Symposium 207

Gut Microbiome and Mucosal or Systemic Dysfunction: Mechanisms, Clinical Manifestations and Interventions

May 19 – 20, 2017
Brisbane Convention & Exhibition Centre
Brisbane, Australia

Abstracts
Poster Abstracts
Abstracts of Invited Lectures
Poster Abstracts

Symposium 207

GUT MICROBIOME AND MUCOSAL OR SYSTEMIC DYSFUNCTION: MECHANISMS, CLINICAL MANIFESTATIONS AND INTERVENTIONS

Brisbane, Australia
May 19 – 20, 2017

Scientific Organization:
G. Holtmann, Brisbane (Australia)

Co-Organization:
W.D. Chey, Ann Arbor (USA)
P.R. Gibson, Melbourne (Australia)
M. Morrison, Brisbane (Australia)
M. Simrén, Gothenburg (Sweden)
N.J. Talley, Newcastle (Australia)
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H.M. Mitchell, Sydney

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Session I

The fundamentals and how to determine what really matters
Prevalent but uncharacterised microbial populations in the human gut microbiome

Philip Hugenholtz, Ph.D.
School of Chemistry & Molecular Biosciences, Australian Centre for Ecogenomics, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia

The structure and function of the human gut microbiome is rapidly being revealed through culture-independent molecular techniques. Despite a great deal of interpersonal variability due to factors such as diet, age and ethnicity, there are a small number of relatively abundant microbial species that are widespread in the human population. Some of these are well known, such as \textit{Faecalibacterium prausnitzii}, but others are as yet uncultured and uncharacterised. In this talk, I will introduce the audience to some of the lesser-known but potentially important members of the human gut microbiome.
Crosstalk between microbiota, pathogens and the innate immune responses

Univ. Prof. Dr. Jan Wehkamp
Medical Department 1, University of Tübingen, Germany

It is nowadays generally accepted that the microbiome is a central driver of chronic inflammatory bowel diseases based on observations from human patients as well as inflammatory rodent models. Many studies focussed on different aspects of microbiota and some there is evidence that the microbiome itself can impact on the host and inflammatory situation. It is also clear that the microbiome is influenced by environmental and genetic factors and is also tightly regulated by host defense molecules such as antimicrobial peptides (defensins et al.). Different lines of investigations showed different complex antimicrobial barrier defects in inflammatory bowel diseases which also influence the composition of the microbiome and generally impact on the microbial-mucosal interface. This talk will discuss some of the mechanisms of different aspects how the microbiome and the host interact and how this can be used therapeutically. Altering the microbiome by using defensins is a potential therapeutic avenue which is currently under development. This emerging research field opens new perspectives and is fastly growing. Many of the mechanisms which alter the host microbial interplay might also be important for other diseases outside the intestine.
Morphology of inflammatory changes of the mucosa: Time to reassess inflammatory responses of the mucosa

Prof. M.M. Walker
School of Medicine & Public Health Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW 2308, Australia

Classical microbial pathogens which infect the gut from oesophagus to rectum have characteristic immune responses which are easily recognized on histology, although biopsies are not always performed and often these are diagnosed clinically or by microbiology. Easily recognised microbes on histology include oesophageal candida and herpes virus (often seen in immunosuppression), cytomegalovirus in the colon in inflammatory bowel disease and visible parasites such as giardia lamblia in the small intestine which cause diarrhoea. Since the relatively recent (1984) description of gastric infection by *Helicobacter pylori* this bacteria is now well recognised as the cause of peptic ulcers and is a declared WHO Class I cancer pathogen\(^1\). The gastric pathology of infection is defined by the Sydney classification. These histologies are often florid, have overt epithelial damage and are principally neutrophil driven, as migration of neutrophils to sites of injury and infection represent a significant change to the tissue microenvironment and are easily recognised on microscopy\(^2\).

Histopathology can recognise disorders of the immune system that lack macroscopic pathology, for example, microscopic colitis, an entity distinguished by presenting symptoms (watery diarrhoea) and characteristic histology with an increase in intraepithelial lymphocytes and/or a collagenous band underlying the epithelium\(^3\). The cause is likely multifactorial involving mucosal immune responses to luminal factors in a genetically predisposed individual, and recently alterations in the microbiome with significantly lower numbers of Akkermansia spp\(^4\) described.

Recent global studies also describe subtle gut pathologies shown to underlie functional gut disorders. These are distinguished by abundant eosinophils and mast cells, which are normally present and responsible for mucosal homeostasis. In excess these cells hallmark innate immune dysregulation and possibly a response to allergens or a disrupted gut microbiome giving rise to patients’ symptoms of abdominal pain and disordered gut motility\(^5\). Duodenal eosinophilia is described in subsets of patients with functional dyspepsia, particularly in those with symptoms of post prandial distress\(^6\). In those with non-constipating irritable bowel syndrome, eosinophil clusters have been noted alongside colonic spirochaetosis\(^7\). Excess mast cells are described in colonic mucosa in irritable bowel syndrome, in some patients with diarrhoea\(^5\).

Histopathology should mirror our increasing understanding of immune dysfunction and dysbiosis in the gut and we need to work in tandem with clinical and molecular colleagues to continually refine our diagnostic acumen and also help target therapies to cause of disease.
References:


Making it all fit – Statistical challenges and new data analysis methods for integrating microbiome data with other clinical measures

Dr. Kim-Anh Lê Cao¹,²
¹The University of Queensland Diamantina Institute, Woolloongabba, Brisbane, Australia
²School of Mathematics and Statistics and Centre for System Genomics, The University of Melbourne, Australia

The advent of high throughput technologies has led to a wealth of biological data coming from different sources, the so-called ‘omics data (transcriptomics for the study of transcripts, proteomics for proteins, metabolomics for metabolites, microbiome, the study of microbial communities). In order to understand biological mechanisms and uncover important biological insights, we need to adopt a holistic and systems biology approach to analyze those complex data.

Univariate statistical approaches have been focusing on identifying key biological variables to explain or model biological conditions or phenotypes by considering each biological variable independently. To shift the univariate analysis paradigm, we have developed several multivariate methods based on Projection to Latent Structure (PLS) to identify a subset of variables - a ‘molecular or microbial signature’. I will first discuss the analytical and statistical challenges due to the inherent properties of microbiome data, including sparse counts, compositional data, batch effects and the need to model microbial communities as a whole.

I will introduce multivariate statistical models we have recently developed for microbiome 16S data to identify and compare bacteria driving changes in their ecosystem and illustrate the potential of such methods in several case studies. Our methods are available in the R package mixOmics (www.mixOmics.org).

Keywords: beta diversity, key indicator species, dimension reduction, feature selection.

Biography (short)

Dr Kim-Anh Lê Cao (The University of Queensland Diamantina Institute, Brisbane Australia, and recently affiliated to The University of Melbourne) is an expert in multivariate statistical methods and novel developments. Since 2009, her team has been working on developing mixOmics dedicated to the integrative analysis of ‘omics' data, including microbiome data, to help researchers mine and make sense of biological big data (http://www.mixOmics.org).
Assessment of symptoms in FGID and IBD studies that target the GI microbiome

Michael P. Jones
Psychology Department, Faculty of Human Sciences, Macquarie University, North Ryde, NSW 2109, Australia

Assessment of gastrointestinal symptoms has traditionally followed the model of clinical examination in which a patient or research subject is asked directly about symptoms, commonly, over the previous 3–6 months. The particular symptoms elucidated and the period covered varies depending on the nature of the disorder and may be determined by consensus criteria such as the Rome criteria for functional gastrointestinal disorders. Much attention has been placed on which symptoms should be measured and in how much detail according to clinical experience and theorized pathophysiological mechanisms. At this level symptom measurement approaches are quite specific and based on science.

Challenges however arise when matching clinical logic with measurement theory. When measuring psychological symptoms it is usual to apply rigorous, quantitative psychometric methods to the development and validation of measurement instruments. Gastrointestinal symptom scales have not traditionally adopted this level of rigor. Exceptions include the gastrointestinal symptom rating scale (1) and some GI-specific quality-of-life scales, such as the Nepean Dyspepsia Index (2). Other symptom questionnaire instruments, such as those used by the Rome criteria, tend to have paid less attention to the instruments used to evaluate symptoms. Potential problems in common symptom evaluation instruments include recall bias (3) and understanding of terminology.

This talk will outline past approaches to symptom measurement and more recent approaches that offer advantages in conforming more closely to principles of measurement theory. A thesis will be offered that measurement tends to be more reliable when conducted prospectively rather than by recall, particularly when over a long period. A number of approaches will be discussed, including paper-based symptom diaries and electronic versions. A new approach termed ecologic momentary assessment (EMA) which evaluates gastrointestinal symptoms, along with other concomitant symptoms, in-the-moment will be outlined. EMA is not well known in gastrointestinal studies yet but shows great promise in understanding sequencing of symptoms with other phenomena, such as mood disturbance or extra-gastrointestinal symptoms. EMA is typically implemented via mobile telephone apps which prompt the patient or research participant to answer a very short, prospective symptom evaluation.
References:


Session II

Microbiome-host interactions
Faecalibacterium prausnitzii and other next-generation probiotics to prevent and to treat gastrointestinal disorders and diseases in humans

Commensal and Probiotics-Host Interactions Laboratory, Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

In 2008, we have identified F. prausnitzii as the first anti-inflammatory commensal bacterium detected on the basis of human clinical data and validated in acute high-dose TNBS colitis model\(^1\). Since this finding, diminished prevalence and abundance of Faecalibacterium prausnitzii have been reported in gastrointestinal disorders as inflammatory bowel disease (IBD)\(^2\) and irritable bowel syndrome (IBS)\(^3\). Today, it is well established that the absence of F. prausnitzii is associated with several human dysbiotic diseases and can be thus considered as a biomarker of human health\(^4\).

F. prausnitzii is thus now a major actor in novel preventive and curative strategies required to prevent and to treat gastrointestinal disorders and diseases. Here, we will present all our last results on F. prausnitzii and human health. We will thus describe its beneficial effects in i) a novel chronic inflammation model\(^5\); ii) in a novel chronic low-grade inflammation model to mimic the disorders observed in IBS patients\(^6,7\); and iii) in acute stress models which are neomaternal separation mice model and partial restrain stress in rats\(^8\). Recent data on the mode of action will be also described including i) the novel gnotobiotic model which led us to the identification of anti-inflammatory metabolites potentially produced by F. prausnitzii\(^9\); and ii) the identification of a potent anti-inflammatory F. prausnitzii MAM (for Microbial Anti-inflammatory Molecule) protein\(^10\).

All these recent results confirm the high potential of F. prausnitzii as a potential next-generation probiotic for both IBS and IBD patients. We will also present potential other next-generation probiotics which are lactobacilli able to produce Ahr agonists recently shown to be protective in murine colitis models\(^11\).

