaluate the microbiome in IBS and IBD. All relevant confounding factors and effect modifiers will be captured and followed over time and longitudinally, which will allow a better understanding of what drives exacerbations of both IBS and IBD given that these are chronic relapsing and remitting diseases. The other strength of the study is the multidisciplinary team that makes up the IMAGINE Network. In particular, the collaboration between psychiatry and gastroenterology allows a careful evaluation of gut brain connections (17) and in particular how the gut microbiome (56) may impact on anxiety and depression in patients with IBD and IBS. Another key component of the multidisciplinary team is the level of patient engagement throughout the proposal. This is a Canadian Institute of Health Research funded grant through the SPOR initiative mandating that priorities are set by patients and they have input into study design. To date, we have 19 patient partners as part of the IMAGINE

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Network and many are involved in the MAGIC cohort study. This strengthens the research, making it more patient focused, and supports knowledge translation of the findings to patients.

Limitations

This study is observational so any associations found may not relate to the causes of IBS and IBD. We will rigorously control for confounding factors and but cannot control for unknown confounders and so cannot draw causal inferences from the data. The MAGIC study will therefore be hypothesis generating and any data relating to the microbiome or diet is likely to need confirmation in randomized controlled trials (RCTs). The IMAGINE Network is committed to develop RCTs to further investigate any promising findings from the MAGIC cohort study. This is the largest study evaluating IBS and IBD, but the data that will be collected is enormous and so any results related to the secondary outcomes of the study need to be interpreted with caution. The adjustment for multiple testing is for the primary outcomes only and given the number of other outcomes that can be evaluated with the data that is generated, it is important to realize that any positive results from the secondary outcomes could be a chance finding related to multiple testing. We will highlight this when reporting the data of more exploratory outcomes being studied. Despite these caveats, the MAGIC study will provide valuable insight into the etiology of IBS and IBD as well as associated psychiatric disorders. Data from this study will also provide strategies for personalized medicine approaches to manage these diseases more effectively.

ACKNOWLEDGEMENTS

We are grateful to Glenda MacQueen for all the support she gave to the IMAGINE Network and the MAGIC study. Her advice was invaluable throughout this project and she will be missed by all of

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

us. We are grateful to all our patient partners and everyone within the IMAGINE Network (see Appendix 1).

FUNDING SOURCES

The IMAGINE Network is supported by a grant from the Canadian Institute of Health Research (Funding Reference Number: 1715-000-001) with funding from several partners. Funding was matched by McMaster University, University of Calgary, University of Alberta, Queen's University, Dalhousie University, Montreal Heart Institute Research Centre, Takeda Pharmaceutical Company, Allergan Incorporated, Alberta Innovates, Research Manitoba, Crohn's and Colitis Canada.

DISCLOSURES

Dr. Moayyedi holds the Audrey Campbell Chair in Ulcerative Colitis Research. Drs. Moayyedi, Bercik and Aida Fernandes have no conflicts of interest.

Dr. Bernstein is supported in part by the Bingham Chair in Gastroenterology. He is on Advisory Boards for AbbVie Canada, Janssen Canada, Takeda Canada, Pfizer Canada. He is a Consultant for Mylan Pharmaceuticals. He is receiving educational grants from AbbVie Canada, Pfizer Canada, Shire Canada, Takeda Canada, Janssen Canada. Speaker's panel for AbbVie Canada, Janssen Canada, Takeda Canada, and Medtronic Canada. Received research funding from AbbVie Canada.

Dr. Rioux receives research funding from Pfizer Inc.

Dr. Dieleman is on the Advisory Boards for Janssen Canada, AbbVie Canada, Pfizer Canada and Takeda Canada.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Dr. Marshall holds a Canada Research Chair (2008-18) and the Arthur J.E. Child Chair and receives travel reimbursement through Illumina for meetings of the Global Economics and Evaluation of Clinical Genomics Sequencing Working Group.

Dr. Vanner was supported by an Educational grant from Allergan.

Sandra Zelinsky is a patient research partner and received a grant from Takeda Canada.

Data Statement

Technical appendix, statistical code, and dataset will be available from a repository (e.g. Dryad data repository) once follow up is complete and main analyses have been published.

Author Statement & Contact Information

All authors have contributed to the conception and design of the protocol. Paul Moayyedi and Aida Fernandes constructed the first draft of the article which was significantly revised by all other authors. All authors have given final approval of the version submitted for publication. Paul Moayyedi acts as guarantor for the article.

Author	Contact Information
Corresponding Author: Paul Moayyedi	1280 Main St. W. HSC 3V3 Hamilton, ON Canada, L8S 4K1 (905) 521 2100 x.76764 moayyep@mcmaster.ca
Glenda MacQueen	Post-humous
Charles N Bernstein	Charles.Bernstein@umanitoba.ca
Stephen Vanner	vanners@hdh.kari.net
Premysl Bercik	bercikp@mcmaster.ca
Karen L Madsen	karen.madsen@ualberta.ca
Michael G Surette	surette@mcmaster.ca
John D Rioux	john.rioux@inflammgen.org
Levinus A. Dieleman	l.dieleman@ualberta.ca
Elena F Verdu	verdue@mcmaster.ca
Russel De Souza	rdesouz@mcmaster.ca
Antony Otley	anthony.otley@dal.ca
Laura Targownik	Laura.Targownik@sinaihealth.ca

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

John Lavis	lavisj@mcmaster.ca
Deborah Marshall	damarsha@ucalgary.ca
Sandra Zelinsky	sandraz.per@gmail.com
Aida Fernandes	fernaa19@mcmaster.ca

Word Count: 5,443

References

1. Thompson WG, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional Gastrointestinal Disease in Canada. Dig Dis Sci 2002; 47: 225-35.
2. Sperber AD, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, Whitehead WE, Dumitrascu DL, Fang X, Fukudo S, Kellow J, Okeke E, Quigley EM, Schmulson M, Whorwell P, Archampong T, Adibi P, Andresen V, Benninga MA, Bonaz B, Bor S, Fernandez LB, Choi SC, Corazziari ES, Francisconi C, Hani A, Lazebnik L, Lee YY, Mulak A, Rahman MM, Santos J, Setshedi M, Syam AF, Vanner S, Wong RK, Lopez-Colombo A, Costa V, Dickman R, Kanazawa M, Keshteli AH, Khatun R, Maleki I, Poitras P, Pratap N, Stefanyuk O, Thomson S, Zeevenhooven J, Palsson OS. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. Gastroenterology. 2020 Apr 12. pii: S0016-5085(20)30487-X. doi: 10.1053/j.gastro.2020.04.014. [Epub ahead of print]
3. Ng SC, Shi HY, Hamidi N et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017; 390: 2769-78.
4. Brandt LJ, Chey WD, Foxx-Orenstein AE, Quigley EMM, Schiller LR, Schoenfeld PS, Spiegel BM, Talley NJ, Moayyedi P. An evidence-based position statement on the

For peer review only

management of irritable bowel syndrome. American Journal of Gastroenterology 2009; 104 (suppl 1): S1-S36.

5. Talley NJ, Abreu MT, Achkar JP, Bernstein CN, Dubinsky MC, Hanauer SB, Kane SV, Sandborn WJ, Ullman TA, Moayyedi P. An evidence-based systematic review on medical therapies in inflammatory bowel disease. Am J Gastroenterol 2011: 106 supp 1: S2-S25.
6. Gralnek IM, Hays RD, Kilbourne A, Naliboff B, Mayer EA. The Impact of Irritable Bowel Syndrome on Health-Related Quality of Life. Gastroenterology 2000; 119: 654-60.
7. Casellas F, Lopez-Vivancos J, Badia X et al. Influence of inflammatory bowel disease on different dimensions of quality of life. Eur J Gastroenterol Hepatol 2001;13:567–72.
8. Bernstein CN, Loftus EV, Ng SC, Lakatos PL, Moum B. Hospitalisations and surgery in Crohn's disease. Gut 2012; 61: 622-9.
9. Peery AF, Crockett SD, Barritt AS, Dellon ES, Eluri S, Gangarosa LM, Jensen ET, Lund JL, Pasricha S, Runge T, Schmidt M, Shaheen NJ, Sandler RS. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States Gastroenterology 2015; 149:1731–1741.
10. Lee C, Doo E, Choi JM, et al. The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis. J Neurogastroenterol Motil. 2017;23(3):349–362. doi:10.5056/jnm16220
11. Mikocka-Walus A, Knowles SR, Keefer L, Graff L. Controversies Revisited: A Systematic Review of the Comorbidity of Depression and Anxiety with Inflammatory Bowel Diseases, *Inflammatory Bowel Diseases*, Volume 22, Issue 3, 1 March 2016; 752–762.
12. Jones R, Latinovic R, Charlton J, Gulliford M. Physical and psychological co-morbidity in irritable bowel syndrome: a matched cohort study using the General Practice Research Database. Aliment Pharmacol Therap 2006; 24: 879-86.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

13. Roy-Byrne PP, Davidson KW, Kessler RC, Asmundson GJ, Goodwin RD et al. Anxiety disorders and comorbid medical illness. Gen Hosp Psych 2008; 30: 208-25.

14. Ford AC, Moayyedi P, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BMR, Quigley EMM. American College of Gastroenterology Monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. American Journal of Gastroenterology 2014; 109: S1-S26.

15. Moayyedi P, Simren M, Bercik P. Evidence-based and mechanistic insights into exclusion diets for IBS. Nature Reviews Gastroenterology & Hepatology 2020 epub DOI: 10.1038/s41575-020-0270-3.

16. Moayyedi P. Update on fecal microbiota transplantation in patients with inflammatory bowel disease. Gastroenterology and Hepatology 2018; 14: 319-22.

17. MacQueen G, Surette M, Moayyedi P. The gut microbiome and psychiatric illness. Journal of Psychiatry and Neuroscience 2017; 42: 75-7.

18. Pittayanon R, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, Moayyedi P. Gut microbiota in patients with irritable bowel syndrome – a systematic review. Gastroenterology 2019; 157: 97-108.

19. Pittayanon R, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, Moayyedi P. Gut microbiota in patients with inflammatory bowel disease – a systematic review. Gastroenterology 2020; in press.

20. www.imaginespor.com

21. Palsson OS, Whitehead WE, van Tilburg MAL, Chang L, Chey W, Crowell MD, Keefer L, Lembo AJ, Parkman HP, Rao SSC, Sperber A, Spiegel B, Tack J, Vanner S, Walker LS,

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Whorwell P, Yang Y; Development and Validation of the Rome IV Diagnostic Questionnaire for Adults. Gastroenterology 2016; 150, Issue 6: 1481-1491.

