## **Defining the Australian Inflammatory Bowel Disease Microbiome – The AIM Study**

**Principal researchers**

Name: Prof Georgina Hold

Position: Professor of Gut Health

Address: Level 2, Clinical Sciences (WR Pitney) Building,

St George Hospital

Kogarah,

NSW 2017

Name: Professor Michael Grimm

Position: Clinical Associate Dean

Address: St George and Sutherland Clinical School

Short Street, Kogarah, NSW 2017

Name: Prof Rupert Leong

Position: Gastroenterologist, Concord Repatriation General Hospital

Address: ACE Unit, Level 1 West

Concord Repatriation General Hospital

Concord

NSW 2139

Australia

**Co-Investigators**

Name: A/Prof Susie Connor

Position: Gastroenterologist

Address: Level 1, Clinical Building, Department of Gastroenterology

Liverpool Hospital

Liverpool NSW 2170

Name: A/Prof Avi Lemberg

Position: Head of Department, Paediatric Gastroenterology Senior Staff Specialist, Sydney

Address: Dept. of Gastroenterology. Sydney Children’s Hospital

High St, Randwick NSW 2031

Australia

Name: Dr Simon Ghaly

Position: Gastroenterologist, St. Vincent’s Hospital, Sydney

Address: 390 Victoria Rd, Darlinghurst

NSW, 2010

Name: Prof Golo Ahlenstiel

Position: Professor of Medicine

Address: Blacktown Clinical School, WSU, Blacktown Hospital, PO Box 792, Seven Hills, NSW 2147, Australia

Name: Prof Paul Pavli

Position: Professor of Gastroenterology

Address: Inflammatory Bowel Disease Research, Lvl 5, Bldg 10

Canberra Hospital, Yamba Drive, Garran ACT 2605

Name: A/Prof Mark Danta

Position: Clinical Academic Gastroenterologist, St. Vincent’s Clinical School, St Vincent’s Hospital and UNSW, Sydney

Address: 390 Victoria Rd, Darlinghurst

NSW, 2010

Name: Prof Hazel Mitchell

Position: Head of Medical Microbiology

Address: School of Biotechnology and Biomolecular Sciences

The University of New South Wales

Randwick, NSW 2052

Name: Dr Claire O’Brien

Position: NHMRC Peter Doherty Research Fellow

Address: Inflammatory Bowel Disease Research, Lvl 5, Bldg 10

Canberra Hospital, Yamba Drive, Garran ACT 2605

Name: Dr Steven Leach

Position: Research Fellow, University of New South Wales

Address: Westfield Research Laboratories, Level 2

Sydney Children’s Hospital

High St, Randwick NSW 2031

Australia

Name: Dr Cath Burke

Position: Lecturer

Address: School of Life Sciences, Faculty of Science

University of Technology Sydney

Ultimo, NSW, 2007

Name: Dr. Valerie Wasinger

Position: Senior Research Officer, University of New South Wales

Address: Bioanalytical Mass Spectrometry Facility

Mark Wainwright Analytical Centre

The University of New South Wales

Kensington

NSW 2052

Australia

Name: Dr Crispin Corte

Position: Gastroenterologist and Senior Clinical Lecturer

Address: AW Morrow Gastroenterology and Liver Centre

Royal Prince Alfred Hospital

Missenden Rd

Camperdown

NSW 2050

Name: Dr Thomas Lee

Position: Staff Specialist

Address: Wollongong Hospital,

Loftus St

Wollongong 2500

Name: Dr Yunki Yau

Position: Senior Scientist, Concord Repatriation General Hospital

Address: ACE Unit, Level 1 West

Concord Repatriation General Hospital

Concord

NSW 2139

Australia

Name: Dr Craig Haifer

Position: Gastroenterologist,

Address: St. Vincent’s Hospital

390 Victoria Rd, Darlinghurst,

NSW 2010

Australia

Name: Dr Steven Tattersall

Position: Gastroenterologist (VMO),

Address: Royal North Shore Hospital

Reserve Road

St Leonards

NSW 2065

Name: Dr Viraj Kariyawasa

Position: Staff Specialist Gastroenterologist,

Address: Blacktown Clinical School, WSU, Blacktown Hospital, PO Box 792, Seven Hills, NSW 2147, Australia

Name: Dr William Bye

Position: Gastroenterologist,

Address: Prince of Wales Hospital, Barker St, Randwick NSW 2031

Name: Dr Dorit Samocha-Bonet

Position: Group Leader, Clinical Insulin Resistance Group

Address: Garvan Institute of Medical Research

384 Victoria Street, Darlinghurst, NSW 2010

Name: Dr Scott Read

Position: Postdoctoral Researcher

Address: Blacktown Clinical School, WSU, Blacktown Hospital, PO Box 792, Seven Hills, NSW 2147, Australia