References:

5. Martin et al. 2014. Inflammatory Bowel Disease.
How does the gut microbiota affect the epigenome?

M.V. Joglekar and A.A. Hardikar on behalf of the Thrifty Jerry Study Group
Diabetes and Islet Biology Laboratory, NHMRC Clinical Trials Centre, University of
Sydney, 92-94 Parramatta Road, Camperdown, Sydney, NSW 2050, Australia

Gut microbes are well-recognised as an environmental factor influencing an
individual’s susceptibility to obesity and Type 2 diabetes. Further studies in a rat model
of multigeneration under-nutrition and nutrient transition (Cell Metabolism, 2015),
demonstrate that:

i) the gut microbiota composition of lean rats differs from obese rats; and
ii) that obese rats benefit metabolically after ingestion of lean rat faeces following coprophagic
feeding behaviour. This corroborates with previous reports suggesting associations
between the gut microbiome and metabolic syndrome (diabetes and obesity). Similar
human studies confirm that metabolic benefits can be derived through faecal
transplants; however, underlying mechanisms are not yet fully elucidated. We
hypothesize that one of the mechanisms is epigenetic modifications of gut epithelial
cells following exposure to microbial metabolites, such as short chain fatty acids
(SCFAs). SCFAs including acetate, propionate and butyrate are end products of
microbial fermentation of dietary carbohydrates, specifically fibres and starches. The
concentration of different SCFAs in the gut depends on the microflora and diet of an
individual. Though these SCFAs are known to impart beneficial effects on gut, liver and
other tissues, their effect on transcriptome and epignome of gut epithelial cells is
largely unknown. We present data demonstrating the potential role of SCFAs on
chromatin conformation (incretin gene expression) as well as small non-coding RNA
expression. We believe that our work sheds light on the basic phenomenon that links
gut microbiota, cellular epigenome and the life-long risk of diabetes and obesity.
A question of culture? Development and differentiation of the mucosal and luminal microbiomes

Dr. Páraic Ó Cuív
The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, Brisbane, QLD 4102, Australia, E-Mail: p.ocuiv@uq.edu.au

Australia has amongst the highest incidences of chronic gut diseases in the world including inflammatory bowel diseases, colorectal cancer and obesity. These diseases are underpinned by specific host genetic, environmental and lifestyle factors however it is now increasingly recognised that the microbial community resident in the gut (gut microbiota) also influence disease risk. Much of our understanding of the diversity and functional capacity of the microbiota has been provided by culture independent 16S rRNA profiling and metagenomic sequencing. These have revealed that the faecal and mucosal associated gut microbiota differ, and that the former is comprised of over 9 million non-redundant genes whose functions are largely unknown. The development of new approaches to isolate and propagate fastidious gut microbes has not kept pace with those of culture-independent approaches nonetheless these often provide the best opportunity to link isolates to function. To address this challenge, we recently developed a new method termed “metaparental mating” that supports the rapid and directed isolation of genetically tractable fastidious gut bacteria. We used this approach to selectively isolate genetically tractable human colonic Firmicutes, including mucosa associated taxa, as these are functionally diverse but significantly underrepresented in existing bacterial culture collections. We have now identified numerous immuno-modulatory bacteria and determined that these functional capacities may be more widespread than previously appreciated. Collectively, this offers new opportunities to link genes with function and transform our understanding of the gut microbiota in gut health.
The role of mucins in shaping the microbiome

Michael McGuckin
Inflammatory Disease Biology and Therapeutics Group, Mater Research Institute – The University of Queensland, Translational Research Institute, 37 Kent St, Woolloongabba, Brisbane, QLD 4102, Australia;
E-Mail: michael.mcguckin@mater.uq.edu.au

All mucosal surfaces have an associated microbiome and all of these surfaces secrete a viscoelastic mucus layer which prevents dehydration, provides lubrication and acts as a biophysical barrier between the mucosal epithelium and microbes. In the gut the mucus layer is very thick, having to defend the mucosa from a large and complex microbial community and the potential presence of pathogens. The major macromolecular constituents of mucus are mucin glycoproteins which homooligomerise into very large molecules that are largely responsible for the biophysical properties of the mucus layer. The mucins are highly glycosylated and these O-glycans mediate molecular interactions with microbes as well as being a source of energy utilised by the microbes living in the outer mucus layer as they degrade the mucus, necessitating continuous replenishment of mucus by secretory cells in the mucosa. The mucus layer also contains and effectively retains and arrays many other types of host molecules capable of reacting with and limiting penetration by microbes including anti-microbial peptides, enzymes and antibodies. In the intestine the major secreted mucin is MUC2 and when this is knocked out in mice a spontaneous dysbiosis and chronic inflammation arises rapidly throughout the intestine but particularly in the colon, demonstrating the importance of mucins in maintenance of intestinal homeostasis and accommodation of the microbiota. Host immunity regulates mucus production and mucin glycosylation thereby retaining a capability to alter the niche occupied by the mucosal microbiota, which in turn can, via secreted metabolites and peptides, modulate the epithelium and underlying immunity. I will discuss how this ancient system functions and how dysregulation of the system can lead to chronic inflammation in the intestine.
How does epithelial cell stress affect the nutrient and physical landscapes of the microbiome?

Simon Keely
Faculty of Health and Medicine, School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia

The intestinal microbiota exists in symbiosis with the host mucosa, offering digestive function, protection and immune-regulation, while benefiting from host-secreted metabolites. Changes to the microbiota community profile are associated with a wide range of chronic digestive diseases although it is not known what causes these changes and it is unclear as to whether this is a consequence or initiating factor of disease pathology.

During mucosal inflammation, vascular tissue damage and the burden of infiltrating immune cells leads to reduced tissue-oxygen tension, where oxygen demand exceeds supply (hypoxia). In response to this hypoxia, cell metabolism shifts from oxidative phosphorylation to glycolysis, as cells attempt to adapt to conserve oxygen during hypoxic stress.

Our work has focused on epithelial hypoxic stress-responses during intestinal inflammation and we have characterised changes to epithelial-derived metabolite signatures during inflammation, particularly metabolites serving as micronutrients for the intestinal microbiota.

Hypothesising that changes to epithelium-derived metabolites during hypoxic inflammation influences host-microbiota interactions, our work aims to identify whether epithelial micronutrients that are secreted during inflammatory stress are sufficient to alter bacterial gene expression and whether these changes contribute to the altered intestinal microbiota associated with intestinal inflammation. Importantly, these changes may be transcriptional in nature, as individual bacteria attempt to adapt to the altered intestinal microenvironment and thus community profiles at the phylum level would not account for changes in the status of the host-microbe relationship.
The induction of dysbiosis in the small intestine promotes allergic sensitisation

G. Burns¹, B. Goggins¹, K. Minahan¹, M.M. Walker², N.J. Talley², P. Foster¹, J. Horvat¹, S. Keely¹
¹School of Biomedical Sciences and Pharmacy, University of Newcastle; ²School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

Introduction: Food allergy is characterised by a T helper type-2 immune response against a food antigen, manifesting as symptoms including nausea, diarrhoea, vomiting or anaphylactic events. It is estimated that 10% of the Australian population have a food allergy, and common allergens include cow’s milk, shellfish and peanuts. Epidemiological studies have identified antibiotics as a significant risk factor for food allergy in infants. We examined how the broad spectrum antibiotic amoxicillin influenced mucosal immune responses to peanut proteins and the development of peanut allergy in mice.

Methods: Balb/C mice were treated daily with 5 mg/kg amoxicillin or PBS for 5 days (days 0–4). On days 5 and 6 animals received 0.2 mg peanut extract or PBS vehicle by oral gavage. Animals were rechallenged with peanut or vehicle by oral gavage on days 11 and 13 and sacrificed on day 16 and immune responses to peanut challenge in blood and intestinal tissues were assessed by protein, mRNA and histological analysis.

Results: The proportion of circulating eosinophils was increased in the blood of mice treated with both antibiotics and peanut. Histological examination revealed an increase in small intestinal eosinophils, predominantly at the villous tips, indicating recruitment to the mucosa. RNA and protein analysis revealed an increase in IL-5 associated with increased Nod-Like Receptor Protein 3 (NLRP3) inflammasome activation.

Discussion/Conclusion: These studies demonstrate that antibiotic treatment prior to food antigen challenge can lead to altered mucosal immune homeostasis, facilitating IL-5-mediated eosinophil recruitment, characteristic of allergic responses. Importantly, we have demonstrated an adjuvant-free model of food sensitisation and small intestinal eosinophilia. These findings contribute to a better understanding of how mucosal disruption by antibiotics contributes to the development of allergic sensitisation and reaction.
Faecal supernatants from patients with diarrhoea predominant IBS disrupt colonic epithelial barrier function and directly affect colorectal afferent nerves

Hannah Wardill1,2, Joanne Bowen2, Nicole Dmochowska1,2, Melissa Campaniello1, Chris Mavrangelos1, Jane Andrews2,3, Sam Costello4, Patrick Hughes1,2
1Centre for Nutrition and Gastrointestinal Disease, South Australian Health and Medical Research Institute (SAHMRI); 2Adelaide Medical School, University of Adelaide; 3IBD Service, Department Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia; 4Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, SA, Australia

Introduction: Irritable bowel syndrome (IBS) is a chronic debilitating disease of the gastrointestinal tract. Despite no overt pathology, the luminal environment is altered in IBS with evidence of microbial dysbiosis, inflammation and protease activity (Hughes et al. Am J Gastroenterol. 2013). However, it is unclear if these changes actively drive symptoms of altered bowel habit and abdominal pain, or simply reflect a consequence of disease. We aimed to characterise the effect of faecal supernatants (FSN) from patients with diarrhoea predominant IBS (IBS-D) on epithelial barrier function and pain sensing colonic pelvic afferent nerves.

Methods: FSNs were prepared from 10 patients with IBS-D (ROME III) and 8 sex/age-matched healthy controls (HC) at 0.3 g/ml ringers solution. Proteolytic and LPS activity were quantified in IBS(D)-FSN and HC-FSN. IBS(D)-FSN, HC-FSN or vehicle ± protease inhibitor cocktail (PIC) were applied apically and basolaterally to healthy mouse distal colon sections mounted into Ussing chambers. Resistance ($R_{TE}$) and conductance were measured for 2 h. IBS(D)-FSN and HC-FSN were applied to high-threshold potentially nociceptive pelvic colonic extrinsic afferents for 5 min and changes in mechanosensitivity determined.

Results: IBS(D)-FSN had increased proteolytic and LPS activity compared to HC-FSN. IBS(D)-FSN decreased $R_{TE}$ and increased conductance compared to both HC-FSN and vehicle, indicative of epithelial barrier dysfunction. This effect was enhanced when FSN was applied basolaterally. Protease inhibition had a modest protective effect on IBS(D)-FSN-induced barrier dysfunction, but failed to completely prevent changes. IBS(D)-FSN but not HC-FSN directly activated high threshold pelvic colonic afferent endings and sensitised them to mechanical stimuli.

Discussion/Conclusion: Mediators present in IBS-D faecal samples impair epithelial barrier integrity and activate high-threshold potentially nociceptive colonic afferent nerves. Further characterisation of the composition of FSN is required to identify therapeutically relevant targets.
Session III

The gastrointestinal microbiome, diet and management of chronic diseases
Microbes affecting gastrointestinal growth and function: Role of the aryl-hydrocarbon-receptor

Sven Pettersson Ph.D., M.D.
Professor in Metabolic Diseases, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 639798, Singapore

The functional organization of human beings are their ability to perform characteristic activities of life, including growth, reproduction, perception, imagination, desire, and thinking. The discovery of bacteria by Koch and Pasteur introduced new species that could affect human beings. In 1991, Lynn Margulis introduced the “Holobiont” meaning that if two or more bionts coexist for a longer period, they form a holobiont, a human being. The holobiont is an evolutionary conserved functional unit, including interorgan crosstalk with dedicated functions to secure nutritional intake, DNA replication and reproduction. It follows that most, if not all organs within the host respond to changes within the microbiome elicited through diet, exercise, behavior and circadian rhythm. The host organ, in turn, reciprocates, by influencing microbiome communities to further support host organ function. My presentation will focus on recent results of microbiome mediated effects on growth and function of the alimentary tract with a focus on the observed bilateral interplay between the AhR receptor signaling function responding to the microbiome mediated signals. The results presented are relevant to intestinal disorders such as IBD and colorectal cancer.
Effect of eradication therapy of *H. pylori* on gut microbiota

Kentaro Sugano
Department of Medicine, Division of Gastroenterology, Jichi Medical University, Tochigi 329-0498, Japan

*H. pylori* (HP) infection is the major cause of peptic ulcer diseases (PUD) and gastric cancer (GC). Hence, international guidelines and IARC have recommended eradication of this organism as a mean to reduce the disease burden resulting from this infection. For eradication, regimens involving proton pump inhibitor (PPI) combined with two or more antibiotics are the mainstay. The success rates of the standard triple therapy, PPI combined with two of the three antibiotics, clarithromycin (CAM), amoxicillin (AM), and nitroimidazole (NM) have been deteriorated due to antibiotic resistance of HP forcing to use more complicated regimens for prolonged periods, which increase the adverse events like diarrhea, indicating that eradication therapy exerts profound influence on gut microbiota. Although resilience of gut microbiota following antibiotic use was reported, prolonged effects of eradication therapy resulting in altered composition of gut microbiota were also reported. It is worrisome that the spread of antibiotic resistance in other members of gut microbes as demonstrated by the expansion of *erm*(B)-positive microbiota after eradication therapy has been documented. The use of potent acid suppressants also influences the gastrointestinal microbiota due to diminished acid sterilization. In this regard, recovery of acid secretion in patients with gastric atrophy after eradication success drastically diminishes the overgrowth of gastric microbiota that might be contributing to reduce GC.