22. Satsangi J, Silverberg MS, Vermeire S, Colombel J-F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006; 55: 749-53.

23. Levine A, Griffiths AM, Markowitz J, Wilson D, Turner D, Russell R, Fell J, Ruemmele F, Walters T, Sherlock M, Dubinsky M, and Hyams JS. Pediatric Modification of the Montreal Classification for Inflammatory Bowel Disease - the Paris Classification. Inflamm Bowel Dis 2011: 17: 1314-21.

24. Ruemmele FM, Hyams JS, Otley A, Griffiths A, Kolho KL, Dias JA, Levine A, Escher JC, Taminiau J, Veres G, Colombel JF, Vermeire S, Wilson DC, Turner D. Outcome measures for clinical trials in paediatric IBD: an evidence-based, expert-driven practical statement paper of the paediatric ECCO committee. Gut. 2015 Mar;64(3):438-46.

25. Shklarov S, Marshall DA, Wasylak T, Marlett NJ. "Part of the Team": Mapping the outcomes of training patients for new roles in health research and planning. Health Expectations, Dec 2017;20:1428-1436

26. Marlett N, Shklarov S, Marshall DA, Santana MJ, Wasylak T. Building New Relationships in Research: A Model of Patient Engagement Research. Qual Life Res 2015 May;24(5):1057-67.

27. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, Armstrong D, Marshall JK, Kassam Z, Reinisch W, Lee CH. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology 2015; 149: 102-9.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

28. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002; 13: 3-9.

29. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willet WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr 2000; 72: 912-2.

30. Northstone K, Ness AR, Emmett PM, Rogers IS. Adjusting for energy intake in dietary pattern investigations using principal components analysis. Eur J Clin Nutr 2006; 62: 931-8. 113.

31. Lau JT, Whelan FJ, Herath I, Lee CH, Collins SM, Bercik P, Surette MG. Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. Genome Med. 2016 Jul 1;8(1):72.

32. Woo TE1,2, Lim R2, Heirali AA3, Acosta N3, Rabin HR2,3, Mody CH, Somayaji R, Surette MG, Sibley CD, Storey DG, Parkins MD. A longitudinal characterization of the Non-Cystic Fibrosis Bronchiectasis airway microbiome. Sci Rep. 2019 May 3;9(1):6871. doi: 10.1038/s41598-019-42862-y.

33. Xu J. Fungal DNA barcoding. Genome. 2016 Nov;59(11):913-932. Epub 2016 Aug 30.

34. Whelan FJ1, Surette MG2,3. A comprehensive evaluation of the sl1p pipeline for 16S rRNA gene sequencing analysis. Microbiome. 2017 Aug 14;5(1): 100.

35. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 Jul;13(7):581-3. doi: 10.1038/nmeth.3869. Epub 2016 May 23.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

36. Schmieder R, Edwards R. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. PLoS One. 2011 Mar 9;6(3):e17288. doi: 10.1371/journal.pone.0017288.

37. Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, Lipson KS, Knight R, Caporaso JG, Segata N6, Huttenhower C. Species-level functional profiling of metagenomes and metatranscriptomes.Nat Methods. 2018 Nov;15(11):962-968. doi: 10.1038/s41592-018-0176-y. Epub 2018 Oct 30.

38. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods. 2012 Jun 10;9(8):811-4. doi: 10.1038/nmeth.2066.

39. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data.PLoS One. 2013 Apr 22;8(4):e61217. doi: 10.1371/journal.pone.0061217. Print 2013.

40. Qu K, Guo F, Liu X, Lin Y, Zou Q. Application of Machine Learning in Microbiology. Front Microbiol. 2019 Apr 18;10:827. doi: 10.3389/fmicb.2019.00827. eCollection 2019.

41. Camacho DM1, Collins KM2, Powers RK3, Costello JC4, Collins JJ5. Next-Generation Machine Learning for Biological Networks. Cell. 2018 Jun 14;173(7):1581-1592. doi: 10.1016/j.cell.2018.05.015. Epub 2018 Jun 7.

42. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S, Fung C, Nikolai L, Lewis M, Coutouly MA, Forsythe I, Tang P, Shrivastava S, Jeroncic K, Stothard P, Amegbey G, Block D, Hau DD, Wagner J, Miniaci J, Clements M, Gebremedhin M, Guo N, Zhang Y, Duggan GE, Macinnis GD, Weljie AM, Dowlatabadi R, Bamforth F, Clive D, Greiner R, Li L, Marrie T, Sykes BD, Vogel HJ, Querengesser L.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007 Jan; 35(Database issue): D521-6.

43. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel JF, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RS, Floyd JA, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot JP, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra HJ, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet. 2011 Mar;43(3):246-52. doi: 10.1038/ng.764. Epub 2011 Feb 6.

44. Rivas MA, Avila BE, Koskela J, Huang H, Stevens C, Pirinen M, Haritunians T, Neale BM, Kurki M, Ganna A, Graham D, Glaser B, Peter I, Atzmon G, Barzilai N, Levine

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

AP, Schiff E, Pontikos N, Weisburd B, Lek M, Karczewski KJ, Bloom J, Minikel EV, Petersen BS, Beaugerie L14, Seksik P, Cosnes J, Schreiber S, Bokemeyer B, Bethge J; International IBD Genetics Consortium; NIDDK IBD Genetics Consortium; T2D-GENES Consortium, Heap G, Ahmad T, Plagnol V, Segal AW, Targan S, Turner D, Saavalainen P, Farkkila M, Kontula K, Palotie A, Brant SR, Duerr RH, Silverberg MS, Rioux JD, Weersma RK, Franke A, Jostins L, Anderson CA, Barrett JC, MacArthur DG, Jalas C, Sokol H, Xavier RJ, Pulver A, Cho JH, McGovern DPB, Daly MJ. Insights into the genetic epidemiology of Crohn's and rare diseases in the Ashkenazi Jewish population. LoS Genet. 2018 May 24;14(5):e1007329. doi: 10.1371/journal.pgen.1007329. eCollection 2018 May.

45. Huang H, Fang M, Jostins L, Umićević Mirkov M, Boucher G, Anderson CA, Andersen V, Cleynen I, Cortes A, Crins F, D'Amato M, Deffontaine V, Dmitrieva J, Docampo E, Elansary M, Farh KK, Franke A, Gori AS, Goyette P, Halfvarson J, Haritunians T, Knight J, Lawrance IC, Lees CW, Louis E, Mariman R, Meuwissen T, Mni M, Momozawa Y, Parkes M, Spain SL, Théâtre E, Trynka G, Satsangi J, van Sommeren S, Vermeire S, Xavier RJ; International Inflammatory Bowel Disease Genetics Consortium, Weersma RK, Duerr RH, Mathew CG, Rioux JD, McGovern DPB, Cho JH, Georges M, Daly MJ, Barrett JC. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature. 2017 Jul 13;547(7662):173-178. doi: 10.1038/nature22969. Epub 2017 Jun 28.

46. Cleynen I, Boucher G, Jostins L, Schumm LP, Zeissig S, Ahmad T, Andersen V, Andrews JM, Annese V, Brand S, Brant SR, Cho JH, Daly MJ, Dubinsky M, Duerr RH, Ferguson LR, Franke A, Gearry RB, Goyette P, Hakonarson H, Halfvarson J, Hov JR, Huang H, Kennedy NA, Kupcinskas L, Lawrance IC, Lee JC, Satsangi J, Schreiber S, Théâtre

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

E, van der Meulen-de Jong AE, Weersma RK, Wilson DC; International Inflammatory Bowel Disease Genetics Consortium, Parkes M, Vermeire S, Rioux JD, Mansfield J, Silverberg MS, Radford-Smith G, McGovern DP, Barrett JC, Lees CW. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet. 2016 Jan 9;387(10014):156-67. doi: 10.1016/S0140-6736(15)00465-1. Epub 2015 Oct 18.

47. Goyette P, Boucher G, Mallon D, Ellinghaus E, Jostins L, Huang H, Ripke S, Gusareva ES, Annese V, Hauser SL, Oksenberg JR, Thomsen I, Leslie S; International Inflammatory Bowel Disease Genetics Consortium; Australia and New Zealand IBDGC; Belgium IBD Genetics Consortium; Italian Group for IBD Genetic Consortium; NIDDK Inflammatory Bowel Disease Genetics Consortium; United Kingdom IBDGC; Wellcome Trust Case Control Consortium; Quebec IBD Genetics Consortium, Daly MJ, Van Steen K, Duerr RH, Barrett JC, McGovern DP, Schumm LP, Traherne JA, Carrington MN, Kosmoliaptsis V, Karlsen TH, Franke A, Rioux JD. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet. 2015 Feb;47(2):172-9. doi: 10.1038/ng.3176. Epub 2015 Jan 5.
48. Vukcevic D, Traherne JA, Næss S, Ellinghaus E, Kamatani Y, Dilthey A Lathrop M, Karlsen TH, Franke A, Moffatt M, Cookson W, Trowsdale J, McVean G, Sawcer S, Leslie S. Imputation of KIR Types from SNP Variation Data. Am J Hum Genet. 2015 Oct 1;97(4):593-607. doi: 10.1016/j.ajhg.2015.09.005.
49. Gourraud PA, Khankhanian P, Cereb N, Yang SY, Feolo M, Maiers M, Rioux JD, Hauser S, Oksenberg J. HLA diversity in the 1000 genomes dataset. PLoS One. 2014 Jul 2;9(7):e97282. doi: 10.1371/journal.pone.0097282. eCollection 2014.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

50. Das S, Abecasis GR, Browning BL. Genotype Imputation from Large Reference Panels. Annu Rev Genomics Hum Genet. 2018 Aug 31;19:73-96. doi: 10.1146/annurev-genom-083117-021602. Epub 2018 May 23.

51. Ivison S, Des Rosiers C, Lesage S, Rioux JD, Levings MK. Biomarker-guided stratification of autoimmune patients for biologic therapy. Curr Opin Immunol. 2017 Dec;49:56-63. doi: 10.1016/j.coi.2017.09.006. Epub 2017 Oct 17.

52. Gao, X.; Song, P. (2010). "Composite likelihood Bayesian information criteria for model selection in high-dimensional data". Journal of the American Statistical Association. 105 (492): 1531–1540. doi:10.1198/jasa.2010.tm09414.