Name: Dr Sudarshan Paramsothy

Position: Staff Specialist Gastroenterologist, Concord Repatriation General Hospital

Address: ACE Unit, Level 1 West, Concord Repatriation General Hospital

Concord, NSW 2139

Name: Dr Astrid Williams

Position: Staff Specialist Gastroenterologist, Liverpool Hospital

Address: Liverpool Hospital

Elizabeth St, Liverpool

NSW 2170

Name: Dr Ramesh Paramsothy

Position: Staff Specialist Gastroenterologist,

Address: Blacktown Hospital

18 Blacktown Rd,

Blacktown NSW 2148

Name:         Dr Kelly Lambert

Position:      Research Fellow / Renal Dietitian

Address:      Department of Clinical Nutrition,

                   Level 5, Block C, Wollongong Hospital

                   Crown Street, Wollongong

​                   NSW 2500

**Statistical Support**

Name: Professor Marissa Lassere

Position: Conjoint Professor with specialist expertise in Health Informatics and Biostatistics

**Pathology Support**

Name: Dr Ewan Miller

Position: Pathologist at St George Hospital

Short Street, Kogarah, NSW 2017

## RESEARCH QUESTIONS

**Primary research questions**

* To define the microbiota signature of IBD in Australia
* To determine key microbiota changes associated with onset of IBD symptoms?
* To define whether longitudinally collected microbiota data can be used to a) predict IBD relapse and b) better inform therapeutic decision-making?
* To determine whether microbial manipulation can improve IBD patient health

**Secondary research questions**

* What is the IBD disease burden in NSW?
* How does the Australian IBD microbiome compare with other populations?

**Background:** Inflammatory bowel disease (IBD) is a global disease challenge and common cause of chronic ill-health among young people in Australia. The inflammatory bowel diseases include Crohn's Disease (CD) and Ulcerative Colitis (UC), chronic idiopathic conditions, for which there is currently no cure. It affects approximately 1 in 250 Australians aged 5 – 40 years, with almost 75,000 Australians having CD or UC, with this number projected to rise to 100,000 within the next 5 years. It is broadly accepted that IBD arises from a dysregulated immune response to alterations in the gut microbiota in genetically susceptible individuals 1. Sufferers can endure numerous attacks or ‘flares’ which can be followed by periods of relative remission, however, the disease trigger remains elusive. All too often IBD confers a lifetime of unpleasant, intrusive and potentially dangerous intestinal inflammation on individuals. Existing treatment modalities are limited by lack of efficacy, toxicity and poor patient acceptability, with high risk of disease recurrence. **Being able to identify people at risk of disease onset**, prior to symptomatology, or by preventing symptom progression would yield significant global social impact and economic benefit and plays to the heart of IBD healthcare, namely to improve patient health.

Until recently most IBD studies have focussed on single time-point sampling – a ‘snapshot in time’ of a patient’s disease 2,3. As we understand more about IBD, it is clear that this approach, whilst yielding valuable information, provides a narrow window into a disease process which is continually evolving and is unique to individual sufferers. This is especially true when considering the role of the gut microbiota, which has been shown to fluctuate greatly over the natural course of an individual’s disease. Two recently published studies have highlighted the strengths of utilising longitudinal assessment of the IBD gut microbiome 4,5. Both studies have provided insight into how the microbiota changes through the natural history of disease and offers potential in terms of determining therapeutic management. However, more studies are needed to validate these findings, both of which assessed European populations. **There is an existing knowledge gap** in terms of defining microbiota changes in IBD in Australia. Different populations have differing genetic risk loci and disease prevalence rates in terms of IBD, they also harbour different gut microbes, in part due to varying environmental exposures and dietary habits 6,7.

**We believe it is timely** to initiate such a study to contribute information on the natural history of gut microbiota changes in IBD in Australia. We will adopt state-of-the-art clinical data and sample collection in a large case-controlled cohort. This will engage our local population of interest and provide the necessary metrics to address our research questions. In so doing, we will generate the requisite infrastructure to prospectively recruit large numbers of new and existing cases of IBD in our target populations, as well as defining a control population, thus creating a powerful predictive analysis tool for the future study of IBD. The ultimate translation of this research will be in 1) the elucidation of microbial changes associated with onset of IBD symptomatology, 2) the identification of an ‘at risk’ microbial signature to allow targeted intervention and 3) the generation of novel predictive models of direct translational utility to the clinic.

The project brings together IBD clinicians from 15 different hospitals including St George, St Vincent’s, Concord, Liverpool, Blacktown, RPA, Sydney Children’s Hospital, Wollongong, Canberra, North Shore as well as an impressive network of translational scientists, molecular biologists, microbiologists, bioinformaticians with an IBD specialist interest from St George and Sutherland Clinical School, UNSW Kensington, UTS, ANU and Sydney Children’s Hospital. This is a new alliance which will act as a focus for IBD research on both a national but also international platform. Creation of the network will allow further investigator-led studies to be initiated including clinical trials. This will facilitate additional larger scale funding applications as well as stakeholder involvement including industry. Through aligning our research capacity, we can maximise our academic excellence, demonstrating world-leading research outcomes and global impact.