In Japan, a massive increase in the number of patients receiving eradication therapy occurred after insurance approval, accelerating the decrease of HP. Despite of this increase, the amount of antibiotics (CAM, AM, NM) prescribed for eradication therapy remained a minor proportion among the entire dispensations. The eradication of HP will contribute reducing PUD or GC, but we need to follow long-term trends of other diseases associated with dysbiosis, such as obesity, inflammatory bowel diseases among the patients who received eradication therapy. Furthermore, we need to pay attention to limit antibiotic use in general as the amount of antibiotics used for eradication accounts for a minor proportion with definite merits.
Pre-, pro- and synbiotics for chronic kidney disease

Katrina Campbell, Ph.D.
Nutrition and Dietetics, Princess Alexandra Hospital, Metro South Health, Ipswich Road, Woolloongabba, Brisbane, QLD 4102, Australia

Dietary modification has long been considered a modifiable risk factor for chronic kidney disease (CKD), and a key management strategy in dialysis and its related complications. However the role of diet is rapidly evolving as our understanding extends to the potential for manipulation of the gut microbiota.

Appreciation for the role of the gut microbiota in the generation of uremic toxins associated with kidney disease and cardiovascular disease risk has emerged over the past decade. Gut dysbiosis is commonly observed in kidney disease populations. This contributes to increased risk of cardiovascular disease through a range of mechanisms including inflammation, hypertension and gut-derived toxins including indoxyl sulphate (IS) and p-cresyl sulphate (PCS).

Recent trials in kidney disease have demonstrated the role of prebiotics, probiotics, and their combination as synbiotics to modulate the composition of the intestinal microbiota. Such therapies have demonstrated to modify the production of IS and PCS. Investigations are underway to explore the potential for mitigating cardiovascular disease risk.

This talk will examine the role of the gut microbiome in shaping kidney disease risk, progression, and associated cardiovascular disease. Further, will discuss the potential for improving the microbial diversity through synbiotics. This highlights an important role of the gut in mediating CVD risk, but also reaffirms the role of the overall quality of the diet in the management of chronic disease.
The microbiome and obesity: Cause, consequence or both?

Andrew Holmes
Charles Perkins Centre, The University of Sydney, Sydney, Australia

Virtually all animals are symbioses that incorporate a tight association with microorganisms, especially bacteria. The roles of microbial communities in the life history of animals are incredibly diverse, but arguably none are more significant than those in nutrition and metabolism. A characteristic of modern society has been industrialization of the food supply chain in ways that have dramatically altered the nutrient environment of humans. It is now widely accepted that the global epidemic in chronic diseases has emerged in lock-step with these changes. This raises the question of the role of our microbiome in nutrition-related chronic disease, of which obesity has the highest profile. Although there is overwhelming evidence that gut microbes influence aspects of energy balance and can drive pathological processes in obesity-related diseases, no robust microbial signature of obesity has been identified. This almost certainly reflects the multifactorial nature of the disease as well as the complexity of both nutrition and the microbiome. The geometric framework is an experimental platform to explore interactions in complex systems. Through its application to diet-host-microbiome interactions in the mouse system we have identified how the nutrient environment of an animal fundamentally shapes feedback processes between host and microbiome that contribute to obesity-related disease. I will discuss the potential for new strategies in management of obesity based on recognition of the microbiome as a key contributor to our body’s adaptation to changes in its nutrient environment.
Reducing the maternal dietary intake of indigestible and slowly absorbed short-chain carbohydrates is associated with improved infantile colic: A proof-of-concept study

M. Iacovou1, E.C. Mulcahy1, H. Truby2, J.S. Barrett1, P.R. Gibson1, J.G. Muir1
1Department of Gastroenterology, Monash University, Melbourne, VIC, Australia
2Department of Nutrition and Dietetics, Monash University, Melbourne, VIC, Australia

Introduction: Infantile colic is a common complaint for which parents seek professional advice. In breastfed infants, mothers are often advised to avoid intestinal-gas-producing foods (e.g., onions and legumes). Anecdotal relief of infantile colic when the mother reduced dietary FODMAPs (Fermentable, Oligo-, Di-, Mono-saccharides and Polyols), prompted assessment of the concept that maternal low FODAMP diet might be efficacious for infantile colic.

Methods: Exclusively breastfeeding mothers and their colicky, typically-developing, healthy infants who met the Wessel Criteria for infantile colic were recruited from the community. After assessment of habitual maternal diet, mothers were provided a 7-day low FODMAP diet. Using the validated Barr diary, crying, fussing, sleeping, feeding and awake-and-content durations were captured at baseline and during the dietary intervention. Analysis was corrected for infant's age. At baseline and at the end of the dietary intervention, breast milk was analysed for FODMAP and microbiota content and infant faecal samples for changes in pH and microbiota.

Results: Eighteen breastfeeding mothers (aged 27–40 years) adhered to the diet that reduced FODMAP intake by about 75%. Infants were of gestational age 37–40 weeks and aged 2–17 weeks. At entry, crying durations were a mean [95% CI] of 142 [106–61] min and fell by 52 [178–120] min (p = 0.005; ANCOVA). Combined crying-fussing durations fell by 73 [301–223] min (n = 13; p = 0.007), as did crying episodes (p = 0.01) and fussing durations (p = 0.011). Infant sleeping, feeding, and awake-and-content durations did not change. Infant faecal pH did not change. Breast milk lactose content was stable and other known FODMAPs were not detected. Abundance and diversity of breast milk and infant faecal microbiota changed.

Discussion/Conclusion: Maternal low FODMAP intake may be associated with a reduction in infantile colic symptoms. Crying-fussing durations reduced greater than the clinical significance of > 25%. A controlled evaluation is needed to assess if microbiota change was an effect of diet.
Oral poster presentation

The prebiotic impact of dietary FODMAPs on intestinal microbiota

E.P. Halmos¹², C.T. Christophersen³⁴, A.R. Bird³, J.G. Muir¹, P.R. Gibson¹
¹Monash University, Melbourne; ²Walter & Eliza Hall Institute, Melbourne; ³CSIRO, Adelaide; ⁴Edith Cowan University, Perth, Australia

Introduction: A low FODMAP diet (LFD) is mainstream treatment for managing irritable bowel syndrome (IBS). However, there are concerns that restricting FODMAPs, particularly oligosaccharides, leads to a loss of prebiotic effects and an ‘at-risk’ bacterial profile. Conversely, FODMAPs are prebiotic, but trials have only investigated oligosaccharide supplements, providing supra-physiological doses where background FODMAP intake has not been considered. This study aimed to re-address the impact of changing dietary FODMAPs on microbiota compared to a habitual diet using findings from a blinded cross-over study (Gut. 2015;64:93–100).

Methods: Twenty-seven IBS and six healthy subjects underwent evaluation of their habitual diet followed by randomisation to 21 days of provided low or typical amounts of FODMAPs ('Australian diet') and matched for other nutrients. Five-day faecal samples were collected at the end of each dietary period, pooled, and analysed for bacterial abundance.

Results: Mean daily oligosaccharide intakes for the habitual diet was 3.8 g, compared with LFD 1.6 g and Australian diet 5.5 g. In relation to the habitual diet, the LFD was associated with decreased total bacteria and absolute but not relative abundance of butyrate-producing Clostridium cluster XIVa and mucus-associated Akkermansia muciniphila. In contrast, the Australian diet was associated with five- and seven-fold increases in those bacteria relative to total, respectively (see Table).

Table. Absolute (Log₁₀ copies 16S rRNA gene/g) and relative abundance (%) of faecal bacteria. Data shown in bold indicate bacteria significantly different to those on the other two diets (p ≤ 0.001; Wilcoxon matched-pairs signed rank test)

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<tr>
<th>Abundance</th>
<th>Bacteria</th>
<th>Australian diet</th>
<th>Low FODMAP diet</th>
<th>Habitual diet</th>
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<tr>
<td></td>
<td>Faecalbacterium praunstzii</td>
<td>1.11 [0.82–1.40]</td>
<td>0.95 [0.69–1.22]</td>
<td>1.29 [0.92–1.66]</td>
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<td></td>
<td>Bifidobacteria spp.</td>
<td>1.33 [0.74–1.92]</td>
<td>0.87 [0.47–1.27]</td>
<td>1.48 [0.79–2.18]</td>
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<tr>
<td></td>
<td>Akkermansia muciniphila</td>
<td><strong>0.10 [0.03–0.16]</strong></td>
<td>0.02 [0.01–0.03]</td>
<td>0.01 [0–0.02]</td>
</tr>
<tr>
<td></td>
<td>Ruminococcus torques</td>
<td>0.04 [0.02–0.06]</td>
<td>0.06 [0.04–0.09]</td>
<td>0.05 [0.02–0.08]</td>
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Values: means [95% CI] for 33 observations (n = 30 for A. muciniphila)

Discussion/Conclusion: A LFD reduces total bacteria non-selectively, but an increase of dietary oligosaccharide intake of only 2.2 g is associated with a strong prebiotic effect. Small increases in dietary FODMAPs are enough to selectively increase beneficial bacteria.
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<td><strong>Intended and unintended alterations of the human microbiome</strong></td>
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State-of-the-Art Lecture

Comparative efficacy of traditional treatments and treatments targeting the microbiome in IBD and IBS

Paul Moayyedi
Division of Gastroenterology, McMaster University, Hamilton, ON L8S 4K1, Canada

The main focus of therapy for both ulcerative colitis (UC) and Crohn’s disease (CD) is to suppress the immune system. Until recently there has been little focus on altering the environment that might be driving the aberrant immune response in the GI tract. The microbiome is a likely driver if the gut immune response and if the microbiome that is hypothetically causing the immune response seen in UC or CD could be changed this may be a useful therapeutic option in inflammatory bowel disease. Fecal microbiota therapy (FMT) has been successful in treating clostridium difficile colitis and small case series have given conflicting results in UC and CD.

There have now been three randomized trials evaluating FMT in over 200 active UC patients. Overall FMT seems to be more effective than placebo although the effect is much more modest than that seen with clostridium difficile with about 25% achieving remission at 6–8 weeks. This seems disappointing but is comparable to remission rates seen with biologic therapies at this time point. However, the numbers of patients studied is small and we are uncertain of how to administer FMT so at the moment this should only be used in the context of a research study. Case series suggest FMT may also be effective in CD and randomized trials are ongoing.