53. Lavis JN, Wilson MG, Grimshaw JM. Evidence Brief: Optimizing Clinical Practice in Ontario Based on Data, Evidence and Guidelines. Hamilton, Canada: McMaster Health Forum, 6 March 2015.

54. Wilson MG, Lavis JN. Dialogue Summary: Optimizing Clinical Practice in Ontario Based on Data, Evidence and Guidelines. Hamilton, Canada: McMaster Health Forum, 6 March 2015.

55. McIntosh K, Reed DE, Schneider T, Dang F, Keshteli AH, De Palma G, Madsen K, Bercik P, Vanner S. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut 2017; 66: 1241-1251.

56. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. Neurogastroenterol Motil 2012; 24: 405-13.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 1: Eligibility criteria

Type	Inclusion Criteria	Exclusion Criteria
IBD	Patients with documented CD, UC or IBD-U, >4 years old	• subtotal colectomy and/or ileostomy patients • major comorbid condition where the projected survival is less than 5 years • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder
IBS	Patients with IBS who have met Rome IV criteria, ≥13 years old **IBS-D** • Normal CBC • Negative tissue transglutaminase antibody if diarrhea the main symptom • Symptoms onset > 45 years old, then negative colonic biopsies for microscopic colitis **IBS-C, IBS-M & IBS-U** • Negative tissue transglutaminase antibody • Symptoms onset > 50 years age, with new symptoms < 1 year duration, then have a negative colonoscopy, CT colonography or Air Contrast Barium Enema. • Normal CBC	• major gastrointestinal surgery (Roux en y, bowel resection), • major comorbid condition, where the projected survival is less than 5 years • drug use that is the major cause of GI symptoms and/or undermines longitudinal compliance, including chronic antibiotic use, narcotic analgesics and substance abuse • narcotic analgesic use causing GI symptoms • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder • GI cancer within 5 years
Healthy Controls	No gastrointestinal symptoms using the ROME IV Questionnaire	• major gastrointestinal surgery (Roux en y, bowel resection) • any major comorbid chronic condition • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder

Table 2: Participant Information Collection

Contact	Screening period		12-month visit	24-month visit	36-month visit	48-month visit
Purpose	**Enrol in Study**		Health status Biosamples Questionnaires	Health status Biosamples Questionnaires	Health status Biosamples, Questionnaires	Health status Biosamples Questionnaires
Information collected	Contact by telephone, email or clinic visit. Explain project Set up study visit(s)	Signed consent Eligibility screening Demographics Medical history Provide urine & stool kit Obtain blood (+DNA) Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 3: List of Patient-answered Questionnaires

SUBGROUP	ADULTS	PEDIATRICS
IBS	• Demographic Questionnaire	• Demographics (exclusions apply)
	Disease Specific	
	• IBS Severity Score	• IBS Severity Score • Rome IV Diagnostic Questionnaire
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) - Age 5-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15 • Euro Quality of Life 5 Level - Age 16 +
	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire
IBD	• Demographic Questionnaire	• Demographics (exclusions apply)
	Disease Specific	

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

	• Short IBD Symptom Inventory	• IMPACT – III - Ages 9-17 • PUCAI (UC); PCDAI (CD)
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) - Age 4-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15
	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire
Healthy controls	• Demographic Questionnaire	• Demographics (exclusions apply)
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) Age 5-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15 • Euro Quality of Life 5 Level - Age 16 +

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire

For peer review only

APPENDIX 1: IMAGINE MEMBERS (alphabetically)

Hamilton:
Premsyl Bercik
John Bienenstock
Stephen Collins
Russel de Souza
Aida Fernandes (Executive Director)
Benicio Frey
Khurram Khan
John Lavis
Paul Moayyedi (PI)
Nikil Pai
Mary Sherlock
Mike Surette
Elena Verdu

Calgary:
Christopher Andrews
Paul Beck
Humberto Jijon
Gilaad G. Kaplan
Glenda MacQueen (post-humous)
Deborah Marshall
Yasmin Nasser
Remo Panaccione
Cynthia Seow
Valerie Taylor

Winnipeg:
Charles Bernstein
Wael El-Matary
Patricia Furer
Jean-Eric Ghia
Lesley Graff
Harminder Singh
John Walker (post-humous)

Edmonton:
Leo Dieleman
Richard Fedorak (post-humous)
Hien Huynh
Dina Kao
Karen Madsen

For peer review only

Puneeta Tandon
Eytan Wine

Kingston:
David Reed
Dean Tripp
Stephen Vanner

Halifax:
Jennifer Jones
Anthony Otley
Johan Van Limbergen

Montreal:
Alain Bitton
Justin Cote-Daigneault
Christophe Faure
Peter Lakatos
John Rioux
Sacha Sidani

Toronto:
Ken Croitoru
Louis Liu
Jeff Meyer
Geoffrey Nguyen
Laura Targownik

Ottawa:
Eric Benchimol
Sanjay Murthy

St John's:
Mark Borgaonkar

Vancouver:
Brian Bressler

Patient Research Partners:
Gail Bellissimo
Sara Blake
Kim Daley
Alysia DeNino
Anny Fernandez

For peer review only

Shauna Jones
Ellen Keunzig
Sophie LeBlanc
Nadine Mackay
Lisa MacNeil
Megan Marsiglio
Kate Mason
Adhiyat Najam
Emma Neary
Shawn Reynolds
Perry Steckly
Amy Van Engelen
Karthika Yogaratnam
Sandra Zelinsky

For peer review only

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-041733.R1
Article Type:	Protocol
Date Submitted by the Author:	05-Sep-2020
Complete List of Authors:	Moayyedi, Paul; McMaster University Faculty of Health Sciences, Medicine MacQueen, Glenda; University of Calgary Bernstein, Charles; University of Manitoba, Gastroenterology Vanner, Stephen; Queen's University Bercik, Premysl; McMaster University Faculty of Health Sciences, Medicine Madsen, Karen; University of Alberta Surette, Michael; McMaster University Faculty of Health Sciences Rioux, John; Universite de Montreal Dieleman, Levinus; University of Alberta Faculty of Medicine and Dentistry Verdú, Elena; McMaster University Faculty of Health Sciences, Division of Gastroenterology de Souza, Russell; McMaster University Faculty of Health Sciences, Department of Clinical Epidemiology & Biostatistics Otley, Anthony; Dalhousie University Targownik, Laura; Mount Sinai Hospital Lavis, John; McMaster University Faculty of Health Sciences Cunningham, Jennifer; McMaster University Faculty of Health Sciences, Population Health Research Institute Marshall, Deborah; University of Calgary, Department of Community Health Sciences Zelinsky, Sandra; University of Calgary, PaCER Innovates Fernandes, Aida; McMaster University Faculty of Health Sciences, Medicine
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Genetics and genomics, Epidemiology, Mental health, Nutrition and metabolism, Immunology (including allergy)
Keywords:	Inflammatory bowel disease < GASTROENTEROLOGY, Functional bowel disorders < GASTROENTEROLOGY, Depression & mood disorders < PSYCHIATRY, Microbiology < NATURAL SCIENCE DISCIPLINES, Nutritional support < GASTROENTEROLOGY

SCHOLARONE™
Manuscripts

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

For peer review only

IMAGINE Network's Mind And Gut Interactions Cohort (MAGIC) Study:
A protocol for a prospective observational multicentre cohort study in Inflammatory Bowel Disease & Irritable Bowel Syndrome

Authors:
Paul Moayyedi[1], Glenda MacQueen (post-humous)[2], Charles N Bernstein[3], Stephen Vanner[4], Premysl Bercik[1], Karen L Madsen[5], Michael G Surette[1], John D Rioux[6,7], Levinus A. Dieleman[5], Elena F Verdu[1], Russell J. de Souza[1], Anthony Otley[8], Laura Targownik[9], John Lavis[10], Jennifer Cunningham[11], Deborah Marshall[2], Sandra Zelinsky[2], Aida Fernandes[1,2], on behalf of the IMAGINE Network

1. Farncombe Family Digestive Health Institute, McMaster University, Hamilton, ON, Canada
2. University of Calgary, AB, Canada
3. University of Manitoba, Winnipeg, MB, Canada
4. Queen's University, Kingston, ON, Canada
5. University of Alberta, Edmonton, AB, Canada (bercikp@mcmaster.ca)
6. Université de Montréal, Montréal, QC, Canada
7. Montreal Heart Institute, Montreal, QC, Canada
8. Dalhousie University, Halifax, NS, Canada
9. University of Toronto, ON, Canada
10. McMaster Health Forum, McMaster University, Hamilton, ON, Canada
11. Population Health Research Institute, Hamilton Health Sciences, McMaster University, Hamilton, On, Canada

Corresponding Author:
Paul Moayyedi
McMaster University Faculty of Health Sciences, Medicine
Hamilton, ON, CAN
Tel: 1 905 521 2100 x 76764
Email: moayyep@mcmaster.ca

Key Words:
Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, prospective observational cohort, microbiome, diet, genetic, microbiome, metabolome

ClinicalTrials.gov Identifier: NCT03131414

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

ABSTRACT (reduce to 250 words)

Introduction: Gut microbiome and diet may be important in irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and comorbid psychiatric conditions but the mechanisms are unclear. We will create a large cohort of patients with IBS, IBD and healthy controls and follow them over time, collecting dietary and mental health information and biological samples, to assess their gastrointestinal (GI) and psychological symptoms in association with their diet, gut microbiome and metabolome.

Methods and Analysis: This five-year observational prospective cohort study is recruiting 8000 participants from 15 Canadian centers. Persons with IBS who are 13 years of age and older or IBD ≥ 5 years will be recruited. Healthy controls will be recruited from the general public and from friends or relatives of those with IBD or IBS who do not have GI symptoms. Participants answer surveys and provide blood, urine, and stool samples annually. Surveys assess disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet. The main outcomes evaluated will be the association between the diet, inflammatory, genetic, microbiome and metabolomic profiles in those with IBD and IBS compared with healthy controls using multivariate logistic regression. We will also compare these profiles in those with active versus quiescent disease and those with and without psychological comorbidity.

Ethics and Dissemination: Approval has been obtained from the institutional review boards of all centres taking part in the study. We will develop evidence-based knowledge translation initiatives for patients, clinicians and policy-makers to disseminate results to relevant stakeholders.