**Research Plan**

Study Type

Nested within a longitudinal cohort study of incident and prevalent cases

This is a longitudinal cohort study which is following IBD patients and controls over 24 months and collecting clinical data, patient reported data and biological samples every 3 months. The data and samples will be analysed to understand whether microbial changes can be correlated with clinical data/symptoms and also factors such as diet and genetics. The samples and the data will not be used for any other study unless we specifically seek additional HREC approval.

Setting/Location

Site #1

Department of Gastroenterology

St George Hospital, Gray Street, Kogarah, NSW 2217

Site #2

Department of Gastroenterology and Liver Services

ACE Unit, Level 1 West, Concord Repatriation General Hospital, Hospital Road, Concord, NSW 2139

Site #3

Department of Gastroenterology

Liverpool Hospital

Corner Elizabeth and Goulburn Streets

Liverpool NSW 2170

Site #4

Department of Gastroenterology and Hepatology

St Vincent’s Hospital, Sydney

390 Victoria Rd, Darlinghurst, NSW 2010

Site #5

Department of Gastroenterology

Sydney Children’s Hospital

High Street, Randwick, NSW 2031

Site #6

Department of Gastroenterology

Blacktown Hospital

18 Blacktown Rd, Blacktown, NSW 2148

Site #7

IBD Research, and, Gastroenterology and Hepatology Unit

Lvl 5, Bldg 10, Canberra Hospital

Yamba Drive, Garran ACT 2605

Site #8

Royal Prince Alfred Hospital

Missenden Rd

Camperdown, NSW 2050

Site #9

Wollongong Hospital,

Loftus St

Wollongong 2500

Site #10

Royal NorthShore Hospital

Reserve Road

St Leonards

NSW 2065

Site #11

Prince of Wales Hospital

Barker St,

Randwick

NSW 2031

Site #12

Westmead Children’s Hospital

Corner Hawkesbury Road and Hainsworth Street,

Westmead

NSW 2145

Duration of Study

Anticipated start date: 01/03/2019

Anticipated finish date: 28/02/2024

**Methodology**

### **Acquisition of cases and controls:** The IBD Research Consortium brings together clinicians from 15 different hospitals including St George, St Vincent’s, Concord, Liverpool, PoW, Blacktown, RPA, Sydney Children’s Hospital, Wollongong, Canberra, North Shore Hospitals, all of whom have sizable IBD patient populations (estimate ~ 10,000 patients). The most recent data, from 2011, shows an estimated IBD prevalence in Australia of ~ 100,000 with NSW having an estimated prevalence of 343/100,000 population which equates to almost one-third of the country’s IBD burden8, with almost 25,000 NSW residents living with IBD with the figure projected to increase by 20% in the next 5 years. Current disease incidence data in NSW, in both adult and paediatric populations are lacking but based on these figures we anticipate 1000 new diagnoses of IBD (Crohn’s disease and ulcerative colitis combined) per annum. The IBD Research Consortium will be the platform for case ascertainment and recruitment at study entry.