Irritable bowel syndrome (IBS) may be caused by a more subtle inflammatory response in the GI tract that causes disorders of motility as well as lowering visceral pain thresholds. The cause of this is uncertain but again the microbiome may be important. There are no randomized trials to support FMT in IBS but there is some evidence that diet may play a role and this may have an effect on the microbiome. Antibiotics such as rifaximin also have an impact on IBS symptoms.
The FODMAP diet – A microbiome targeted intervention and what could be the consequences?

M. Chen
Department of Gastroenterology and Hepatology, The First University Hospital of Sun Yat-sen University, Guangzhou, China

FODMAP family is composed of short-chain carbohydrates indicating fermentable oligosaccharides, disaccharides, disaccharides, monosaccharides and polyols, which are poorly absorbed in intestine. Low FODMAPs diet may improve the symptom of IBS and IBD by reducing small intestinal water volume, colonic hydrogen and methane production and changing intestinal transit time. However, short chain fatty acid (SCFA), one of the productions of FODMAPs, is important to gut healthy. The restriction of FODMAPs diet not only result in the inadequate nutrient intake but also have potential adverse effect from altered gut microbiota.

There is an inconsistent result about the effect of low FODMAPs diet on microbial flora. Staudacher et al. found that there was lower absolute and relative abundance of Bifidobacteria in low FODMAPs diet vs controls but no changes in faecal SCFA. While Halmos et al. reported that there was a reduction of absolute abundance of total bacteria with a decline in butyrate-producing bacteria. However, the study did not show a lower relative abundance of bifidobacteria. A study based on 454 pyrosequencing found that no effect of low FODMAPs diet on the overall diversity and abundance of specific bacterial groups.

Based on these studies, there seems an interesting paradox. When Bifidobacteria as probiotic supplement is supplied to IBS patients, some degree of symptom improvement may be observed. While conflict to this, the low FODMAP diet has clinical efficacy but significantly reduces luminal Bifidobacteria concentration. These may be because of the multifactorial etiology of IBS, the heterogeneity of symptoms and the complex and diverse nature of the microbiome. Therefore, there is still much to know with respect to the low FODMAP diet on the luminal gut microbiota. The potential long-term impact of low FODMAP diet on gut microbiota and health require further evaluation.
PRO: Faecal microbiota transplantation (FMT) – Ready for prime time

Prof. Michael A. Kamm
Professor of Gastroenterology, St. Vincent’s Hospital Melbourne and University of Melbourne, Melbourne, VIC 3065, Australia

Transplanting the entire enteric microbiota from a healthy individual to another person as a therapy is not new, but has only recently been validated as an effective strategy and therapy in controlled clinical trials.

In a landmark study, Van Noor and colleagues from the Netherlands randomized patients with resistant Clostridium difficile infection to treatment with vancomycin alone, vancomycin with lavage, or vancomycin, lavage and faecal microbiota transplantation (NEJM 2013). The last therapy demonstrated convincingly the major efficacy of this treatment with “cure” in over 90% of patients. Others studies have demonstrated the long-term benefit of this treatment in achieving “cure” without recurrence.

Inflammatory bowel diseases results from an immune response to enteric gut microorganisms (microbiota). Whether this relates to single pathogens, or is an upregulated response to normal enteric content with loss of tolerance, is unknown.

There are now four randomized controlled trials of FMT in active ulcerative colitis (Moayyedi (Canada) et al., Gastroenterology. 2015; Rossen et al. (Amsterdam), Gastroenterology. 2015; Paramsothy et al. (Australia) Lancet. 2017; and Costello et al. (Australia) ECCO 2017). The study from the Netherlands of low intensity treatment was negative, the other three larger studies demonstrated clear benefit with similar magnitude positive treatment effects.

In our own study (Paramsothy et al, Lancet 2017) steroid-free clinical response (Mayo ↓ ≥ 3 points or 50%) was achieved in 54% of FMT-treated patients versus 23% receiving placebo; steroid-free clinical remission (Mayo frequency + bleeding ≤ 1) in 44% versus 20%, steroid-free endoscopic remission (Mayo 0 / 1) in 32% versus 10%, and steroid-free clinical + endoscopic remission in 27% versus 8% respectively. These results are of a similar magnitude to biologic therapy for active ulcerative colitis.

FMT for Clostridium difficile and active ulcerative colitis has changed the treatment paradigm. It remains to be established as an effective therapy in Crohn’s disease, pouchitis, functional gut disorders and non-gut disorders.
Session V

The gastrointestinal microbiome and extraintestinal diseases
Distinct oral and fecal community profiles enriched in opportunistic pathogens in rheumatoid arthritis patients

Helen Benham, Vanessa Anne Lakis, Kate Ormerod, Paraic O’ Cuiv, Muralidhara Maradana, John Wood, Lisa Nagl, Nishta Rammouth, Clare Owens, Joshua Daly, Nancy Lachner, Tim Wells, Mark Morrison, Philip Hugenholtz, Kim-Anh Lê Cao, and Ranjeny Thomas

The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba QLD 4102, Australia

Background: In the Rheumatoid Arthritis (RA) prodrome, genetic predisposition intersects with environmental risk factors, such as smoking, periodontal disease and respiratory infection. We hypothesized that specific microbial taxa (operational taxonomic units, OTUs) from the oral and fecal microbiota differentiate between RA and healthy controls (HC).

Methods: We characterized a prospective cohort of RA probands, first degree relatives (FDR) and HC. Probands met RA criteria. FDRs included parents, full siblings or offspring of an RA proband; HC were drawn from the community. From all individuals, we obtained demographics, medical history, epidemiological questionnaires and tissue collections. After DNA extraction from tongue and fecal swabs, we undertook targeted 16S rRNA gene sequencing using Illumina MiSeq then used standard QIIME workflows, visualizations with the Phyloseq R package and multivariate statistical analysis using the mixOmics R package. From a subset of samples, complete genomic microbial DNA was extracted and shotgun sequenced to study microbiota composition and existing microbial metabolism.

Results: 116 RA patients, 63 FDR and 43 HC matched for age and gender were recruited. 56% of RA, 4% of FDR and 0% of HCs were ACPA autoantibody positive. 47% RA patients, 30% FDR and 37% HC had ever smoked. Based on multivariate analyses, oral and faecal microbiota were altered in RA relative to HC. The oral community profile in RA was enriched in opportunistic pathogens of lung and periodontium including Rothia mucilaginosa, Porphyromonas, Veillonella, Atopobium and Actinomyces spp. The fecal community profile in RA patients was enriched in E. coli, Pseudomonas aeruginosa and Alistipes, while Clostridiales, particularly members of the Lachnospiraceae, were depleted relative to HC. By univariate analysis, the abundance of particular oral and fecal OTUs was strongly associated with environmental risk factors such as smoking, history of tooth decay or respiratory infection. The oral and faecal OTU profile of some FDR segregated with the RA patients and some segregated with HC.

Conclusions: We demonstrate distinct oral and fecal community profiles in RA patients relative to HC, which are influenced by environmental risk factors. Compound genetic and environmental risks may promote inhospitable mucosal environments for commensals abundant in HC, creating niches for opportunistic pathogens before and after the development of RA.
Friend or foe: The microbiome in liver disease

Assoc. Prof. Leon Adams
School of Medicine and Pharmacology, University of Western Australia, Verdun St., Nedlands, Perth, WA 6009, Australia

The direct flow of venous blood from the intestine to the liver, places it in a unique position to be influenced by microbiota residing in the gut. An altered gut microbiome (aka dybiosis) and its metabolites appear to play a key role in promoting as well as protecting from liver injury in the setting of liver disease. Gut dysbiosis has been demonstrated to enhance liver injury in a range of animal models including non-alcoholic fatty liver (NAFLD), Concanavalin A acute liver injury and bile duct ligation cholestasis. The development of gut dysbiosis may be an important inciting factor by promoting intestinal permeability and leading to increased exposure of the liver to harmful gut derived pathogen associated molecular patterns (PAMPs) such as bacterial DNA, lipopolysaccharide (LPS) and peptidoglycan. Gut derived PAMP’s interact with CD14 and toll-like receptors (TLR’s) to activate the innate immune system and inflammatory pathways within the liver. Emerging evidence also suggests that liver macrophages (Kupffer cells) may directly interact with bacteria and act as a firewall to clear bacteria from the systemic circulation.[1, 2] Additional proposed mechanisms of microbiome associated liver injury include the bacterial metabolism of substances within the gut. Bacterial fermentation of undigested dietary carbohydrate produces hepatotoxins such as ethanol as well as short chain fatty acids, which have been implicated in modulating lipid metabolism and immune function. Gut bacteria also play a key role in the metabolism of bile acids (BA’s) with dybiosis altering the BA pool with numerous effects including potentiation of the development of hepatocellular carcinoma in an animal model of carcinogenesis.[3] In apparent contrast, gut derived bacterial metabolites may also be protective against liver injury by influencing the hepatic transcriptome and susceptibility to drug-induced hepatotoxicity or by altering the bile-acid pool in cholestatic liver disease.[4, 5] Notably, germ-free mice are more susceptible to toxin induced liver fibrosis highlighting a potential protective role for commensal bacteria.[6]

Data from humans demonstrates alterations of the gut microbiome in association with liver injury in NAFLD, and enrichment of bacteria of buccal origin in patients with cirrhosis.[7, 8] The composition of the gut microbiome also predicts complications and mortality in patients with cirrhosis.[9, 10] Thus, the gut microbiome appears an attractive therapeutic target to reduce liver injury in chronic liver disease, however successful translation of this approach is still awaited.

References:


Oral poster presentation

Gut microbiota composition and behaviour problems in early childhood

Amy Loughman\textsuperscript{1}, Martin O'Hely\textsuperscript{2}, Fiona Collier\textsuperscript{2}, Michael Conlon\textsuperscript{3}, Christos Symeonides\textsuperscript{4}, Anne-Louise Ponsonby\textsuperscript{4} and Peter Vuillermin\textsuperscript{2,4} on behalf of the Barwon Infant Study Investigator Group

\textsuperscript{1}RMIT University, Melbourne, VIC, Australia
\textsuperscript{2}Deakin University, Geelong, VIC, Australia
\textsuperscript{3}CSIRO, Adelaide, SA, Australia
\textsuperscript{4}Murdoch Childrens Research Institute, Parkville, VIC, Australia

Introduction: The gut microbiome has been demonstrated to have an association with brain development and function, predominantly in animal models. It is considered likely that the functional properties of the gut microbiome mediate the relationship between diet quality and mental health in both adults and children. There is a dearth of human studies regarding associations between the gut microbiota and child behaviour.

This study aimed to explore predictive relationships between infant faecal microbiota composition at 12 months and behavioural outcomes at 2 years of age in a longitudinal cohort study in the Barwon region of Victoria, Australia.

Methods: Faecal samples were collected from infants at 12 months of age. 16S sequencing was conducted using the Illumina MiSeq platform. When children were aged 2 years, parents completed the Child Behavior Checklist (CBCL), a well-validated 99-item questionnaire of problem items from which subscales of Internalising, Externalising and Total Problems are generated. Analysis was conducted using Calypso and R.

Results: CBCL scores were classified as ‘normal range’ or ‘elevated’ on the basis of borderline-clinical cut-off scores on any of the three subscales. Only 22 of the 217 participants for whom microbiome and behavioural data were available were classified as having ‘elevated’ behavioural problems (Internalising, Externalising or Total Problems). The microbiota diversity was significantly higher in participants with ‘normal range’ versus ‘elevated’ CBCL scores (Shannon Index). Random forest analysis revealed evidence that the two groups could be distinguished on the basis of the abundance of 22 separate OTUs.