For Peer Review Only

Strengths and Limitations

- This is the largest observational study evaluating the microbiome in inflammatory bowel disease and irritable bowel syndrome.
- The patients' disease type and activity are well characterized with detailed information on diet and mental health.
- The degree of patient engagement is another strength of the study.
- The microbiome and diet assessment are conducted once per year and may not correlate with disease flare-ups.
- As with all observational studies, any association may not be causal and will need evaluation in randomized controlled trials.

INTRODUCTION

Two thirds of the population experience significant gastrointestinal (GI) symptoms at some point in their life (1). One of the most common GI disorders is irritable bowel syndrome (IBS), affecting up to 10% of persons worldwide, depending on the definition (2). Another GI disorder that is associated with significant health care resources is inflammatory bowel disease (IBD) which affects approximately 0.3% of the world's industrialized population (3).

The cardinal features of IBS include chronic abdominal pain over 3 months per year related to a change in bowel habit and the disease can present at any age (2,4). IBD is a term encompassing two distinct but related diseases: ulcerative colitis (UC) and Crohn's disease (CD). The cardinal symptom of UC is bloody diarrhea whilst in CD abdominal pain is a more prominent symptom, but

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

diarrhea is a feature as well (5). IBD has the highest incidence in second and third decades of life and are lifelong relapsing and remitting diseases. Growth impairment can be an issue in children with CD.

Both IBS and IBD significantly impact quality of life (6, 7) and often surgery is needed in patients with IBD (8). IBS and IBD also account for significant health care spending in the developed world with many countries spending billions of dollars per annum (9). Persons with IBS and IBD have higher rates of anxiety and depression compared to the general population (10, 11) and those with other chronic diseases (12). The corollary is also true; persons with anxiety and depression have more GI symptoms compared to healthy controls (13). Therapy for IBS has traditionally focused on drugs that alter motility or visceral sensitivity of the GI tract and although various interventions are superior to placebo, the overall impact on symptoms is only modest (14). Therapy for IBD has traditionally focused on drugs that inhibit the exaggerated pro-inflammatory immune response, however only 50% of the patients achieve clinical remission, whereas clinical relapses are common.

There is evidence that the gut microbiome and diet are important in IBS (15), IBD (16), and comorbid psychiatric conditions (17). There is however a need for more longitudinal prospective data on this interaction in patients with IBD and IBS compared with healthy controls. We have conducted systematic reviews in both IBS (18) and IBD (19); although there are numerous case control studies exploring the gut microbiome in these conditions, the median sample size is around 20 per arm and in all cases the sample size was insufficient to deal with the multiple testing issues that relate to microbiome research in humans. In addition, inter-individual differences of the gut microbiome are large. Large sample sizes and longitudinal sampling within the same individuals

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

over time are therefore needed to evaluate the interaction between diet, the microbiome, IBS, IBD and associated mental health issues.

The Inflammation, Microbiome, and Alimentation: Gastro-Intestinal and Neuropsychiatric Effects (IMAGINE) (20) (see Appendix 1) Strategy for Patient Oriented Research (SPOR) Network is conducting a five-year multicenter prospective observational cohort study, Mind And Gut Interactions Cohort (MAGIC). It will explore the interaction between the diet, microbiome and the host associated with IBS and IBD in order to better target treatment of IBD and IBS and the psychiatric disorders associated and affecting these diseases. The overarching hypothesis of this study is that IBS and IBD are driven by a perturbation of the gut microbiome and the associated host immune response. Alterations in the gut microbiome may also drive anxiety and depression associated with these GI disorders and these psychological factors may in turn influence gut symptoms and its microbiome. These mechanisms may also have a genetic predisposition.

Aims

The main aim of the MAGIC study conducted through the IMAGINE SPOR Network is to create a large cohort of patients with IBS, IBD and healthy controls and follow these individuals over time, assessing disease activity, diet, mental health and demographic information using validated questionnaires and collecting annual stool, urine and blood samples, to correlate GI and psychological symptoms with an individual's genetic variants, diet, and gut microbiome, as well host and microbiome metabolic products in stool, urine and serum.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Primary aims for baseline data

1. We will compare the gut microbiome and metabolomic profile between CD, UC, IBS and healthy controls. The main analyses will be between a specific disorder and healthy controls.

2. We will compare the gut microbiome and metabolomics profile of participants with active versus quiescent disease within CD, UC and IBS.

3. We will compare the gut microbiome and metabolomic profile of participants with and without psychiatric co-morbidity for each of CD, UC, IBS and healthy controls.

Primary aims for longitudinal data

1. Microbiome, metabolomic, genetic, inflammatory markers, dietary, disease phenotype, psychiatric comorbidity and demographic predictors of failure of therapy for each of UC, CD, and IBS.

2. Comparison of microbiome, metabolomic, genetic, dietary and demographic factors in IBD of participants who remain in clinical remission over 2 years versus those with recurrent active disease (UC and CD analyzed separately).

3. Comparison of gut microbiome, metabolomic, genetic, dietary and demographic factors in participants with IBS with mild/inactive disease (based on IBS-Symptom Severity Score (SSS)) compared with those with ongoing active disease. IBS will be evaluated overall and also within subgroups independently.

Secondary aims for baseline data

1. To compare dietary patterns between patients with CD, UC, IBS and healthy controls.

2. To compare genetic risk factors between CD, UC, IBS and healthy controls.

3. To compare gut microbiome, metabolomic, genetic, dietary and demographic factors in

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

participants with IBD with quiescent inflammatory disease with and without concomitant IBS (defined by fecal calprotectin <50 mcg/g and subthreshold IBD symptom activity score but who have active IBS symptoms on IBS-SSS).

4. To compare the prevalence of mood and anxiety disorders in participants with each disorder against rates in healthy controls.

5. To compare the dietary, gut microbiome and metabolomics profile between participants with or without anxiety (CD, UC, IBS, healthy controls analyzed separately).

6. To compare the dietary, gut microbiome and metabolomics profiles between participants with and without depression (CD, UC, IBS, healthy controls analyzed separately).

7. To determine whether high rates of early adverse experiences are associated with history of more severe disease or treatment resistance in participants with CD, UC or IBS.

8. To examine the association between symptom severity and multiple domains of function in participants with CD, UC or IBS.

9. To develop models describing how factors such as microbiome, metabolome, diet, genes and psychiatric symptoms interact in CD, UC, and IBS.

Secondary aims for longitudinal data

1. To compare health care resource use between CD, UC, IBS and healthy controls.

2. To compare work productivity between CD, UC, IBS and healthy controls.

3. To compare baseline dietary and gut microbiome and urinary metabolome profiles and inflammatory markers in participants that develop anxiety during follow up versus those who did not have anxiety at any time (CD, UC, IBS and healthy controls analyzed separately).

4. To compare baseline dietary and gut microbiome and urinary metabolome profiles in participants

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

that develop depression during follow up versus those that did not have depression at any time (CD, UC, IBS and healthy controls analyzed separately and if appropriate combined).

7. To compare gut microbiome, urinary metabolome, genetic, dietary and demographic factors in IBS at baseline in those that change their IBS subtype during follow up and those that continue with the same IBS subgroup.

8. To compare which dietary, mental health, gut microbiome and metabolomics profiles precede a clinical relapse in patients with IBD and how these parameters are different in patients with active disease versus those who remain in clinical remission.

METHODS

Design

The IMAGINE MAGIC study is a prospective observational cohort study that is recruiting 2000 participants with each of CD, UC, and IBS and also 2000 healthy participants in 15 centers across Canada. Assessment includes psychological status, dietary intake, gut microbiome, urinary metabolomic profile, inflammatory markers, genotype, health-related quality of life, and health care resource use and associated costs. The cohort and healthy controls will be followed annually for up to 4 years after the baseline study enrollment.

At each visit, the participant provides blood, urine, and stool samples as well as complete questionnaires assessing disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet. Table 1 summarizes participant information collected at each visit.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Participants

A total of 8000 participants will be recruited, 2000 for each for healthy volunteers, IBS, UC and CD. Inclusion and exclusion criteria are outlined in Table 1.

Healthy participants over the age of 4 will be recruited from the relatives, spouses and friends of IBS and IBD cases taking part and also through advertisement for healthy volunteers. IBS and IBD patients will primarily be recruited from gastroenterology clinics at participating centres.

IBS: Persons who meet Rome IV criteria and are 13 years of age or older are enrolled (Table 1). Persons with IBS are categorized into diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), IBS with mixed bowel habits (IBS-M), or unclassified with IBS (IBS-U) (21). Patients with IBS that also have celiac disease will be eligible provided they continue to have symptoms after six months of a gluten free diet and their tissue transglutaminase antibody has returned to normal.

IBD: Persons with either CD or UC over the age of 4 years are enrolled regardless of whether the disease is active or in remission (Table 1). Persons with unclassified IBD (IBD-U) are included. The Montreal Classification is used for adult CD and UC patients (22), and the Paris classification (23) for pediatric IBD. The research coordinator conducts a chart review to confirm the date of diagnosis and maximal phenotype at time of enrolment using the Montreal Classification.

Data Collection

All participants attend a baseline, 12, 24, 36 and 48-month visit. Sources of information for each patient are blood, urine and stool samples, questionnaires, and chart review (Table 2).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Participants complete a questionnaire to obtain age, sex, gender identity, education level attained, ethnic heritage, smoking/alcohol/drug history, comorbidities, medication and therapies, menstrual status at baseline. The study research coordinator records height, weight, BMI and disease-related information (24). Participants answer a series of validated questionnaires to assess disease activity, quality of life, physical pain, lifestyle factors, psychological status, and diet at each study visit. (Table 3). 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.

Healthcare resource use data and associated costs regarding physician visits, clinical procedures, imaging procedures, hospitalizations, emergency room visits, and medication use are collected by linking the participants to provincial administrative data from the Canadian Gastro-Intestinal Epidemiology Consortium (CanGIEC) (https://cangiec.ca).

Biosamples

A stool sample is collected for fecal microbiome, inflammatory markers and short chain fatty acids. A urine sample is collected for metabolomics, and blood samples are taken for DNA isolation as well as for serum for inflammatory markers (ie cytokines, chemokines, hsCRP, lipopolysaccharide) and metabolomic profile (e.g. tryptophan metabolites, growth factors such as brain-derived neurotrophic factor, neurotransmitters such as GABA and serotonin, and stress hormones such as cortisol) at each study visit. All biosamples are shipped to Population Health Research Institute (PHRI) for storing at -80 degrees Celsius.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Data Management

Questionnaires are completed electronically using tablets during the clinic with the option to complete the any questionnaires remaining after clinic visit at home on a computer using an e-mail link. The user questionnaires are available in the REDCap platform stored at a central database collection center, PHRI, at McMaster University. Study staff will review surveys within two weeks of receipt and highlight any missing answers that suggests a problem in completing the survey. These issues will be discussed with the PI, site lead and study team. The staff contact participants up to three times by phone e-mail or in person at a regular study visit to remind them to complete questionnaires and to acquire answers to missing items.