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| --- | --- |
| Study Population | There will be 2 major groups recruited for study (see figure)   1. **Patients with IBD (Crohn’s disease and ulcerative colitis)**   IBD cases will be collected across all recruiting centres over 24 months and used to calculate contemporary rates of annual disease incidence. Cases will be identified through a designated clinician and pathologist at every site. Patients will be identified through IBD multi-disciplinary meetings or by screening referral letters prior to scheduled hospital attendance e.g. clinic lists. Diagnosis by the “Copenhagen Criteria” and phenotyped according to the Montreal Classification to CD, UC, IBD-U. Diagnosis of IBD will be confirmed as per recognised criteria by local clinical team, and patients phenotyped and consented. Detailed clinical data for accurate phenotyping of disease will be collected including history of disease, treatment exposures and complications – see appendix CD and UC phenotyping data sheets.  Disease activity will be assessed, in adults, using the Crohn’s disease activity index in Crohn’s – Remission <150 (without corticosteroids), mild-mod 151-300 and severe >301. The Mayo score will be used for UC (remission ≤2 with no subscore >1 without corticosteroids; mild – mod 3-6; severe 7-12). See appendix.  For children, using the paediatric Crohn’s disease activity index, Remission <12.5 and paediatric UC disease activity, Remission < 10 will be used.   1. **Controls without IBD:**   Healthy controls will be recruited from a) partner/spouses and family members living with the IBD case participants and b) population controls. Exclusion criteria for healthy controls include: 1) Probiotics within 1 month of sampling, 2) Antibiotic, corticosteroid or immunosuppressive therapy within 3 months of sampling 3) Any active infections 4) Gastroenteritis or international travel within 1 month of sampling 5) Irritable bowel syndrome, bowel surgery or autoimmune disease 6) Pregnancy    The primary analyses will be independent for Crohn’s disease and ulcerative colitis and will utilise a combined pool of healthy controls. Recruited at 1:1 we will have 500 cases and 500 controls. |
| Recruitment/ case identification and consent | The **AIM** study will be advertised through national and local media, social media, a poster campaign and letters / leaflets directed to patients (‘patient’ includes parent/carer from here on throughout protocol) at participating centres. We have 2 versions of the poster, one for IBD patients and one for healthy volunteers. We intend to display both posters within patient areas at each clinical site which will include gastroenterology waiting rooms where IBD patients and their families are attending for appointments. Both IBD patient and healthy control posters will be on display as family relatives are a relevant control group and are usually highly committed based on their wish to support their family member with IBD. We also plan to work with the national IBD charity – Crohns Colitis Australia who have agreed to help us make their members aware of the study. We plan to send the posters to CCA for inclusion on their website or for them to email their members directly.  We also plan to recruit healthy volunteers from outside of the hospital environment including within UNSW. Separate UNSW specific ethical approval will be sought for this purpose.  Investigators will review the suitability of patients scheduled to have colonoscopies and/or attending IBD clinics and will recruit those suitable at the point of contact and provide further written information on the study. Patients determined to require colonoscopy as standard care by physicians and/or attending inflammatory bowel disease clinic will be informed of the study by the investigators during consultation.  The study will be introduced to potential participants by an investigator during a clinic/study visit. The purpose and requirements of the study will be explained (with the assistance of an interpreter as required). If a patient expresses interest in finding out more about the study, they will be given the Participation Information Sheet to take away and read. They will also be encouraged to discuss this with their GP and family. The investigator may ask permission to call the person in a few days to ask whether they have further queries.  If the participant wishes to join the study, they will be asked to complete the consent form at the next suitable opportunity, during which any questions regarding the study will be answered before they give consent. For patients, the written informed consent process will provide approvals in consenting individuals for access to current and future medical records, electronic input of clinical and research data, cross-linkage to centrally held databases, secure storage of clinical data on study databases, and importantly for future collection of biological materials (e.g. genomic DNA, whole blood, serum, stool, urine, biopsies & surgical specimens) for the purpose of genetic analysis (including whole-genome sequencing) and metagenomic microbiota sequencing. Age-appropriate materials will be provided throughout for paediatric patients. The consent process builds on and consolidates established processes across the various partner clinical sites in adults and paediatric settings, but for the purposes of **AIM**, this process will be achieved using a new purpose-written ethical approval/consent process.  Refusal to participate will not result in any discrimination or reduction in the patient's level of care or any other penalty.  Control participants for this study will be recruited from a) spouses/partners and family members living with the IBD affected study participant during the IBD participant’s clinical consultation, and b) populations controls recruited through poster advertisements and word-of-mouth. Upon consent, control participants will provide biological samples throughout the study at the same time points as IBD patients. For relatives of IBD patients this may mean recruitment at the same clinic appointment that their IBD affected family member is being seen as they are most likely to accompany their IBD affected family member to regular IBD follow-up encounters.  Below depicts the sampling strategy for both IBD patients and control participants.      **Data Linkage**  We are requesting permission from participants to undertake linkage of AIM study data with state-owned databases including Cancer Registry and NSW State Health records. This linkage is to allow us to further interrogate the valuable dataset we will have collected to allow for outcome data analysis to be undertaken such as assessing cancer incidence rates associated with IBD. The data linkage aspect of the study is not completely defined and will be developed at a later date. This linkage will require an additional HREC application to be made to the Population Health HREC. This application will be made in conjunction with the Centre for Health Record Linkage (CHeReL). Data linkage will not be required in the early stages of the project therefore this additional ethics process will commence later in 2018. |
| Number of patients  We aim to obtain a total of 1000 participants over the 5-year period of this study. The participants will be from four groups:   1. Group 1: Patients with Crohn’s disease (n=250) 2. Group 2: Patients with ulcerative colitis (n=250) 3. Group 3: Spouses/partners/family members (n=250) 4. Group 4: Population controls (n=250)   According to an *a priori* power analysis, based on previously published measures of microbial community structure (alpha diversity), assuming a standard deviation of 44% of the group mean and an alpha error probability of 0.5 and 80% power, we need to recruit 190 participants per group. Therefore, with group sizes of 250 we will recruit sufficient to deal with losing a proportion of recruits through the study duration. | |