Discussion/Conclusion: These results are compatible with a prospective association between the variation in infant gut microbiota composition at 12 months of age and subsequent child behavioural problems. Further investigation will be conducted to determine bacterial taxonomic associations, possible confounding and mediating relationships of other environmental and biological predictors, and mechanisms underlying this relationship.
Oral poster presentation

Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis

Lukas Bajer¹, Miloslav Kverka², Martin Kostovcik², Peter Macinga¹, Jiri Dvorak², Zuzana Stehlikova², Jan Brezina¹, Julius Spicak¹, Pavel Drastich¹
¹Institute for Clinical and Experimental Medicine, Prague, Czech Republic
²Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Introduction: Primary sclerosing cholangitis (PSC) is a progressive disorder of biliary tree which can lead to end-stage liver disease, liver transplantation or even death. Colitis accompanying PSC is considered to be a phenotype of IBD (inflammatory bowel disease) distinct from ulcerative colitis (UC). Our aim was to compare the gut bacterial microbiota of patients with PSC and UC.

Methods: Stool samples were prospectively collected and relevant clinical data obtained from 106 study participants, 43 PSC patients with (n = 32) or without (n = 11) concomitant IBD, 32 UC patients, and 31 healthy controls (HC). Sequencing of the 16S rRNA gene including the V3 and V4 regions was performed on Illumina MiSeq platform to cover low taxonomic levels. Data were further processed in QIIME employing MaAsLin and LEfSe tools for analysis of the output data.

Results: Microbial profiles in both PSC and UC were characterized by low bacterial diversity and significant change in global microbial composition. Rothia, Enterococcus, Streptococcus, Veillonella, and three other genera were markedly overrepresented in PSC regardless of concomitant IBD. Rothia, Veillonella and Streptococcus were tracked to the species level to identify Rothia mucilaginosa, Streptococcus infantus, S. alactolyticus, and S. equi along with Veillonella parvula and V. dispar. PSC was further characterized by decreased abundance of Adlercreutzia equolificans and Prevotella copri. Decrease in genus Phascolarctobacterium was linked to presence of colonic inflammation regardless of IBD phenotype. Akkermansia muciniphila, Butyricicoccus pullicaecorum and Clostridium colinum were decreased in UC along with genus Roseburia. Unclassified Actinomycetes species were markedly increased in overlap syndrome of autoimmune hepatitis (AIH) and PSC. Low levels of serum albumin were significantly correlated with enrichment of order Actinomycetales.

Discussion/Conclusion: PSC was characterized by microbial features independent of concomitant IBD. Several bacterial taxa clearly distinguished IBD phenotypes (PSC-IBD and UC) as well as PSC from PSC/AIH.
Session VI

Targeting the mucosal microbiome and mucosal immune responses
The function of M cells in host-microbe interactions

H. Ohno
Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences (IMS), Yokohama, Japan

The gastrointestinal mucosa is always exposed to enormous numbers of commensal microbiota as well as food-borne microorganisms and their products. Our body does not unconditionally allow habitation of these microorganisms. To prevent translocation of the xenobiotic substances, epithelial cells lining along the mucosal surface serve as the barrier that segregates inside and outside of our body. Intestinal epithelium, especially Goblet cells, secrete antimicrobial peptides to chemically prevent their access to the epithelial barrier. Furthermore, a large amount of IgA is secreted into the intestinal lumen to prevent attachment of microbes to the epithelium. A large number of lymphocytes accumulate in the intestine to establish the gut immune system. The gut immune system consists of inductive and effector sites. The former is called gut-associated lymphoid tissue (GALT), such as Peyer’s patches and isolated lymphoid follicles. Specialized epithelial M cells localizing in follicle-associated epithelium (FAE) covering lymphoid follicles of GALT significantly contribute to mucosal immunosurveillance by taking up and transferring mucosal particulate antigens to antigen-presenting cells underneath FAE. We have identified bacteria-uptake receptors expressed by M cells, including Spi-B and prion protein, which are important for induction of antigen-specific IgA response. We also found that a transcription factor Spi-B is critical for differentiation of mature M cells. Loss of mature M cells due to the absence of Spi-B resulted in attenuated immune response to Salmonella enterica serovar Typhimurium. These data indicated that M cell-dependent antigen-uptake plays non-redundant role in the induction of immune responses to mucosal pathogens.
The mucosal microbiome of the upper GI tract and its role in FGIDs

Erin R. Shanahan
Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, University of Queensland, Woolloongabba, Brisbane, QLD 4102, Australia

Studies of the gastrointestinal microbiome typically focus on the large bowel and luminal contents such as the stool. However, it is likely that the mucosa-associated microbiota (MAM), in close proximity to host tissues, is critically important in the host-microbe interactions linked to health and disease. While the largest surface area of the gut is that of the small intestine, the microbiome of this difficult to sample region remains poorly understood. The duodenum in particular has long been considered sterile, with bacteria only present due to cross contamination or luminal overgrowth. However, utilising the specialized Brisbane Aseptic Biopsy forceps, we have overcome issues of contamination and demonstrated the presence of a duodenal mucosal microbiota. Our studies have also investigated the MAM in patients with functional dyspepsia and irritable bowel syndrome, disorders associated with low level inflammation. We reveal a potential role for the small intestinal microbiota, and particularly microbial load, in driving symptoms in these patients. Using culture based methods, we have isolated mucosal bacteria from duodenal biopsies, and are investigating survival in the duodenal niche, along with host-microbe interactions. An improved understanding of the small intestinal microbiome will enable investigation of the interplay between microbes, inflammation and gut permeability that drives chronic gastrointestinal disorders.
The gut-lung axes and IBD

K.M. Fock
National University of Singapore, Singapore

Chronic airway diseases, asthma and chronic obstructive pulmonary disease (COPD) and inflammatory bowel disease (IBD) are inflammatory diseases that affect the gastrointestinal tract and airways. There are similarities in disease pathogenesis, environmental risk factors as well as generic susceptibility to suggest a complex interplay between IBD and airway diseases. Lung involvement in IBD patients is increasingly recognized and 40–60% of IBD have airway involvement that are subclinical but detected by investigations. Conversely Swedish asthmatic patients showed increased incidence of CD and UC. Likewise, in COPD patients the incidence of both CD and UC were significantly increased. Moreover, the gastrointestinal tract and bronchial tree share a common embryonal origin in the primitive gut with similar anatomical structure. The lymphoid tissue in the submucosal layer is composed of antigen-presenting cells and lymphocytes capable of releasing pro-inflammatory cytokines that have important role in innate and immune defense. A number of bacteria appeared in the intestine before they were detected in the respiratory tract. In disease states such as both COPD and IBD the microbiomes of lung and intestines have changes in the dominant species and a reduction in diversity without decrease in microbial numbers. It is uncertain if these changes are the consequence or mechanism of inflammation. These observations have led to the hypothesis of gut-lung cross-talk. The mechanism for gut-lung interaction has not been identified, although autoimmunity, immune cell homing factors and disruption of microbiome have been postulated. Understanding the nature of organ cross-talk in airway disease and IBD could contribute to the elucidation of etiology of these inflammatory diseases and identify therapeutic strategies in the future.
The microbiome, autophagy and IBD

Dr. Jakob Begun
Mater Research Institute, University of Queensland, South Brisbane, QLD 4101, Australia

Autophagy was initially described as a homeostatic process required for starvation survival in eukaryotic cells and has emerged as ubiquitous process required for a broad range of cellular activities. Autophagy has been implicated in processes as diverse as programmed cell death, carcinogenesis, and cellular defence against intracellular pathogens. The role of autophagy in inflammatory bowel disease (IBD) was first revealed through genome wide association studies identifying several autophagy genes. Genetic and immunologic studies have also implicated pathways involved in microbial defence including innate and adaptive immune functions. Recent research has revealed the complex regulatory pathways controlling autophagy and elucidated the important role that autophagy plays in cellular interactions with the microbiome, and perturbations that can lead to the maladaptive inflammation that characterises inflammatory bowel disease. Of particular interest is the defects in antibacterial autophagy, which appear to be linked to inappropriate bacterial handling by the epithelium in animal models of IBD and patients with IBD. The therapeutic potential of this pathway and the precise relationships between the microbiome, the innate and adaptive immune system, autophagy, and IBD pathogenesis remain to be fully realised, although significant progress has been made.
Characterising local and systemic immune response in functional dyspepsia

Anh Do1,2, Yuwen Li1, Erin R. Shanahan1,2, Natasha Koloski3,4, Teressa Hansen3, Simon Keely3,4, Marjorie M. Walker3,4, Nicholas J. Talley3,4, Gerald Holtmann1,2
1The University of Queensland, 2Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Woolloongabba, Brisbane; 3The University of Newcastle, 4Hunter Medical Research Institute, New Lambton Heights, Newcastle, NSW 2305, Australia

Functional dyspepsia (FD) is a common disorder with two main subcategories: postprandial distress and epigastric pain. FD patients suffer from chronic abdominal discomfort and pain in the upper gastrointestinal tract and frequently overlap with irritable bowel syndrome (IBS). The pathogenic mechanism of the disease is not well understood, yet recent studies have revealed novel immune activation mechanisms, causing a low-grade inflammation that may drive the disease mechanism in both FD and IBS. A broad examination in immunological responses both locally and systemically in FD and FD/IBS patients will help to clarify the pathology underpinning observed symptoms.

A cohort of 150 patients presenting with FD or FD/IBS were recruited in this study. GI symptoms were assessed utilizing SAGIS (Structured Assessment of Gastrointestinal Symptoms Instrument), standard nutrient challenge test (NCT) and gastric emptying test (GET). Peripheral blood mononuclear cells (PBMCs) and granulocytes were isolated and analysed for cytotoxic T cells, helper T cells, Th1, Th2, Th17 and gut-homing markers (α4, β7 and CCR9) among naïve/memory T cells. A remarkable increase of gut-homing markers on T-cells was observed in both FD and FD/IBS patients, which correlated with NCT score. In patients with postprandial pain, the percentage of eosinophils was positively correlated with delayed GE. Assessment of immune activation in patient’s small bowel showed increase in both CCR4 and CCR6 on circulating CD4+ T-cells and CXCR3 on lamina propria CD4+ T-cells, which associated with postprandial distress symptoms. Investigation of 13 pro-inflammatory and anti-inflammatory cytokines of in PBMCs and granulocytes culture also indicated increase of specific cytokines by PBMCs and granulocytes in some subsets of patients. In summary, the alteration in T-cell profile both systemically and locally reflected GI symptoms in FD and FD/IBS patients. There were also distinct differences between FD and FD-IBS overlap in relation to specific T-cell subpopulations. Further investigation on these subpopulations will help to reveal the cause of FD and FD/IBS.
Esophageal dysbiosis in Barrett’s esophagus and esophageal adenocarcinoma

Thi-My-Tam Nguyen¹, Erin Shanahan¹²³, Lutz Krause¹, David C. Whiteman⁴, Bradley J. Kendall²³⁴, Luke F. Hourigan²⁵, Andrew P. Barbour³, Gerald Holtmann²³⁶, Mark Morrison¹ and Michelle Hill¹

¹The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Woolloongabba QLD 4102 Australia; ²Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Woolloongabba QLD 4102 Australia; ³School of Medicine, The University of Queensland, Translational Research Institute, Woolloongabba QLD 4102 Australia; ⁴QIMR Berghofer Medical Research Institute, Brisbane City QLD 4006 Australia; ⁵Gallipoli Medical Research Institute, School of Medicine, The University of Queensland, Greenslopes Private Hospital, Brisbane QLD 4120 Australia; ⁶Faculty of Health and Behavioral Sciences, The University of Queensland, St Lucia QLD 4072 Australia

Introduction: Before the mid-1970s, esophageal adenocarcinoma had represented less than 5% of all esophageal cancer cases. Now esophageal adenocarcinoma represents almost half of all cases; making it one of the most rapidly increasing cancers among the Western populations. The current risk factors for esophageal adenocarcinoma and the premalignant condition Barrett’s esophagus cannot explain why an individual progresses from Barrett’s esophagus or the cause for such a rapid shift in esophageal adenocarcinoma incidences. One possible, yet understudied risk factor for disease progression may be the microbiome. Here we investigate the correlation between the microbiome of esophageal adenocarcinoma and the premalignant conditions gastroesophageal reflux disease and Barrett’s esophagus.