Patient and Public Involvement

The research proposed in IMAGINE was informed by patients. Patient perspectives were sought in identifying priorities for strategic research funding opportunities. Diet, researching the gut microbiome, and mental health were initially identified as key priorities through this process. Through the Crohn's and Colitis Canada (CCC) "Gutsy Learning Series" these priorities were further defined with 289 lay participants in-person and on-line with IBD who gave feedback on what they felt were the most important research topics, identifying fecal transplants, diet and mental health as key priorities. Furthermore, through a workshop organized by the Canadian Digestive Health Foundation (CDHF) prior to this IMAGINE SPOR application, a key message was that IBS patients are concerned about using current pharmacological therapies to relieve their symptoms and prefer approaches that correct the imbalances they perceive to be the root cause of their disorder rather than taking drugs. Patients with IBS are concerned with the long-term use of powerful and sometimes expensive agents and would like more exploration of the factors that are driving the disease. One of the major areas this

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

patient group wanted more research on was the role of diet and also the role that gut bacteria (and probiotics) play in driving IBS. There is remarkable congruence in the research priorities of the IBD and IBS patient communities. The IMAGINE SPOR proposal was informed by these priorities and seeks to address them by exploring the diet-gut microbiome-relationship and how this influences GI and mental symptoms of IBS and IBD.

IMAGINE Patient Research Partners were involved in the development of the MAGIC demographic questionnaire. They also served to pilot test the online questionnaires and provide feedback on user experience and feasibility.

IMAGINE Patient Partners have been directly involved with study design and recruitment of subjects in IMAGINE and will support capacity development for patient engagement more broadly. In person interviews are being conducted by our Patient Partners to identify strategies to improve recruitment and retention rates. Also, this network of patient-engaged researchers is communicating knowledge derived from the study to healthcare professionals, policy makers, and other patients.

A key component of the IMAGINE research program is developing capacity for patient engagement, patient preferences for informing treatment choices and working with our Patient Partners to improve our recruitment rates and long-term retention of IMAGINE participants. We have a unique opportunity to integrate patient engagement as recommended by SPOR by leveraging our innovative Patient and Community Engagement Research (PaCER) program based at the University of Calgary (25, 26). PaCER is designed to promote new roles for patients and family members in health care and health culture through engagement in research. PaCER provides opportunities for patients to be involved in

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

the development and conduct of research designed to affect the lives of patients living with IBD and IBS. Involving patients and families in research is an opportunity to increase the capacity to anticipate problems, manage their condition as a partner in their health care team, and to support other patients and families.

DATA MONITORING AND ETHICS

This is an observational study with no intervention mandated by the protocol and so there is no external data safety and monitoring board. Research ethics approval has been obtained for all 15 sites involved in the study. The protocol was approved on May 31, 2017 with an approval number 2017-3000-GRA with the last amendment to date being on June 25, 2019. 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.

SAMPLE SIZE AND ANALYSES

Sample size

UC, CD and IBS cases will be analyzed. A sample size of 2000 cases in each disease group and 2000 healthy controls will have 90% power to detect a probability of 0.547 that an observed abundance in the disease group is more than the observed abundance in the control group using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.001 two-sided significance level (to adjust for multiple testing). This sample size also assumes 20% data dropout. The probability of 0.547 was derived from 75 IBD cases that we have obtained from pilot studies (27).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Analyses

Primary and secondary aims will be evaluated through multivariate logistic regression with further details regarding how diet, microbiome, metabolomics and genetics will be analysed below.

Diet Analyses

We will use principal component analysis to derive dietary patterns from self-reported semi-quantitative food-frequency questionnaires, and understand the differences in dietary patterns across participants with UC, CD, IBS, and healthy controls; and for active versus non-active disease within each disease group, as outlined in the primary and secondary aims. Briefly, the purpose of PCA and/or machine learning is to reduce large and complex high-dimensional data into fewer dimensions — in this case comprehensive FFQ data (containing up to 150 items or more) is reduced to 2 or 3 dietary patterns (i.e., foods commonly consumed together) that explain the greatest amount of dietary variability within the reported eating habits of the cohort (28). The number of dietary patterns to be retained for subsequent analysis will be based on visual inspection of Scree plots in conjunction with eigenvalues, and principal component interpretability (29). To account for differences in total energy intake between participants, dietary pattern scores will be adjusted to the mean total population caloric intake using the residual method (28). Associations between a participant's adherence to a specific PCA-derived dietary pattern (e.g., Western or plant-based) and UC, CD, IBS and its disease activity will be quantified using logistic regression (case vs control) with appropriate adjustment for covariates (e.g., BMI, age, sex, etc.). For machine learning, dietary patterns will be derived using unsupervised methods that require little to no input or direction by the researcher. The use of both PCA and unsupervised machine learning methods will allow for validation of the derived diet patterns. Our experience suggests that the PCA and machine learning

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

derided patterns will be largely similar (30).

In an exploratory analysis, of particular interest for the IBS group, we will collect data using a supplementary fermentable oligo-di-monosacharides and polyols (FODMAP) Questionnaire to capture foods rich in: a) oligosaccharides, including fructans and galacto-oligosaccharides; b) disaccharides, including lactose; c) monosaccharides, including fructose; d) polyols. These data will be used to better understand "trigger foods" or dietary components that are likely to produce symptoms in participants with IBS.

Microbiome Processing and Analyses

All stool samples will be processed in one lab for consistency. Frozen samples sent from each site will be thawed on ice in an anaerobic environment, mixed thoroughly with a sterile spatula. Two aliquots of 0.3g will be transferred to DNA extraction buffer for molecular analysis. Three aliquots of 1.8 mL will be biobanked at -80^{o}C. DNA will be extracted using established methods (31). Total bacterial load will be measured by quantitative PCR of the 16S rRNA gene. Microbial community profiling will be carried out by amplification and paired-end Illumina sequencing of the v3-v4 region of the 16S rRNA gene for bacteria (31, 32) and the Internal Transcribed Region of the ribosomal genes (ITS) for fungi (33). Microbiome profiles will be processed through in house bioinformatic pipelines (34) incorporating dada2 (35) to generate amplicon sequence variants (ASVs). As sequencing and library construction costs decrease, it will be feasible to carry out shotgun metagenomics on a significant portion of the stool samples. 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. Metagenomic sequencing libraries will

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

be constructed using NEBNext Ultra DNA Library kits with modifications to reduce reaction volume. Libraries will be sequenced to ~15,000,000 reads per sample (150nt paired-end) on an Illumina NovaSeq. After filtering and trimming for sequence quality and primer removal, DeconSeq (36) will be performed on the remaining reads in order to remove reads of human DNA. Genes and functional predictions and comparisons across sample groups will be computed using HUMAnN2 (37) and MetaPHlAn (38) for functional microbiome analysis.

Microbiome analysis will include α-diversity metrics for each sample (observed species, chao1, Shannon diversity) and β-diversity measures will be used to compare diversity between samples. For the latter, centered log-ratio transformation of the read count data will be carried out to account for the compositional nature of microbiome data (39) and visualized using Aitchison principal-component analysis (40). Statistical analyses will be carried out in R using PhyloSeq (41), and ALDEx2 (42) in R. Using generalized linear mixed models (GLMM), we will identify microbial taxa and/or genes associated with disease phenotypes/progression, response to treatment, genotype, diet and other measured parameters. The large number of samples will also allow for application of machine learning methods such random forest and support vector machine methods (43, 44).

Metabolomics Processing and Analyses

Urinary metabolomic profiles will be determined and analyzed together with microbial and dietary profiles to identify relationships and associations with disease status and clinical phenotypes/response to therapies. Urinary metabolic profiles will be analyzed by ^{1}H-NMR on a 4-channel Varian INOVA 600 MHz NMR spectrometer. Assignment of endogenous urinary metabolites will be done using Chenomx NMR Suite 8.5 (Chenomx Inc, Edmonton, Canada) and

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

online databases (HMDB) (45). Metabolite concentrations will be $\log_{10}$ transformed to normalize data prior to statistical analyses. Metabolome association study analyses will be done using multiple linear regression models in the R Project for Statistical Computing (R program). Projection-based principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), orthogonal partial-least squares (OPLS) analysis will be performed using R program.

Genetic Analyses

In terms of genetic analyses, genomic DNA samples will be tested using two different approaches: 1) genome-wide genotyping to capture common genetic variation and enable genome-wide association studies (GWAS) and 2) whole exome sequencing (WES) will primarily be used to capture rare genetic variation and identify non-synonymous coding variants as potential causal variants. Both datasets will be used to identify genetic risk variants associated with disease status or clinical phenotypes/outcomes detailed above (e.g. response to therapy); both as previously described (46-49). These data will also be used to impute the genetic variation at the highly polymorphic Human Leukocyte Antigens (HLA) and Killer cell Immunoglobulin-like Receptor (KIR) genes (50, 51), as these are key determinants of the host's immune response and genetic risk factors for many inflammatory diseases.

For the statistical analyses, following rigorous quality control of the genotype/sequence data, whole genome imputation of the dataset using a relevant public reference panel (e.g. 1000 Genomes, Haplotype Reference Panel, TOPMed, etc.) (52, 53) will be performed followed by principal components analysis (PCA). Principal components will be tested for phenotype association (using logistic regression with study indicator variables included as covariates) and evaluated for their

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

impact on the genome-wide test statistics using λ (the genomic control inflation factor based on the median χ2) after genome-wide association of the specified principal component. Association testing as well as binary and linear genotype– phenotype analyses will be done with PLINK and multinomial and ordinal regression analyses with a custom program, Trinculo. Survival analysis and risk prediction will be done with R using the packages "survival" and "Mangrove", respectively. For integrated biomarker discovery (54), this genetic data will also be integrated with other biomarker data generated from the various IMAGINE platforms, in order to select those that estimate a large association with clinical outcomes (e.g. response to therapy), in order to create the best subset of predictors. Variable selection will be based on mathematical criteria for model selection, i.e., the Bayesian Information Criteria (BIC), and expert a priori (e.g., clinical knowledge, preliminary evidence). The selection of the model will be in the context of logistic regression, using the candidate biomarkers as covariates and drug response (positive or negative) as the outcome. The BIC has been proven to lead to less over-fitting of the model to data compared to other less conservative approaches (55). This will reduce type-I errors and lead to increased robustness of the results.