| Key Inclusion Criteria  Participants must be:   1. Aged between 6 to 80 years old at study entry 2. Able to give informed consent/assent 3. Confirmed CD or UC (Copenhagen criteria (adults) and PCDAI and PUCAI (paediatrics)) (physician global assessment) or newly-diagnosed 4. For control groups, no history of gastrointestinal inflammation or IBD | |
| Key Exclusion Criteria   1. Female participants must not be pregnant or breast-feeding at time of recruitment into study 2. Unable to provide informed consent (patient or parent/carer) or comply with follow-up | |
| Study Participation Requirements | 1. *Patients with Inflammatory Bowel Disease*   Male and female (nonpregnant) patients with either ulcerative colitis or Crohn's disease undergoing follow-up colonoscopy for disease activity assessment will have samples of large bowel mucosa, peripheral blood, urine and stool collected at colonoscopy (stool and urine collection tubes/instructions will be provided in advance, so patients can bring samples to their colonoscopy appointment).  IBD patients who are eligible for the study but are not requiring colonoscopy, will be invited to participate in the study by local research teams either at the current clinic visit, a future scheduled clinic visit or a separate visit to the local clinical research facility. At this appointment, peripheral blood, urine oral swab and stool will be collected (stool and urine collection tubes/instructions will be provided in advance, so patients can bring samples to their appointment).  All IBD patients will be asked to complete a series of validated questionnaires detailing patient reported outcomes/environmental exposures as well as dietary habits. Completion of the questionnaires will be requested at study entry and at the end of 12 and 24 months. A limited patient reported measure of disease activity will also be requested alongside 3 monthly stool sampling. The study database for the AIM study has been developed within REDCAP – a secure encrypted database. The database contains all clinical data collection tools, baseline demographic forms, patient self-completed questionnaires. During clinic or study appointments, the study clinical team will complete the various data forms within REDCAP. Study participants will be given the option to complete their forms electronically (via a unique participant link – sent via email) or if they prefer forms can be completed on paper copy which will be subsequently transferred to the database.  To allow further detailed analyses of the gut microbiota, patients will be asked to provide oral swab and stool samples, for microbiota analysis and measurement of host protein levels including faecal calprotectin, every 3 months for 24 months. Patients will be asked to self-collect samples at home (following previously provided instructions) and return to the central processing lab (MRC at St George Hospital) in postage paid envelopes. In the event that patients experience an increase in symptoms, they will be advised to contact their IBD clinical team as per usual clinical management, but they will be requested to provide additional oral swab and stool samples during the episode of flare (defined by a standardised clinical definition as documented in the clinical data collection tool), in addition to the 3-monthly regular request.   1. *Population controls*   (Nonpregnant) partner/spouse and family members of case participants will be invited to participate in the study as controls by local research teams when they accompany their IBD affected family member to clinic appointments. Population controls will be recruited through publicity and word of mouth and recruited at clinical research facilities in specific AIM study clinics. Upon consent, peripheral blood, urine, oral swab and stool will be collected in the same manner as IBD patients, (stool and urine collection tubes/instructions will be provided in advance, so participants can bring samples to their next follow-up appointment).  The study database for the AIM study has been developed within REDCAP - a secure encrypted database. The database contains all clinical data collection tools, baseline demographic forms, patient self-completed questionnaires. During clinic or study appointments, the study clinical team will complete the various data forms within REDCAP. Study participants will be given the option to complete their forms electronically (via a unique participant link – sent via email) or if they prefer forms can be completed on paper copy which will be subsequently transferred to the database. All healthy controls will be asked to complete a series of validated questionnaires detailing participant reported outcomes/environmental exposures and dietary habits. Completion of the questionnaire will be requested at study entry and at the end of 12 and 24 months.  To allow further detailed analyses of the gut microbiota, participants will be asked to provide oral swab and stool samples, for microbiota analysis and measurement of host protein levels including faecal calprotectin, every 3 months for 24 months. Participants will be asked to self-collect samples at home (following previously provided instructions) and return to the central processing lab (MRC at St George Hospital) in postage paid envelopes. A limited participant reported measure of wellness will also be requested alongside 3 monthly stool sampling.  Significant discussion within the AIM study clinical/scientific committee was undertaken in relation to the 3 monthly sample collection strategy. Whilst requesting participant samples every 3 month was felt to be quite onerous on participants, the purpose of regular sampling was to aim to establish a comprehensive understanding of a participants gut microbiota and associated reported outcomes/environmental exposures and host responses to act as a baseline for patients who experience a disease flare. By regularly sampling we increase the chances of collecting samples prior to symptom exacerbation thus maximising on pre-symptomatic sample collection. |
| *Sample and Data Collection*  For participants undergoing colonoscopies, up to six additional intestinal tissue samples will be collected along with the biopsy samples that are routinely taken during this procedure for clinical management. Approximately 20ml of blood (4 teaspoons) will also be collected along with the blood tests that are routinely taken at this time. In addition, patients will be asked to provide a urine sample (50 ml) in individual sterile collection tubes, an oral swab, and a stool sample will be collected by the participant before commencing bowel preparation the previous night (collection kit/instructions provided in advance).  For participants attending IBD outpatient clinics or participants attending a clinical research facility, 30ml of blood (6 teaspoons), 50 mL of urine, oral swab, and a stool sample will be collected when they initially enter the study. Collection kit/instructions will have been provided in advance of the appointment.  After collection, all samples will be processed and stored in a –80oC freezer. Blood samples will be de-identified/coded and stored in each clinical location until they are batch transferred for analysis to one of the named analysis partners.  