Methods: 50 biopsy tissue samples were collected from 30 individuals with confirmed gastroesophageal reflux disease (n = 10), Barrett’s esophagus (n = 10), and esophageal adenocarcinoma (n = 10). Samples taken from Barrett’s esophagus and esophageal adenocarcinoma patients were collected from different sites of the esophagus; termed matched squamous and columnar for Barrett’s esophagus, and matched stomach and tumor for esophageal adenocarcinoma. The total DNA was extracted from the samples and used to produce PCR amplicon libraries of the V6–V8 hypervariable regions of Bacterial 16S rRNA genes, which were subjected to Illumina-based sequencing. Data analysis was performed using QIIME, Graphpad Prism and Calypso.

Results: Comparison between the study groups revealed esophageal disease was associated with altered microbial community composition but not alpha diversity. Higher relative abundance in Veillonella-affiliated sequences was observed in esophageal adenocarcinoma samples, with a commensurate lower abundance in the Streptococcus OTUs, correlating with severity in reflux disease. In addition, we observed a higher abundance of Prevotella in gastroesophageal reflux disease samples compared to both tissue types from esophageal adenocarcinoma patients,
and columnar tissue with a trend in squamous tissue from patients with Barrett’s esophagus. For matched normal and disease tissue from the same participant, microbial compositional differences were found for Barrett’s esophagus in an undetermined genus from the family Streptococcaceae.

**Discussion/Conclusion:** Our findings reveal microbial differences are manifest between Barrett’s esophagus and esophageal adenocarcinoma tissue. As studies looking into the microbiome in esophageal disease is limited, increasing our understanding of Barrett’s esophagus and esophageal adenocarcinoma is vital. Currently it is not possible to predict which patients with Barrett’s esophagus are likely to progress to esophageal adenocarcinoma; understanding the etiology and microbial pathogenesis of esophageal reflux disease may potentially contribute to improvements in treatment and/or management of Barrett’s esophagus patients.
Oral poster presentation

Reduced abundance of *Faecalibacterium prausnitzii* in the terminal ileum mucosa-associated microbiome correlates with increased small intestinal permeability in chronic liver disease

Ashok S. Raj1,5, Erin R. Shanahan1,2, Cuong D. Tran3, Purnima Bhat4, Linda M. Fletcher1,5, Mark Morrison2, Gerald Holtmann1,5, Graeme A. Macdonald1,5

1Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia; 2University of Queensland Diamantina Institute, Translational Research Institute, University of Queensland, Brisbane, Australia; 3Health and Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia; 4Australian National University School of Medicine, Australian National University, Canberra, Australia, 5School of Medicine, Translational Research Institute, University of Queensland, Brisbane, Australia

Introduction: Chronic liver disease (CLD) is associated with dysbiosis of the stool microbiota, but little is known about the mucosal microbiota of the terminal ileum, some of which may be beneficial for mucosal integrity. Our aim was to evaluate for dysbiosis of the terminal ileum mucosal microbiota and relationships with small intestinal permeability and disease severity in subjects with CLD.

Methods: Subjects with and without CLD, undergoing routine colonoscopy were prospectively recruited. Those with mucosal inflammation or functional bowel disease were excluded. Bacterial DNA was sequenced from mucosal biopsies taken from the terminal ileum, using Illumina® Miseq. Small intestinal permeability was measured by the plasma ratio of lactulose:rhamnose (L:R), and hepatic stiffness by Transient Elastography. The presence of the metabolic syndrome was assessed by the IDF/AHA/NHLBI 2009 consensus criteria. Statistical analysis was performed by SPSS v22 and Calypso version 5.2

Results: 21 subjects with CLD (male:female 15:12; age 40–76 years) and 25 controls (M:F 13:12; age 36–73 years) were assessed. In CLD subjects, there was a strong inverse correlation between small intestinal permeability and the relative abundance of *Faecalibacterium prausnitzii* (r = -0.79, p = 0.015, corrected for multiple comparisons, Fig. 1). As a community, the microbial composition of CLD was similar to controls, with no significant separation on redundancy analysis (p = 0.71), and similar microbial diversity (Shannon index, p = 0.68). There was no effect of hepatic stiffness or the metabolic syndrome on the terminal ileum microbiota in CLD subjects (p > 0.05).
Small intestinal permeability and Faecalibacterium prausnitzii

Figure 1: The correlation between small intestinal permeability and the relative abundance of *Faecalibacterium prausnitzii* in the terminal ileum of CLD subjects. (Abbreviations: L:R, Lactulose:Rhamnose ratio)

**Discussion/Conclusion:** In CLD, reduced abundance of *Faecalibacterium prausnitzii* in the terminal ileum mucosa may be implicated in the pathogenesis of increased small intestinal permeability.
Session VII

What are the challenges and opportunities of future approaches to maintain or improve health and wellbeing by targeting the microbiome?
The current and future assessment and targeted modulation of the gastrointestinal microbiome in the clinical setting

Eamonn M.M. Quigley, M.D.
Division of Gastroenterology and Hepatology, Lynda K and David M Underwood Center for Digestive Disorders, Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX, USA

The advent of what has been referred to as the “microbiome revolution” presents new opportunities for the development of novel approaches to the diagnosis and management of gastrointestinal and systemic disorders. “Mining” the gut microbiome for signatures that might be of diagnostic or prognostic import, on the one hand, and for novel therapeutics, on the other, is being actively pursued in many laboratories and clinical research units around the world. While these approaches offer much promise and are now buttressed by an impressive portfolio of basic science research, formidable barriers exist and should restrain those who are in danger of overpromising the immediate appearance of new diagnostics and therapeutics. Those who seek to use microbial patterns in fecal or other samples to guide diagnosis or select therapy must confront considerable hurdles before they even extract a sample: the intrinsic variability of the human gut microbiome, the likely presence of multiple confounding factors ranging from diet and other lifestyle influences to medications and the heterogeneity of the very disease that they choose to study. Sampling itself poses further dilemmas; who, how, what, and how often? Next comes a choice of methodologies: high throughput sequencing, metagenomics and metabolomics, to mention just some of the options, all provide important, if different, information which may or may not be relevant to the clinical or research question. Those who focus on microbiome modulation face these, as well as additional (industrial, regulatory and safety, for example) obstacles in bringing new therapies into the clinic. In microbiome therapeutics the problem lies not in a scarcity of options but in a paucity of quality products and high-quality clinical evidence; fundamental issues that must be addressed if progress is to be made. There are exciting developments out there: bacteria-derived small molecules, genetic modulation and, above all, the lessons that can be learned from fecal microbial transplantation.
The host genes and associations with the microbiome: Implications for targeted interventions

Prof. Siew C. Ng
Department of Medicine and Therapeutics, Division of Gastroenterology and Hepatology, State Key Laboratory of Digestive Disease, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Host genetics and intestinal microbes can both contribute to an individual’s overall susceptibility to intestinal diseases including inflammatory bowel disease (IBD). Over 200 genetic variants have been associated with risk for IBD via affecting barrier function or autophagic clearance of intracellular bacteria. Most of the evidence relating to possible causal genes points to an essential role for host defence against infection in IBD. Moreover, 70% of the genetic loci that are associated with IBD are also shared by a variety of autoimmune diseases suggesting that a variety of complex autoimmune diseases share common environmental influences with IBD. The rising incidence of IBD globally and in newly industrialized countries suggest the critical role played by environment in the pathogenesis of IBD. Many of these environmental factors including mode of birth, breast feeding, smoking, hygiene, infections, antibiotics, diet and stress are all known to cause gut microbial perturbations. It is also increasingly recognised that early life is a crucial period in which genetic environmental influences are most paramount.

It has been shown that interactions between genetic (ATG16L1/NOD2) and microbial factors cooperate to promote beneficial immune responses. Secondly, an interaction between a specific virus infection and a mutation in the Crohn’s disease susceptibility gene Atg16L1 induces intestinal pathologies in mice. Disease localisation or complication may also be a major determinant of the IBD-associated gut microbiota composition. Traditional antibiotics, probiotics, and prebiotics appeared to have limited efficacy. Hence, the host:microbe interface holds an important role in transformative medicine for IBD which involved targeting the microbiome via targeted immune modulation, organism-directed antibiotics, and host:microbial population modulators. A better understanding of gene-microbiota interactions that precede the onset of IBD and their interaction with host genetics can lead to new IBD therapeutics and the potential of microbial prevention strategies as well as microbial markers that can predict patient outcome.
Next generation microbiology: Monitoring the resistome by shot gun metagenomics to fight the spread of antimicrobial resistance

Ingo B. Autenrieth, Silke Peter, Matthias Willmann
Institute of Medical Micobiology and Hygiene, Tübingen, Germany

Humans are populated by microbiomes with important physiological roles. However, the gut microbiome is also a reservoir for facultative pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis* or *Pseudomonas aeruginosa*. These facultative pathogens are cause of severe infections in immunocompromised and hospitalized patients. This is of particular importance when these microbes are resistant to antimicrobials.

Due to inappropriate use of antibiotics, a dramatic increase of antibiotic (pan)-resistant pathogens has evolved. In addition to exertion of selection pressure for antibacterial resistant pathogens, antibiotics damage the microbiome and promote rapid evolution of antibiotic-resistant bacterial pathogens, most likely through an intensified transfer of mobile genetic elements harbouring antibiotic resistance genes. To reduce selection pressure and disruption on microbiomes, novel measures are required to limit the spread of antibiotic resistant pathogens in order to prevent a putative “post-antibiotic era”. Both narrow-spectrum antibiotics along with strategies to decolonize patients from antibiotic resistant pathogens, and novel diagnostic strategies including shot gun metagenomics and precision medicine are required.

Trials conducted by the German Center of Infection Research (DZIF) aim at monitoring the resistome representing all antimicrobial resistance genes carried by microorganisms within a given microbiome. Metagenomics and next-generation-sequencing provide a powerful approach to study microbiome, pathogenome (all bacterial genes encoding pathogenicity factors), and resistome. We used a novel algorithm for the determination of antibiotic selection pressure which can be applied in clinical settings to compare therapeutic regimes regarding their effect on the intestinal resistome. This strategy will provide important information for clinicians to choose antibiotics with low selective force contributing to diminish spread of resistance and a reduced burden of infections with multidrug resistant pathogens.
The clinician’s perspective of needs and opportunities in developing countries

Uday C. Ghoshal, Professor
Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India; E-Mail: udayghoshal@gmail.com

Of the several challenges in the developing countries, lack of adequate foods, frequent occurrence of infectious diseases including gastrointestinal infection associated with acute and chronic diarrhea, intestinal malabsorption and malnutrition, are a few worth mentioning. In several of these maladies, gut microbiota has been reported to play an important role. For example, extraction of calorie from the foods depends on dominance of Firmicutes, which is particularly important in the face of scarcity of foods. Tropical sprue, a condition primarily reported from developing countries in the tropics is well known to be associated with dysbiosis, malabsorption and malnutrition. Recently, a similar condition, named environmental enteropathy, which is associated with gut dysbiosis, has been reported from Bangladesh; environmental enteropathy has been shown to result in growth failure with consequent morbidity and mortality among children. Dysbiosis has also been postulated to reduce response to several vaccines to prevent infectious diseases that pose significant burden in the developing countries.