Subgroup Analyses

There are a number of subgroup analyses planned. In particular, we will analyze the primary and secondary outcomes by sex. We will strive to ensure representative enrollment of men and women with a wide range of life experience and at different life stages. When women are surveyed or otherwise evaluated, we will take note of past, anticipated and ongoing pregnancies, obtain a menstrual history, and use instruments that are sensitive to the influence of gender on outcomes.

The pediatric population (IBD cases aged 4-18 years of age) is also an important group to study. All

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

the primary and secondary outcomes described above will also be evaluated specifically in the pediatric population. This includes predictors of success and failure of therapy for IBD. Children developing IBD are predominantly treated from the time of first presentation at academic centres rather than in community practice. Hence the pediatric collaborators in this proposal offer access to the broad spectrum of IBD, including prior to alteration of the microbiome by any therapy. We will evaluate predictors of success and failure of these therapies for IBD over time in the pediatric population.

We will evaluate subtypes of IBS; constipation predominant, diarrhea predominant and mixed patterns. We will assess the microbiome, metabolomics, genetic, demographic and dietary predictors of response to therapies for IBS. Predictors of response to a low FODMAPs diet will help inform a RCT of low FODMAPs diet in IBS, and in particular we will evaluate whether responders are more likely to initially be taking a diet that is very rich in FODMAPs and how this response relates to their microbiome compositional and functional changes, affected by diets. A subgroup analysis will be performed of those between the ages of 13 to 17 compared to adult patients. We will also evaluate those with stable celiac disease and compare results to those without this disorder.

DISSEMINATION

Our approach to dissemination involves developing evidence-based knowledge translation initiatives for research that is ready for prime time – for each of category of our three research partners (patient, clinicians and policymakers). For patients, we plan to create a 'white label' version of the McMaster Optimal Aging Portal (https://www.mcmasteroptimalaging.org) that focuses specifically on supporting self-management and more generally informed decision-making for GI disease. This

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

involves 1) identifying existing Evidence Summaries and preparing new ones to provide patients with the key messages from scientific research (typically high-quality systematic reviews) that's ready to be acted on; 2) identifying existing Web Resource Ratings and preparing new ones to help patients identify the free health resources on the internet that are based on scientific research; 3) identifying existing Blog Posts and preparing new ones that provide commentaries for patients about on what the scientific research on a topic actually means and on why good science matters; and 4) identifying existing patient decision aids to help patients (and clinicians working with patients) to engage in shared decision-making.

We will work with network partners (e.g. CDHF and CCC) to determine the best online platform for patient-focused products, to develop inclusion criteria, and to ensure that their online resources focused on GI disease meet the high standard necessary to be captured and ranked highly in the Web Resource Ratings. We plan to prepare 52 new Evidence Summaries, 24 Blog Posts, and 400 Web Resources Ratings for the 'white label' portal. We will also be working with network partners to ensure high rates of use of the white-label, GI disease-focused content site for patients.

The patients, family members and caregivers who live with the chronic conditions covered by the IMAGINE Network will be able to use the portal to find information that aids them in managing their conditions and making related health decisions. The content produced by the McMaster Optimal Aging Portal has been shown to be effective in informing health consumers of quality heath information related to aging but not in other domains. We plan to conduct a randomized controlled trial (which will be detailed in a separate protocol) to assess how the online resources for patients,

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

provided through the white label website, changes patient behaviour in regards to using information and making evidence-informed health decisions.

For clinicians, we will follow an approach that emerged from a comprehensive review of the literature on scaling up effective clinical interventions (summarized in an evidence brief) (56) and a stakeholder dialogue involving the key policymakers, stakeholders and researchers focused on supporting scale up of effective clinical practices in Ontario (summarized in a dialogue summary) (57): 1) supporting dynamic efforts to identify GI disease-related clinical practices to be optimized and the causes of underlying problems, using both empirical approaches like systematic reviews and theoretical approaches like the Behaviour Change Wheel and the Theoretical Domains Framework; 2) using rigorous processes to select and implement approaches to optimizing clinical practices that address the underlying causes of problems (e.g., audit and feedback, financial incentives; and 3) monitoring, evaluating and reviewing the approaches selected to optimize clinical practices. We plan to support two approaches: 1) prioritizing clinical interventions to be scaled up by engaging network members using an explicit process; and 2) scaling up effective clinical interventions by developing and executing a scale-up plan, both of which will build on what has been learned from related work at the McMaster Health Forum, and will rely on the frequent engagement of key members of the IMAGINE SPOR Network in doing so.

For policymakers, we will use our tried and tested approach to supporting evidence-informed policymaking, which means: 1) preparing an evidence brief on a pressing need for scale-up and the factors hindering that achievement (e.g., nurses' scope of practice, existing financial incentives, lack of multidisciplinary teams), options for scaling up, and key implementation considerations (which

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

includes an equity lens brought to bear on particularly vulnerable groups); 2) convening a stakeholder dialogue that brings together key policymakers, patient and clinical leaders and researchers who can consider the research evidence alongside the other factors that drive decision-making; and 3) preparing a dialogue summary, disseminating the evidence brief and dialogue summary, conducting personalized briefings to senior leaders in the system, and supporting their efforts to act on what they learned.

We will enhance this approach by convening citizen panels to capture the insights and values of a diverse group of citizens, with different types of lived experience with the issue at hand, in a panel summary, the key messages of which would be included in the evidence brief informing the stakeholder dialogue. We plan to address two topics using this approach, one focused on reducing emergency-department usage in people with IBD, and one focused on framing the work led by IMAGINE in the context of rapid learning health systems for specific conditions (including those addressed by the IMAGINE Network). For each topic we will conduct consultations with a steering committee and key informants to define the terms of reference for evidence briefs, identify stakeholders and potential dialogue invitees, and review the outputs (briefs, summaries, and evaluation reports).

ARTICLE SUMMARY

Strengths

Studies that have evaluated the microbiome in IBS (18) and IBD (19) have been small and underpowered. These studies usually did not evaluate disease phenotype in detail and have not assessed diet (18, 19), which can be an important effect modifier (58). This will be the largest

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

observational study published to date that is sufficiently powered to evaluate the microbiome in IBS and IBD. All relevant confounding factors and effect modifiers will be captured and followed over time and longitudinally, which will allow a better understanding of what drives exacerbations of both IBS and IBD given that these are chronic relapsing and remitting diseases. There is also the possibility of pooling data with similar cohorts to provide more robust data on microbiome changes over time in these diseases (59). The other strength of the study is the multidisciplinary team that makes up the IMAGINE Network. In particular, the collaboration between psychiatry and gastroenterology allows a careful evaluation of gut brain connections (17) and in particular how the gut microbiome (60) may impact on anxiety and depression in patients with IBD and IBS. Another key component of the multidisciplinary team is the level of patient engagement throughout the proposal. This is a Canadian Institute of Health Research funded grant through the SPOR initiative mandating that priorities are set by patients and they have input into study design. To date, we have 19 patient partners as part of the IMAGINE Network and many are involved in the MAGIC cohort study. This strengthens the research, making it more patient focused, and supports knowledge translation of the findings to patients.

Limitations

This study is observational so any associations found may not relate to the causes of IBS and IBD. We will rigorously control for confounding factors and but cannot control for unknown confounders and so cannot draw causal inferences from the data. The MAGIC study will therefore be hypothesis generating and any data relating to the microbiome or diet is likely to need confirmation in randomized controlled trials (RCTs). The IMAGINE Network is committed to develop RCTs to further investigate any promising findings from the MAGIC cohort study. This is the largest study

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

evaluating IBS and IBD, but the data that will be collected is enormous and so any results related to the secondary outcomes of the study need to be interpreted with caution. The adjustment for multiple testing is for the primary outcomes only and given the number of other outcomes that can be evaluated with the data that is generated, it is important to realize that any positive results from the secondary outcomes could be a chance finding related to multiple testing. We will highlight this when reporting the data of more exploratory outcomes being studied. Despite these caveats, the MAGIC study will provide valuable insight into the etiology of IBS and IBD as well as associated psychiatric disorders. Data from this study will also provide strategies for personalized medicine approaches to manage these diseases more effectively.

ACKNOWLEDGEMENTS

We are grateful to Glenda MacQueen for all the support she gave to the IMAGINE Network and the MAGIC study. Her advice was invaluable throughout this project and she will be missed by all of us. We are grateful to all our patient partners and everyone within the IMAGINE Network.

FUNDING SOURCES

The IMAGINE Network is supported by a grant from the Canadian Institute of Health Research (Funding Reference Number: RN279389 – 35803) with funding from several partners. Funding was matched by McMaster University, University of Calgary, University of Alberta, Queen's University, Dalhousie University, Montreal Heart Institute Research Centre, Takeda Pharmaceutical Company, Allergan Incorporated, Alberta Innovates, Research Manitoba, Crohn's and Colitis Canada.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

DISCLOSURES

Dr. Moayyedi holds the Audrey Campbell Chair in Ulcerative Colitis Research. Drs. Moayyedi, Bercik and Aida Fernandes have no conflicts of interest.

Dr. Bernstein is supported in part by the Bingham Chair in Gastroenterology. He is on Advisory Boards for AbbVie Canada, Janssen Canada, Takeda Canada, Pfizer Canada. He is a Consultant for Mylan Pharmaceuticals. He is receiving educational grants from AbbVie Canada, Pfizer Canada, Shire Canada, Takeda Canada, Janssen Canada. Speaker's panel for AbbVie Canada, Janssen Canada, Takeda Canada, and Medtronic Canada. Received research funding from AbbVie Canada.

Dr. Rioux receives research funding from Pfizer Inc.

Dr. Dieleman is on the Advisory Boards for Janssen Canada, AbbVie Canada, Pfizer Canada and Takeda Canada.

Dr. Marshall holds a Canada Research Chair (2008-18) and the Arthur J.E. Child Chair and receives travel reimbursement through Illumina for meetings of the Global Economics and Evaluation of Clinical Genomics Sequencing Working Group.

Dr. Vanner was supported by an Educational grant from Allergan.