Collected urine samples will be aliquoted/divided, de-identified and stored in a –80oC freezer. Urine samples will be batched and transported to analysis laboratories e.g. Bioanalytical Mass Spectrometry Facility UNSW for proteomic/metabolomic analysis. The de-identified samples will be stored in a secure –80oC freezer for the duration of the study.  Collected oral swabs and stool samples will be processed to assess microbiota profiles. Collected oral swabs and stool samples will be posted from participant homes to the Microbiome Research Centre laboratories at UNSW, St George Hospital. Postage will be using pre-paid envelope. Analysis will include nucleic acid content assessment using metagenomic and metatranscriptomic approaches as well as proteomic/metabolomic analyses which will be conducted in collaboration with Bioanalytical Mass Spectrometry Facility UNSW or similar facility. Stool samples will also be used to determine faecal calprotectin levels and other novel faecal markers.  Mucosal samples, collected at clinically indicated colonoscopy, in addition to the routine biopsy samples taken during the colonoscopy procedure of consented participants, will be stored in a –80oC freezer at the recruiting hospitals. Subsequently samples will be de-identified, collated, and transported to the Microbiome Research Centre at UNSW, St George Hospital for assessment of gut microbiota profiles. The de-identified samples will then be stored in a secure –80oC freezer at the MRC for the duration of the study.  Clinical/phenotyping information will be de-identified and recorded on paper proformas and a computer-based database for statistical analysis.  Local research teams will extract detailed baseline phenotyping of patients from participant interviews and review of clinical records/local databases. The minimum baseline dataset will incorporate:   * age, gender, ethnicity, date of diagnosis, and smoking status * Past medical history, current medications, surgical interventions, family history of IBD and colorectal cancer, * Full blood count, U&E, LFTs, albumin, CRP, ESR, B12, folate, Fe studies, vitamin D * Stool cultures and clostridium toxin; faecal calprotectin * Disease location and disease behaviour (Clinical activity indices for all IBD patients and additional Endoscopic scoring for IBD patients undergoing colonoscopy. Both documents are attached to the ethics application.)   Baseline phenotyping of control subjects will be generated from participant interviews. The minimum baseline dataset will incorporate:   * age, gender, ethnicity, date of diagnosis, and smoking status * Past medical history, current medications, surgical interventions, family history of IBD and colorectal cancer,   All blood and tissue samples will be collected in either Gastroenterology Ambulatory suites or blood collection units by qualified personnel. Urine, oral swabs and stool will be self-collected by patients at home using collection packs provided in advance.  We would also like to access previously collected pathology specimens for the purpose of understanding microbial changes that are relevant to IBD. For example, to track whether particular microbial signatures can be seen in previously collected clinical specimens. This will be undertaken through collaboration with Pathologist Dr Ewan Miller who will identify previously collected pathology specimens from our study cohort, in the form of paraffin embedded tissue blocks and provide sections for analysis to characterise microbial signatures.  *Questionnaires*  Participants will be asked to fill in a Food Frequency Questionnaire and a food avoidance questionnaire online in one of their visits to the clinic (or they can be given a user login and password to fill in the questionnaire at home). These questionnaires are aiming to evaluate the participants’ usual eating and drinking habits over the last 12 months (e.g. What type of spread or oil did you usually put on your bread? with the following answers to select from: none, butter, butter-margarine blends [e.g. Devondale Extra Soft or Dairy Soft, Western Star spreadable varieties], margarine, olive oil).  Participants will be asked to record every food item they eat in a specialised smartphone application for 5 days (4-week days and 1 weekend day, not necessarily consecutive). If they are not smartphone users, they will be provided with a 5-day diet diary which they will be asked to fill in and return in their next visit to the clinic (See attached). Participants will complete this at study entry, at 12 and 24 months.  All participants will also complete a limited participant reported outcomes measures score (attached to ethics application) which will be returned with their 3 monthly stool and oral samples.  Participants will also be asked to complete a lifestyle factor questionnaire during their visits to the clinic (or they can be given a user login and password to fill in the questionnaire at home). This lifestyle questionnaire is aiming to evaluate the participants’ usual lifestyle habits over the last 12 months (e.g. level of exercise, sleep habits, travel habits, use of health supplements and complementary medicines. A paper-based format will be available for participants who cannot complete it online (See attached). Participants will complete this at study entry, at 12 and 24 months.  For all participants in this study, the researchers may access their hospital records for demographic and/or clinical information solely for the purposes of this study. | |
| **Analysis Plan**  **Blood samples:** Blood samples will be used to a) analyse genome-wide SNP data which will allow us to incorporate genetic risk profiles into our analyses. Previous work has shown this to be useful in sub-classifying disease. While testing for genetic signatures predisposing to flare will be underpowered in the current collection, the samples will be valuable to contribute to international collaborative efforts, b) to assess the immune cell compliment of blood (measuring immune cell types) and also their function (measuring cytokine/soluble factor production) also referred to as immune cell activity.  **Urine samples:** Urine samples will be used to study systemic proteomic/metabolomic profiles in relation to various demographic and clinical and microbial parameters.  **Stool, oral swabs and mucosal biopsy samples.** Microbial nucleic acids (DNA and RNA) will be extracted from oral swabs and stool samples collected in preservation buffer using standardised, locally validated protocols and snap-frozen mucosal biopsies9-13. We will undertake a combination of 16S profiles and full meta-genomic sequencing of samples from each individual in the study. The 16S profiles are the most widely used (and inexpensive) means for quantifying microbial diversity and will allow our data to be easily integrated with published data. The additional metagenomic profiles will produce high-resolution species classification, assay variation within individual bacterial genes and survey non-bacterial organisms (e.g. viruses and fungi). This will enable us to ask a variety of additional questions beyond primary analyses of diversity, such as whether a particular strain (classified by genetic variants) within a species has the potential to produce flares. We will also future-proof our study collection by also ensuring collected samples are suitable for RNA-based analyses including transcriptomics approaches. Currently these approaches are expensive and out of budget scope but we anticipate costs reducing in the next few years as technology advances. Stool samples will also be used to measure faecal calprotectin levels and metabolomics profiles including short chain fatty acid levels.  