The current fiction is, could manipulation of gut microbiota help to solve some of these issues? As treatment of tropical sprue is well known to correct malnutrition, the expectation is not entirely illogical. However, a randomized controlled trial of short-course rifaximin among Malawian children with tropical enteropathy casted doubt on this expectation as could not reduce frequency of diarrheal or improve intestinal permeability. Several studies, however, showed that gut microbiota manipulation using probiotics was useful in preventing acute diarrhea. Another important issue worth mentioning cutting-down the cost of treatment of diseases such as inflammatory bowel disease using fecal microbial transplantation in the resource-limited nations in the era of biologicals. Though futuristic, gut microbiota and its manipulation has several opportunities in the developing countries for preventing and treating several diseases.
A type 1 diabetes associated core microbiome driven by interleukin 2 pathway and MHC genetic variation

J.A. Mullaney, J.E. Stephens, C. Fong, B.E. Geeling, E.E. Hamilton-Williams
The University of Queensland Diamantina Institute, Brisbane, QLD, Australia

Changes in the gut microbiota has been implicated in the pathogenesis of many autoimmune conditions including type 1 diabetes (T1D). We performed an analysis of associations between the gut microbiota and T1D genetic risk using a mouse model of T1D. We identified a core microbiome associated with the T1D susceptible NOD strain and we demonstrate that disease protective alleles at the Idd3/5 (IL2, Ctl4, Slc11a1 and Acadl) loci, all of which mediate profound protection from T1D are associated with shifts in the microbiome. Comparison of the intestine of type 1 diabetes susceptible NOD mice with protected strains revealed subclinical pathology in the NOD intestine including increased immune cell infiltrates, reduced goblet cell mucous production and reduced Paneth cell anti-microbial peptide production, which were partly corrected by protective alleles of the MHC and Idd3/5. Immunotherapeutic administration of interleukin-2, mimicking the effects of the protective Idd3 allele, was able to reduce gut inflammation in NOD mice and shift the microbiota. These findings demonstrate for the first time that T1D associated genetic variants that restore immune tolerance to islet antigens also result in functional changes in the gut immune system and resultant changes in the microbiota.
Oral poster presentation

Postoperative Crohn’s disease recurrence is associated with specific changes in the faecal microbiome – Potential pathogenic and protective roles

Amy L. Hamilton¹, Michael A. Kamm¹,², Shu-Mei Teo³, Peter De Cruz¹, Emily K. Wright¹, Hai Feng⁴,⁵, Kathryn J. Ritchie¹, Joseph J.Y. Sung⁵, Carl D. Kirkwood⁴, Michael Inouye³

¹St. Vincent’s Hospital and University of Melbourne, Melbourne, Australia; ²Imperial College, London, UK, ³Centre for Systems Genomics, University of Melbourne, Melbourne, Australia; ⁴Murdoch Children’s Research Institute, Melbourne, Australia, ⁵Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Introduction: Crohn’s disease usually recurs after “curative” resection. This may relate to specific microbial populations playing a pathogenic role.

Methods: Faecal samples were obtained peri-operatively (baseline), 6, 12 and 18 months after surgery from 130 patients in the POCER study. Endoscopic recurrence was assessed (Rutgeerts Score ≥ i2) at 18 months. The V2 region of the 16S rRNA gene was sequenced using Illumina MiSeq. Data were processed using the QIIME pipeline and analysed for diversity and differential abundance at genus level using R. Hierarchical cluster analysis was performed on relative abundance at family level; each cluster was assessed for outcome at 18 months.

Results: Alpha diversity increased significantly after surgery (all patients, baseline vs 18 months (p = 0.048). At six months, diversity was greater for patients who remained in remission vs. recurrence (p = 0.04). Overall bacterial composition (β diversity) differed between recurrence and remission at 18 months (p = 0.008), as well as over time (all patients and all samples: baseline, six, 12 and 18 months; p = 0.001). Nine genera (four from the order Clostridiales, two from Lactobacillales and Bacteroidales) were differentially abundant between disease recurrence compared to remission. Comparison of endoscopic outcomes at 18 months based on cluster analysis resulted in six distinct groups based on predominance of certain bacterial families (Table 1).
Table 1: Odds ratio for recurrence in the six identified clusters based on hierarchical cluster analysis.

Discussion/Conclusion: In faecal samples from postoperative patients, dominance of the bacterial family Lachnospiraceae (group one) is associated with lower rates of recurrence at 18 months, whereas dominance of Enterobacteriaceae (group four) is associated with disease recurrence. This may be as a result of higher relative populations of enteric pathogens such as *Proteus*, *Serratia* and *Escherichia* in samples within this cluster. The specific bacterial genera associated with disease recurrence mirror the results of the cluster analysis, including decreases within the orders Clostridiales and Lactobacillales, and increased abundance of Bacteroidales.
List of Chairpersons, Speakers and Scientific Organizers

**Assoc. Prof. Leon Adams**  
School of Medicine and Pharmacology  
University of Western Australia  
Verdun Street  
Nedlands, WA 6009  
Australia

**Dr. Katrina Campbell**  
Nutrition and Dietetics  
Princess Alexandra Hospital  
199 Ipswich Rd.  
Woolloongabba, QLD 4102  
Australia

**Prof. Jane Andrews**  
Department of Gastroenterology & Hepatology  
Royal Adelaide Hospital  
North Terrace  
Adelaide, SA 5000  
Australia

**Prof. Ingo B. Autenrieth**  
Medizinische Mikrobiologie  
Universitätsklinikum Tübingen  
Elfriede-Aulhorn-Str. 6  
72076 Tübingen  
Germany

**Prof. Minhu Chen**  
Department of Gastroenterology and Hepatology  
The First Affiliated Hospital  
Sun Yat-sen University  
58 Zhongshan Road II  
510080 Guangzhou  
China

**Dr. Lukas Bajer**  
Hepatogastroenterology  
IKEM Prague  
Videnska 9  
140 21 Praha 4  
Czech Republic

**William D. Chey, M.D.**  
Professor of Internal Medicine  
Division of Gastroenterology  
University of Michigan  
3912 Taubman Center, SPC 5362  
Ann Arbor, MI 48109  
USA

**Dr. Jakob Begun**  
Mater Research Institute  
University of Queensland  
Aubigny Bldg., Level 3  
Raymond Terrace  
South Brisbane, QLD 4101  
Australia

**Dr. Anh Do**  
PAH, Department of Gastroenterology & Hepatology  
Translational Research Institute  
University of Queensland  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

**Prof. Emad M. El-Omar**  
St. George Clinical School  
University of New South Wales  
Level 2 Clinical Sciences  
Kogarah, NSW 2217  
Australia

**Grace Burns**  
Priority Research Centre for Digestive Health & Neurogastroenterology  
School of Medicine & Public Health  
The University of Newcastle  
HMRI Bldg., Level 2 East Wing  
New Lambton Heights, NSW 2305  
Australia
Prof. Timothy Florin  
Inflammatory Bowel Diseases Group  
Mater Research Institute  
University of Queensland  
Translational Research Institute  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Prof. Kwong M. Fock  
National University of Singapore  
21 Lower Kent Ridge Rd.  
Singapore 119077  
Singapore

Dr. Judith Gapasin-Tongo  
St. Luke's Medical Center  
RM. 804 Tower Cathedral Heights  
279 E. Rodriquez Sr. Avenue  
1102 Quezon City  
Philippines

Prof. Uday C. Ghoshal  
Sanjay Postgraduate Institute  
of Medical Sciences  
Rae Bareli Road  
Lucknow, Uttar Pradesh 226014  
India

Prof. Peter R. Gibson  
Department of Gastroenterology  
The Alfred Hospital  
Monash Medical School Bldg.  
Monash University  
Melbourne, VIC 3800  
Australia

Dr. Marcus Gray  
Gehrmann Laboratory of Queensland  
St. Lucia  
Brisbane, QLD 4072  
Australia

Prof. Michael Grimm  
St George and Sutherland  
Clinical School  
University of NSW  
Gray St.  
Kogarah, NSW 2217  
Australia

Dr. Emma P. Halmos  
Department of Gastroenterology  
Monash University  
99 Commercial Rd.  
Melbourne, VIC 3004  
Australia

Amy L. Hamilton  
St. Vincent's Hospital  
University Department of Medicine  
Department of Gastroenterology  
Victoria Parade  
Melbourne, VIC 3065  
Australia

Prof. Geoffrey Hebbard  
Gastroenterology and Hepatology  
Royal Melbourne Hospital  
300 Grattan St.  
Parkville, VIC 3050  
Australia

Assoc. Prof. Andrew Holmes  
Discipline of Microbiology  
Faculty of Science  
University of Sydney  
Rm 4113, Charles Perkins Centre  
Sydney, NSW 2006  
Australia

Prof. Gerald Holtmann  
Princess Alexandra Hospital  
University of Queensland  
199 Ipswich Road  
Brisbane, QLD 4102  
Australia

Dr. Philip Hugenholtz  
School of Chemistry &  
Molecular Biosciences  
Australian Centre for Ecogenomics  
University of Queensland  
St Lucia, QLD 4072  
Australia
Marina Iacovou  
Department of Gastroenterology  
Monash University –  
FODMAP Initiative  
The Alfred Centre  
Level 6, 99 Commercial Road  
Melbourne, VIC 3004  
Australia

Dr. Mugdha V. Joglekar  
Diabetes and Islet Biology  
NHMRC Clinical Trials Centre  
Faculty of Medicine  
University of Sydney  
Level 1, Medical Foundation Bldg.  
92-94 Parramatta Road,  
Camperdown, NSW 2050  
Australia

Prof. Michael P. Jones  
Psychology Department  
Faculty of Human Sciences  
Macquarie University  
North Ryde, NSW 2109  
Australia

Prof. Michael A. Kamm  
St. Vincent's Hospital  
University Department of Medicine  
Department of Gastroenterology  
Victoria Parade  
Melbourne, VIC 3065  
Australia

Dr. Simon Keely  
Faculty of Health and Medicine  
School of Biomedical Sciences and Pharmacy  
University of Newcastle  
University Drive  
Callaghan, NSW 2308  
Australia

Dr. Bradley Kendall  
Department of Gastroenterology and Hepatology  
Princess Alexandra Hospital  
199 Ipswich Road  
Brisbane, QLD 4102  
Australia

Dr. Philippe Langella  
Micalis Institute  
Laboratory of Commensals and Probiotics-Host Interactions  
Allée de Vilvert  
78352 Jouy-en-Josas  
France

Dr. Kim-Anh Lê Cao  
Translational Research Institute  
University of Queensland  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Prof. Rupert W.L. Leong  
Gastroenterology and Liver Services  
Concord Hospital  
University of Sydney  
Sydney, NSW 2139  
Australia

Assoc. Prof. Peter Lewindon  
Department of Gastroenterology  
Lady Cilento Children’s Hospital  
501 Stanley Street  
Brisbane, QLD 4101  
Australia

Amy Loughman  
RMIT University  
PO Box 71  
Bundoora, VIC 3083  
Australia

Emeran A. Mayer, M.D.  
Department of Gastroenterology  
Ronald Reagan UCLA Medical Center  
757 Westwood Plaza  
Los Angeles, CA 90095  
USA