Sandra Zelinsky is a patient research partner and received a grant from Takeda Canada.

Data Statement

Technical appendix, statistical code, and dataset will be available from a repository (e.g. Dryad data repository) once follow up is complete and main analyses have been published.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Author Contributions

PM, GM, CNB, SV, PB, KLM, MGS, JDR, LAD, EFV, RDS, AO, LT, JL, JC, DM, SZ and AF have contributed to the conception and design of the protocol and obtained funding. PM, MGS, JDR, KLM and RDS provided statistical support. JDR led the genetic, MGS the microbiome, KLM the metabolomic, GM the psychiatric, CNB the inflammatory bowel disease, PB and SV the irritable bowel syndrome, LAD, EFV and RDS the diet, AO the pediatric, JL the knowledge translation, DM and SV the patient engagement and JC the data management portions of the protocol. PM and AF constructed the first draft of the article which was significantly revised by all other authors. CNB, SV, PB, KLM, MGS, JDR, LAD, EFV, RDS, AO, LT, JL, JC, DM and SZ have provided informatics support, given critical revisions to the manuscript and approved the final version of the manuscript submitted for publication. PM acts as guarantor for the article.

Author contact Information

Author	Contact Information
Corresponding Author: Paul Moayyedi	1280 Main St. W. HSC 3V3 Hamilton, ON Canada, L8S 4K1 (905) 521 2100 x.76764 moayyep@mcmaster.ca
Glenda MacQueen	Post-humous
Charles N Bernstein	Charles.Bernstein@umanitoba.ca
Stephen Vanner	vanners@hdh.kari.net
Premysl Bercik	bercikp@mcmaster.ca
Karen L Madsen	karen.madsen@ualberta.ca
Michael G Surette	surette@mcmaster.ca
John D Rioux	john.rioux@inflammgen.org
Levinus A. Dieleman	l.dieleman@ualberta.ca
Elena F Verdu	verdue@mcmaster.ca
Russell J de Souza	rdesouz@mcmaster.ca
Anthony Otley	anthony.otley@dal.ca
Laura Targownik	Laura.Targownik@sinaihealth.ca
John Lavis	lavisj@mcmaster.ca
Jennifer Cunningham	jennifer.cunningham@phri.ca
Deborah Marshall	damarsha@ucalgary.ca
Sandra Zelinsky	sandraz.per@gmail.com
Aida Fernandes	fernaa19@mcmaster.ca

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Competing Interests

There are no competing interests for any author.

Word Count: 5,630

References

1. Thompson WG, Irvine EJ, Pare P, Ferrazzi S, Rance L. Functional Gastrointestinal Disease in Canada. Dig Dis Sci 2002; 47: 225-35.
2. Sperber AD, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, Whitehead WE, Dumitrascu DL, Fang X, Fukudo S, Kellow J, Okeke E, Quigley EM, Schmulson M, Whorwell P, Archampong T, Adibi P, Andresen V, Benninga MA, Bonaz B, Bor S, Fernandez LB, Choi SC, Corazziari ES, Francisconi C, Hani A, Lazebnik L, Lee YY, Mulak A, Rahman MM, Santos J, Setshedi M, Syam AF, Vanner S, Wong RK, Lopez-Colombo A, Costa V, Dickman R, Kanazawa M, Keshteli AH, Khatun R, Maleki I, Poitras P, Pratap N, Stefanyuk O, Thomson S, Zeevenhooven J, Palsson OS. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. Gastroenterology. 2020 Apr 12. pii: S0016-5085(20)30487-X. doi: 10.1053/j.gastro.2020.04.014. [Epub ahead of print]
3. Ng SC, Shi HY, Hamidi N et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017; 390: 2769-78.
4. Brandt LJ, Chey WD, Foxx-Orenstein AE, Quigley EMM, Schiller LR, Schoenfeld PS, Spiegel BM, Talley NJ, Moayyedi P. An evidence-based position statement on the

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

management of irritable bowel syndrome. American Journal of Gastroenterology 2009; 104 (suppl 1): S1-S36.

5. Talley NJ, Abreu MT, Achkar JP, Bernstein CN, Dubinsky MC, Hanauer SB, Kane SV, Sandborn WJ, Ullman TA, Moayyedi P. An evidence-based systematic review on medical therapies in inflammatory bowel disease. Am J Gastroenterol 2011: 106 supp 1: S2-S25.
6. Gralnek IM, Hays RD, Kilbourne A, Naliboff B, Mayer EA. The Impact of Irritable Bowel Syndrome on Health-Related Quality of Life. Gastroenterology 2000; 119: 654-60.
7. Casellas F, Lopez-Vivancos J, Badia X et al. Influence of inflammatory bowel disease on different dimensions of quality of life. Eur J Gastroenterol Hepatol 2001;13:567–72.
8. Bernstein CN, Loftus EV, Ng SC, Lakatos PL, Moum B. Hospitalisations and surgery in Crohn's disease. Gut 2012; 61: 622-9.
9. Peery AF, Crockett SD, Barritt AS, Dellon ES, Eluri S, Gangarosa LM, Jensen ET, Lund JL, Pasricha S, Runge T, Schmidt M, Shaheen NJ, Sandler RS. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States Gastroenterology 2015; 149:1731–1741.
10. Lee C, Doo E, Choi JM, et al. The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis. J Neurogastroenterol Motil. 2017;23(3):349–362. doi:10.5056/jnm16220
11. Mikocka-Walus A, Knowles SR, Keefer L, Graff L. Controversies Revisited: A Systematic Review of the Comorbidity of Depression and Anxiety with Inflammatory Bowel Diseases, *Inflammatory Bowel Diseases*, Volume 22, Issue 3, 1 March 2016; 752–762.
12. Jones R, Latinovic R, Charlton J, Gulliford M. Physical and psychological co-morbidity in irritable bowel syndrome: a matched cohort study using the General Practice Research Database. Aliment Pharmacol Therap 2006; 24: 879-86.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

13. Roy-Byrne PP, Davidson KW, Kessler RC, Asmundson GJ, Goodwin RD et al. Anxiety disorders and comorbid medical illness. Gen Hosp Psych 2008; 30: 208-25.

14. Ford AC, Moayyedi P, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BMR, Quigley EMM. American College of Gastroenterology Monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. American Journal of Gastroenterology 2014; 109: S1-S26.

15. Moayyedi P, Simren M, Bercik P. Evidence-based and mechanistic insights into exclusion diets for IBS. Nature Reviews Gastroenterology & Hepatology 2020 epub DOI: 10.1038/s41575-020-0270-3.

16. Moayyedi P. Update on fecal microbiota transplantation in patients with inflammatory bowel disease. Gastroenterology and Hepatology 2018; 14: 319-22.

17. MacQueen G, Surette M, Moayyedi P. The gut microbiome and psychiatric illness. Journal of Psychiatry and Neuroscience 2017; 42: 75-7.

18. Pittayanon R, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, Moayyedi P. Gut microbiota in patients with irritable bowel syndrome – a systematic review. Gastroenterology 2019; 157: 97-108.

19. Pittayanon R, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, Moayyedi P. Gut microbiota in patients with inflammatory bowel disease – a systematic review. Gastroenterology 2020; 158: 930-946 doi:10.1053/j.gastro.2019.11.294

20. www.imaginespor.com

21. Palsson OS, Whitehead WE, van Tilburg MAL, Chang L, Chey W, et al. Development and Validation of the Rome IV Diagnostic Questionnaire for Adults. Gastroenterology 2016; 150: 1481-1491.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

22. Satsangi J, Silverberg MS, Vermeire S, Colombel J-F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006; 55: 749-53.

23. Levine A, Griffiths AM, Markowitz J, Wilson D, Turner D, et al. Pediatric Modification of the Montreal Classification for Inflammatory Bowel Disease - the Paris Classification. Inflamm Bowel Dis 2011: 17: 1314-21.

24. Ruemmele FM, Hyams JS, Otley A, Griffiths A, Kolho KL, et al. Outcome measures for clinical trials in paediatric IBD: an evidence-based, expert-driven practical statement paper of the paediatric ECCO committee. Gut 2015; 64: 438-46.

25. Shklarov S, Marshall DA, Wasylak T, Marlett NJ. "Part of the Team": Mapping the outcomes of training patients for new roles in health research and planning. Health Expectations, Dec 2017;20:1428-1436

26. Marlett N, Shklarov S, Marshall DA, Santana MJ, Wasylak T. Building New Relationships in Research: A Model of Patient Engagement Research. Qual Life Res 2015 May;24(5):1057-67.

27. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, Armstrong D, Marshall JK, Kassam Z, Reinisch W, Lee CH. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology 2015; 149: 102-9.

28. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002; 13: 3-9.

29. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willet WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr 2000; 72: 912-2.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

30. Northstone K, Ness AR, Emmett PM, Rogers IS. Adjusting for energy intake in dietary pattern investigations using principal components analysis. Eur J Clin Nutr 2006; 62: 931-8. 113.

31. Lau JT, Whelan FJ, Herath I, Lee CH, Collins SM, Bercik P, Surette MG. Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. Genome Med. 2016 Jul 1;8(1):72.

32. Woo TE1,2, Lim R2, Heirali AA3, Acosta N3, Rabin HR2,3, Mody CH, Somayaji R, Surette MG, Sibley CD, Storey DG, Parkins MD. A longitudinal characterization of the Non-Cystic Fibrosis Bronchiectasis airway microbiome. Sci Rep. 2019 May 3;9(1):6871. doi: 10.1038/s41598-019-42862-y.

33. Xu J. Fungal DNA barcoding. Genome. 2016 Nov;59(11):913-932. Epub 2016 Aug 30.

34. Whelan FJ1, Surette MG2,3. A comprehensive evaluation of the sl1p pipeline for 16S rRNA gene sequencing analysis. Microbiome. 2017 Aug 14;5(1): 100.

35. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 Jul;13(7):581-3. doi: 10.1038/nmeth.3869. Epub 2016 May 23.

36. Schmieder R, Edwards R. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. PLoS One. 2011 Mar 9;6(3):e17288. doi: 10.1371/journal.pone.0017288.

37. Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, Lipson KS, Knight R, Caporaso JG, Segata N6, Huttenhower C. Species-level functional profiling of metagenomes and metatranscriptomes.Nat Methods. 2018 Nov;15(11):962-968. doi: 10.1038/s41592-018-0176-y. Epub 2018 Oct 30.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

38. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods 2012; 9: 811-4. doi: 10.1038/nmeth.2066.

39. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. Front Microbiol. 2017; 8: 2224. doi:10.3389/fmicb.2017.02224.

40. Martino C, Morton JT, Marotz CA, et al. A Novel Sparse Compositional Technique Reveals Microbial Perturbations. mSystems. 2019; 4: e00016-19. doi:10.1128/mSystems.00016-19

41. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data.PLoS One. 2013; 8: e61217. doi: 10.1371/journal.pone.0061217.

42. Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome. 2014; 2: 15. doi:10.1186/2049-2618-2-15.

43. Qu K, Guo F, Liu X, Lin Y, Zou Q. Application of Machine Learning in Microbiology. Front Microbiol 2019; 10: 827. doi: 10.3389/fmicb.2019.00827. eCollection 2019.

44. Camacho DM, Collins KM, Powers RK, Costello JC, Collins JJ. Next-Generation Machine Learning for Biological Networks. Cell 2018;173: 1581-1592. doi: 10.1016/j.cell.2018.05.015.

45. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, et al. HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007 Jan; 35(Database issue): D521-6.

For peer review only

46. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet. 2011; 43: 246-52. doi: 10.1038/ng.764.

47. Rivas MA, Avila BE, Koskela J, Huang H, Stevens C, et al. Insights into the genetic epidemiology of Crohn's and rare diseases in the Ashkenazi Jewish population. LoS Genet 2018; 14: e1007329. doi: 10.1371/journal.pgen.1007329.

48. Huang H, Fang M, Jostins L, Umićević Mirkov M, Boucher G, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature 2017; 547: 173-178. doi: 10.1038/nature22969.

49. Cleynen I, Boucher G, Jostins L, Schumm LP, Zeissig S, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet 2016; 387: 156-67. doi: 10.1016/S0140-6736(15)00465-1.

50. Goyette P, Boucher G, Mallon D, Ellinghaus E, Jostins L, et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet 2015; 47: 172-9. doi: 10.1038/ng.3176.

51. Vukcevic D, Traherne JA, Næss S, Ellinghaus E, Kamatani Y, et al. Imputation of KIR Types from SNP Variation Data. Am J Hum Genet. 2015 Oct 1;97(4):593-607. doi: 10.1016/j.ajhg.2015.09.005.

52. Gourraud PA, Khankhanian P, Cereb N, Yang SY, Feolo M, et al. HLA diversity in the 1000 genomes dataset. PLoS One. 2014; 9: e97282. doi: 10.1371/journal.pone.0097282. eCollection 2014.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

53. Das S, Abecasis GR, Browning BL. Genotype Imputation from Large Reference Panels. Annu Rev Genomics Hum Genet. 2018; 19: 73-96. doi: 10.1146/annurev-genom-083117-021602.

54. Ivison S, Des Rosiers C, Lesage S, Rioux JD, Levings MK. Biomarker-guided stratification of autoimmune patients for biologic therapy. Curr Opin Immunol. 2017; 49: 56-63. doi: 10.1016/j.coi.2017.09.006.

55. Gao X, Song P. Composite likelihood Bayesian information criteria for model selection in high-dimensional data. Journal of the American Statistical Association. 2010; 105: 1531–1540. doi:10.1198/jasa.2010.tm09414.

56. Lavis JN, Wilson MG, Grimshaw JM. Evidence Brief: Optimizing Clinical Practice in Ontario Based on Data, Evidence and Guidelines. Hamilton, Canada: McMaster Health Forum, 6 March 2015.

57. Wilson MG, Lavis JN. Dialogue Summary: Optimizing Clinical Practice in Ontario Based on Data, Evidence and Guidelines. Hamilton, Canada: McMaster Health Forum, 6 March 2015.

58. McIntosh K, Reed DE, Schneider T, Dang F, Keshteli AH, De Palma G, Madsen K, Bercik P, Vanner S. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut 2017; 66: 1241-1251.

59. Yilmaz, B., Juillerat, P., Øyås, O. *et al.* Microbial network disturbances in relapsing refractory Crohn's disease. Nat Med 2019; 25: 323–336. https://doi.org/10.1038/s41591-018-0308-z

60. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. Neurogastroenterol Motil 2012; 24: 405-13.

For peer review only

Table 1: Eligibility criteria

Type	Inclusion Criteria	Exclusion Criteria
IBD	Patients with documented CD, UC or IBD-U, >4 years old	• subtotal colectomy and/or ileostomy patients • major comorbid condition where the projected survival is less than 5 years • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder
IBS	Patients with IBS who have met Rome IV criteria, ≥13 years old **IBS-D** • Normal CBC • Negative tissue transglutaminase antibody if diarrhea the main symptom • Symptoms onset > 45 years old, then negative colonic biopsies for microscopic colitis **IBS-C, IBS-M & IBS-U** • Negative tissue transglutaminase antibody • Symptoms onset > 50 years age, with new symptoms < 1 year duration, then have a negative colonoscopy, CT colonography or Air Contrast Barium Enema. • Normal CBC	• major gastrointestinal surgery (Roux en y, bowel resection), • major comorbid condition, where the projected survival is less than 5 years • drug use that is the major cause of GI symptoms and/or undermines longitudinal compliance, including chronic antibiotic use, narcotic analgesics and substance abuse • narcotic analgesic use causing GI symptoms • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder • GI cancer within 5 years
Healthy Controls	No gastrointestinal symptoms using the ROME IV Questionnaire	• major gastrointestinal surgery (Roux en y, bowel resection) • any major comorbid chronic condition • difficulties with communication, including unable to communicate in English or French • diagnosis of schizophrenia • diagnosis of eating disorder

Table 2: Participant Information Collection

Contact	Screening period		12-month visit	24-month visit	36-month visit	48-month visit
Purpose	**Enrol in Study**		Health status Biosamples Questionnaires	Health status Biosamples Questionnaires	Health status Biosamples, Questionnaires	Health status Biosamples Questionnaires
Information collected	Contact by telephone, email or clinic visit. Explain project Set up study visit(s)	Signed consent Eligibility screening Demographics Medical history Provide urine & stool kit Obtain blood (+DNA) Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires	Medical history Obtain urine, stool, blood Questionnaires

For peer review only

Table 3: List of Patient-answered Questionnaires

SUBGROUP	ADULTS	PEDIATRICS
IBS	• Demographic Questionnaire	• Demographics (exclusions apply)
	Disease Specific	
	• IBS Severity Score	• IBS Severity Score • Rome IV Diagnostic Questionnaire
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) - Age 5-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15 • Euro Quality of Life 5 Level - Age 16 +
	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire
IBD	• Demographic Questionnaire	• Demographics (exclusions apply)
	Disease Specific	

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

	• Short IBD Symptom Inventory	• IMPACT – III - Ages 9-17 • PUCAI (UC); PCDAI (CD)
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) - Age 4-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15
	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire
Healthy controls	• Demographic Questionnaire	• Demographics (exclusions apply)
	GI Symptoms	
	• PROMIS Scale 5a – (GI Belly Pain) • PROMIS Scale 6a – (GI Diarrhea) • PROMIS Scale 9a – (GI Constipation) • PROMIS Scale 13a – (GI Gas & Bloating) • Leeds Dyspepsia Questionnaire	• PedsQL GI Symptoms Scale- (stomach pain and hurt; discomfort when eating; heartburn & reflux; nausea & vomiting; gas & bloating; constipation; blood in BM; diarrhea; worry about stomach aches; worry about BM) Age 5-17
	General Quality of Life	
	• Euro Quality of Life	• Euro Quality of Life Youth - Age 8-15 • Euro Quality of Life 5 Level - Age 16 +

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

	Psychological	
	• Patient Health Questionnaire • PROMIS-29 – (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Pain Interference & Intensity) • Generalized Anxiety Disorder (GAD-7) • Perceived Stress Scale • Adverse Childhood Experiences • Brief Resiliency Survey • Pain Catastrophizing Scale	• PROMIS-25 - (Physical Function Mobility; Anxiety; Depressive Symptoms; Fatigue; Peer Relations; Pain Interference) - Age 5-17 • Brief Resiliency Survey - Age 12-17 • Pain Catastrophizing Scale - Age 8-17 • Revised Child Anxiety and Depression Scale - Age 6-17
	Productivity	
	• Work Productivity & Activity Index	
	Diet	
	• Food Frequency Questionnaire • FODMAP Questionnaire	• Food Frequency Questionnaire • FODMAP Questionnaire

For peer review only

APPENDIX 1: IMAGINE MEMBERS (alphabetically)

Hamilton:
Premsyl Bercik
John Bienenstock
Stephen Collins
Russel de Souza
Aida Fernandes (Executive Director)
Benicio Frey
Khurram Khan
John Lavis
Paul Moayyedi (PI)
Nikil Pai
Mary Sherlock
Mike Surette
Elena Verdu

Calgary:
Christopher Andrews
Paul Beck
Humberto Jijon
Gilaad G. Kaplan
Glenda MacQueen (post-humous)
Deborah Marshall
Yasmin Nasser
Remo Panaccione
Cynthia Seow
Valerie Taylor

Winnipeg:
Charles Bernstein
Wael El-Matary
Patricia Furer
Jean-Eric Ghia
Lesley Graff
Harminder Singh
John Walker (post-humous)

Edmonton:
Leo Dieleman
Richard Fedorak (post-humous)
Hien Huynh
Dina Kao
Karen Madsen

For peer review only

Puneeta Tandon
Eytan Wine

Kingston:
David Reed
Dean Tripp
Stephen Vanner

Halifax:
Jennifer Jones
Anthony Otley
Johan Van Limbergen

Montreal:
Alain Bitton
Justin Cote-Daigneault
Christophe Faure
Peter Lakatos
John Rioux
Sacha Sidani

Toronto:
Ken Croitoru
Louis Liu
Jeff Meyer
Geoffrey Nguyen
Laura Targownik

Ottawa:
Eric Benchimol
Sanjay Murthy

St John's:
Mark Borgaonkar

Vancouver:
Brian Bressler

Patient Research Partners:
Gail Bellissimo
Sara Blake
Kim Daley
Alysia DeNino
Anny Fernandez

For peer review only

Shauna Jones
Ellen Keunzig
Sophie LeBlanc
Nadine Mackay
Lisa MacNeil
Megan Marsiglio
Kate Mason
Adhiyat Najam
Emma Neary
Shawn Reynolds
Perry Steckly
Amy Van Engelen
Karthika Yogaratnam
Sandra Zelinsky

For peer review only