Based on current availability there is a possibility that sequencing of patient samples may be done by a sequencing service provider overseas including companies in China. If this is the case, then a specific contract will be in place to cover the process which will indicate that samples will be sequenced but that all analysis and data generated will be sent back to the AIM study research team. No identifiable data will accompany samples sent for sequencing.  **Paraffin embedded tissues.** We will use fluorescent microscopy techniques and sequencing approaches to analyse microbial signatures of previously collected pathology specimens. We have developed and published protocols for visualising microbes within patient paraffin embedded tissues which can help us build up a picture of how microbes impact on host health. We also intend to develop molecular techniques to allow us to apply sequencing approaches (as described above) to these archival samples. This is an area of method development as techniques are currently insufficiently sensitive to make this feasible for microbial analysis – however we hope that improvements, within the lifetime of this study will make this a possibility.  **Eating habits and dietary analysis**  Dietary data collection and nutritional analysis will be overseen by Dr Samocha-Bonet (Diabetes & Metabolism Division, Garvan Institute of Medical Research and St Vincent’s Clinical School, UNSW) with additional support from Dr Kelly Lambert (Department of Clinical Nutrition, Wollongong Hospital). Analysis will initially look for associations between dietary habits and clinical reported measures. However, a more in-depth analysis looking to correlate dietary habits with microbiota changes and clinical reported measures will be undertaken. | |
| Statistical Considerations/Data analysis | We will initially produce descriptive analyses of the distribution of measured parameters in the study cohort. These will be presented as mean/sd for normally distributed variables, and median/interquartile range for those not normally distributed. We will present similar statistics for the subjects in whom flares did and did not occur and will assess the differences between these populations using a t-test for normally distributed or Mann–Whitney–Wilcoxon test for non-normally distributed parameters. We will then examine the flare rates of the groups firstly in univariable analyses (assessing the difference in proportions of the groups experiencing flare), and then in multivariable Cox models in which we will consider as potential confounders baseline variables including disease type, location, behaviour, smoking status, therapy, CRP and faecal calprotectin.  We will test for associations between measured parameters and microbiome measures, listing all phyla, genera, species or pathway that correlate significantly (Benjamini-Hochberg FDR < 0.05) with measured parameters under a Kruskal-Wallis test.  We will generate two plots, each showing all samples on the first two principal coordinates of clades (species) and pathways (KO terms), coloured by measured parameters. Principal coordinates will be calculated using the Bray–Curtis distance.  **Longitudinal analysis approach.** All samples will be stored for a similar length of time, prior to extraction, to reduce potential bias introduced through storage.In patients who flare, gut microbiota comparisons will be made between samples collected before, during and after flare and compared with baseline. |
| Ethical Considerations | There is no proposed incentive/payment (eg. movie tickets, food vouchers) or reimbursement (eg travel expenses) to participants.  Information collected from research subjects will be included in their medical file/medical records. This information will be identifiable, as it is needed for hospital/clinic records, for management of patient care and the provision of source documents for data quality assurance. Identifiable information will be retained onsite. Only de-identified data will be entered into the study proformas and applicable de-identified documents taken out from the site where necessary.  The de-identified data is potentially re-identifiable only onsite where a subject list will be retained by the investigator on the hospital computer network. The de-identified information is needed to maintain anonymity of subjects and eliminate bias in the data analysis of the study.  All data will be coded with a patient specific study number which will comprise of a site reference and a patient specific ID. The researchers will not receive the name or address details of the patient and will be unable to identify the patient from the study code.  Potential is AIM-xxxx(site+patient)-yyy(sampletype and number)  The patient would be able to be identified onsite in order to contact them regarding pertinent study information, sample reminders, as well as to review medical records and test results to ensure that data collected is correct and accurate.  At any time, participants can choose to withdraw from all or any individual sample collection without effect on their standard of care or relationship with the study staff. Participants can also withdraw their consent and/or request for no further involvement in the study. In this situation it will be possible to remove existing samples from the study, but existing data and sample analysis data will remain in the study. |
| Safety Considerations | This research project involves collecting extra tissue and blood samples during ‘standard of care’ colonoscopic procedures. No patient will undergo colonoscopy for the sake of this research study alone. The increased risk of bowel perforation from taking extra biopsies during colonoscopy is low. Urine and stool samples, that are requested for the research study, may or may not be in addition to standard of care tests. However, all equipment and instructions relating to patients self-providing these samples will be provided.  Therefore, the risk of harm or discomfort to study participants is minimal: Redness and swelling of the veins due to longer duration of vein access during blood collection (inflammation); Perforation in the bowel from extra biopsy sample collection. |
| Investigator obligations | All study materials will be maintained at the respective clinical centres involved in recruiting study participants. The study database is password protected and will only be accessible by researchers.  Study proformas will be locked in a cabinet only accessible by researchers. Study identifiers will not be removed at the completion of the study. All study related documents will be archived appropriately after completion in accordance with applicable laws and regulations.  Copies of all study documents will be retained by the investigators in a secure and safe location for thirty years following completion. These documents will be retained for a longer period however, if required by regulatory requirements. |
| Funding | Funding for the study is through a GESA collaboration grant, the Microbiome Research Centre and local hospital funds. Additional funding applications will be sought as they are identified. |
| Dissemination | The AIM study (and associated funding) will be promoted through institutional websites, scientific presentations at national and international meetings and annual reports and publicity arising from the AIM study.  We will communicate the results directly back to our patient population through our website, social media, regional meetings and pamphlets / newsletters. We will look to work with charitable backers including IBD charities to highlight the importance of the disease area and our research efforts. |
| Conflict of Interest | No conflicts of interest have been identified at this point in time. |

**AIM Study Schedule**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Assessments and Recordings** | **Screening + Baseline (Day 0)** | **3 months** | **6 months** | **9 months** | **12 months** | **15 months** | **18 months** | **21 months** | **24 months** |
| Medical History/Baseline Health/Baseline Medications  **All participants** | x |  |  |  |  |  |  |  |  |
| Basic Demography  **All participants** | x |  |  |  |  |  |  |  |  |
| Inclusion/exclusion criteria  **All participants** | x |  |  |  |  |  |  |  |  |
| Participant information + informed consent  **All participants** | x |  |  |  |  |  |  |  |  |
| Disease activity scoring and disease phenotype assessment (disease and age dependent)  **IBD patients only** | x |  |  |  | x |  |  |  | x |
| Blood sample collected | x |  |  |  | x |  |  |  | x |
| Urine sample collected | x |  |  |  | x |  |  |  | x |
| Endoscopic scoring\*  **All participants** |  |  |  |  |  |  |  |  |  |
| Patient Reported Outcome Measures (PROM)  **IBD patients only** | x | x | x | x | x | x | x | x | x |
| Dietary Questionnaire (including Food Intolerance Short Questionnaire + Food intake diary+ SF-36)  **All participants** | x |  |  |  | x |  |  |  | x |
| Impact III questionnaire  **IBD patients only** | x |  |  |  | x |  |  |  | x |
| Baseline Demographics Adult/Children (self-reported)  **All participants** | x |  |  |  |  |  |  |  |  |
| Stool and Oral sample collection (home collection and posted to MRC laboratory) | x |  |  |  | x |  |  |  | x |

\*Endoscopic scoring is applicable to any study participants who undergo colonoscopy as part of their clinical management. The scoring will be completed for each colonoscopy.

Green = Study team completed/requested (urine sample)

Orange = Participant completed

Grey = Not completed

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