Prof. Michael McGuckin  
Mater Research Institute  
Translational Research Institute  
University of Queensland  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia
Prof. Hazel M. Mitchell  
School of Biotechnology and Biomolecular Sciences  
University of New South Wales  
Sydney, NSW 2052  
Australia

Prof. Paul Moayyedi  
Division of Gastroenterology  
McMaster University  
1280 Main St. W.  
HSC-3V3  
Hamilton, ON L8S 4K1  
Canada

Prof. Mark Morrison  
Translational Research Institute  
University of Queensland  
Diamantina Institute  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Dr. Jane A. Mullaney  
Translational Research Institute  
University of Queensland  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Prof. Siew C. Ng  
Department of Medicine and Therapeutics  
Institute of Digestive Diseases  
Chinese University Hong Kong  
Ngan Shing Street, Shatin, NT  
Hong Kong

Thi-My-Tam Nguyen  
Translational Research Institute  
University of Queensland  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Dr. Claire O’Brien  
ANU College of Medicine, Biology and Environment  
Australian National University  
Florey Building  
54 Mills Rd.  
Acton, ACT 2601  
Australia

Dr. Paraic O Cuiv  
Translational Research  
University of Queensland  
Diamantina Institute  
37 Kent Street  
Woolloongabba, QLD 4102  
Australia

Dr. Hiroshi Ohno  
Laboratory for Intestinal Ecosystem  
RIKEN Center for Integrative Medical Sciences (IMS)  
1-7-22 Suehiro  
Tsurumi, Yokohama 230-0045  
Japan

Prof. Sven Pettersson  
Lee Kong Chian School of Medicine  
Nanyang Technological University  
50 Nanyang Avenue  
Singapore 639798  
Singapore

Prof. Elizabeth Powell  
Department of Gastroenterology and Hepatology  
Princess Alexandra Hospital  
199 Ipswich Rd.  
Woolloongabba, QLD 4102  
Australia

Eamonn M.M. Quigley, M.D.  
Professor of Medicine  
Gastroenterology and Hepatology  
The Methodist Hospital  
6565 Fannin Street  
Houston, TX 77030  
USA
Dr. Ashok S. Raj
School of Medicine
University of Queensland
5/36 Kitchener Street
Coorparoo, QLD 4151
Australia

Prof. Stuart Roberts
Department of Gastroenterology and Hepatology
The Alfred Centre
99 Commercial Rd,
Melbourne, VIC 3004
Australia

Prof. Magnus Simrén
Department of Internal Medicine & Clinical Nutrition
Institute of Medicine
Sahlgrenska Academy
University of Gothenburg
40530 Gothenburg
Sweden

Dr. Erin R. Shanahan
Department of Gastroenterology and Hepatology
Princess Alexandra Hospital
University of Queensland
37 Kent Street
Woolloongabba, QLD 4102
Australia

Prof. Kentaro Sugano
Department of Medicine
Division of Gastroenterology
Jichi Medical University
3311-1 Yakushiji, Shimotsuke
Tochigi 329-0498
Japan

Prof. Nicholas J. Talley
Faculty of Health and Medicine
University of Newcastle
University Drive
Callaghan, NSW 2308
Australia

Prof. Ranjeny Thomas
Translational Research Institute
University of Queensland
Diamantina Institute
37 Kent Street
Woolloongabba, QLD 4102
Australia

Prof. Gene Tyson
Australian Centre for Ecogenomics
University of Queensland
Molecular Biosciences Bldg.
University of Queensland
St Lucia, QLD 4072
Australia

Prof. Marjorie M. Walker
Anatomical Pathology
School of Medicine and Public Health
Faculty of Health and Medicine
University of Newcastle
University Drive
Callaghan, NSW 2308
Australia

Prof. Robyn Ward
Faculty of Medicine
Biomedical Sciences
UQ Oral Health Centre
University of Queensland
Building 883, Herston Rd
Herston, QLD 4006
Australia

Dr. Hannah Wardill
Centre for Nutrition and Gastrointestinal Disease
University of Adelaide
North Terrace Campus
Adelaide, SA 5005
Australia

Prof. Jan Wehkamp
Innere Medizin I
Universitätsklinikum Tübingen
Otfried-Müller-Str. 10
72076 Tübingen
Germany
POSTER ABSTRACTS

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Intestinal dysbiosis in patients with short bowel syndrome dependent on total parenteral nutrition is reflected by altered metabolome in faeces

Lukas Bajer¹, Martin Kostovcik², Jaromir Hradecky³, Tomas David⁴, Petr Wohl¹, Julius Spicak¹, Pavel Drastich¹, Monika Cahova¹
¹Institute for Clinical and Experimental Medicine, Prague, Czech Republic
²Institute of Microbiology of the ASCR, Prague, Czech Republic
³University of Chemistry and Technology, Prague, Czech Republic
⁴Faculty of Science, Charles University, Prague, Czech Republic

Introduction: Patients with short bowel syndrome (SBS) exhibit substantial disturbances in gut microbiota composition, which implicates significant alterations of intestinal metabolome. The aim of this study was to perform genetic and metabolomic analysis of stool samples collected from SBS patients totally dependent on parenteral nutrition (TPN).

Methods: We analyzed the stool samples from 8 healthy individuals and 8 patients with SBS dependent on TPN. Faecal microbiota composition was assessed by sequencing of variable V3 and V4 regions of 16S rRNA gene using Illumina MiSeq TM platform. Library preparation, template preparation and template sequencing was performed according to manufacturer’s protocols. Obtained data were filtered by quality and length and processed for alpha and beta diversity analyses using QIIME software package. SCFA profile was measured using solid phase microextraction (SPME) coupled to gas chromatography and high resolution mass spectrometry employing time of flight mass analyzer (Pegasus GC-HRT; LECO, USA). D-lactate content was determined using Megazyme kit.

Results: Weighted Unifrac analysis revealed significant differences between control and TPN subjects. In healthy controls, most abundant phylum was Firmicutes (64.2 ± 7.5%), followed by Bacteroidetes (22.5 ± 9.1%) and Actinobacteria (8.9 ± 4.5%). Proteobacteria were found only in one control sample. In TPN group, Firmicutes represented 66.4 ± 29% of microbiota but Bacteroidetes were absent and Actinobacteria significantly reduced (1.6 ±%). Proteobacteria were found in all samples (23.6 ± 15%). The most abundant metabolites in control stool samples were short - chain fatty acids (SCFA): acetate, propionate and butyrate. In the TPN group, lactate predominated significantly, while SCFA were absent in the intestinal content of these patients.

Discussion/Conclusion: Long-term dependence on total parenteral nutrition results in dysbiosis of the intestine residuum characterized by extinction of Bacteroidetes and appearance of Proteobacteria. This shift was reflected by the changes in the composition of prevailing metabolites in stool.
Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis

Lukas Bajer¹, Miloslav Kverka², Martin Kostovcik², Peter Macinga¹, Jiri Dvorak², Zuzana Stehlikova², Jan Brezina¹, Julius Spicak¹, Pavel Drastich¹
¹Institute for Clinical and Experimental Medicine, Prague, Czech Republic
²Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Introduction: Primary sclerosing cholangitis (PSC) is a progressive disorder of biliary tree which can lead to end-stage liver disease, liver transplantation or even death. Colitis accompanying PSC is considered to be a phenotype of IBD (inflammatory bowel disease) distinct from ulcerative colitis (UC). Our aim was to compare the gut bacterial microbiota of patients with PSC and UC.

Methods: Stool samples were prospectively collected and relevant clinical data obtained from 106 study participants, 43 PSC patients with (n = 32) or without (n = 11) concomitant IBD, 32 UC patients, and 31 healthy controls (HC). Sequencing of the 16S rRNA gene including the V3 and V4 regions was performed on Illumina MiSeq platform to cover low taxonomic levels. Data were further processed in QIIME employing MaAsLin and LEfSe tools for analysis of the output data.

Results: Microbial profiles in both PSC and UC were characterized by low bacterial diversity and significant change in global microbial composition. Rothia, Enterococcus, Streptococcus, Veillonella, and three other genera were markedly overrepresented in PSC regardless of concomitant IBD. Rothia, Veillonella and Streptococcus were tracked to the species level to identify Rothia mucilaginosa, Streptococcus infantus, S. alactolyticus, and S. equi along with Veillonella parvula and V. dispar. PSC was further characterized by decreased abundance of Adlercreutzia equolifaciens and Prevotella copri. Decrease in genus Phascolarctobacterium was linked to presence of colonic inflammation regardless of IBD phenotype. Akkermansia muciniphila, Butyricicoccus pullicaecorum and Clostridium colinum were decreased in UC along with genus Roseburia. Unclassified Actinomyces species were markedly increased in overlap syndrome of autoimmune hepatitis (AIH) and PSC. Low levels of serum albumin were significantly correlated with enrichment of order Actinomycetales.

Discussion/Conclusion: PSC was characterized by microbial features independent of concomitant IBD. Several bacterial taxa clearly distinguished IBD phenotypes (PSC-IBD and UC) as well as PSC from PSC/AIH.
Effect of native and acetylated high amylose maize starch on fecal pH, short chain fatty acid concentrations and fecal microbiota in a cohort of non-stunted and stunted children in southern India

R. Balamurugan\textsuperscript{a}, S. Pugazhendhi\textsuperscript{a}, B.M. Gowri\textsuperscript{a}, D. Tamiulselvan\textsuperscript{a}, E. Mortimer\textsuperscript{b}, G. Gopalsamy\textsuperscript{b}, R.J. Woodman\textsuperscript{b}, R. Meng\textsuperscript{b}, M. Manary\textsuperscript{c}, H.J. Binder\textsuperscript{d}, I. Brown\textsuperscript{b}, G.P. Young\textsuperscript{b}, B.S. Ramakrishna\textsuperscript{a}

\textsuperscript{a}Christian Medical College, Vellore, India; \textsuperscript{b}Flinders University of South Australia, Adelaide, Australia; \textsuperscript{c}Washington University School of Medicine, St. Louis, MO, USA; \textsuperscript{d}Yale University School of Medicine, New Haven, CT, USA

Introduction: The prebiotic benefits of dietary resistant starches (RS) may derive from microbial fermentation and subsequent changes in luminal pH and short chain fatty acid (SCFA) concentrations. Our aim was to compare the effects of two types of RS, a high amylose maize starch (HAMS) or an acetylated HAMS (HAMSA), on fecal indicators of fermentation in stunted and non-stunted children in southern India.

Methods: A non-randomized sequential crossover feeding study with 20 children (10 stunted and 10 non-stunted) aged 2–5 years was conducted in Tamil Nadu, India. They consumed biscuits containing HAMS (20 g/day) every day for two weeks followed by a 2-week washout period and then HAMSA biscuits (20 g/day) for 2-weeks. Fecal samples for pH and SCFA concentrations were collected on day 0 and on 12 subsequent occasions at 3–4 day intervals.

Results: At entry, stunted children had lower fecal SCFA concentrations compared to non-stunted. Fecal fermentation parameters were altered by both RS. Both led to a fall in pH ($p < 0.01$) but in stunted children HAMS led to a greater fall than HAMSA ($p < 0.04$). Both RS increased fecal acetate ($p < 0.05$) but non-stunted children had higher acetate than stunted with HAMSA ($p < 0.01$). HAMS led to greater increase in fecal propionate than HAMSA ($p = 0.03$) without any differences between stunted and non-stunted children. HAMS increased fecal butyrate ($p < 0.001$) as did HAMSA ($p = 0.025$). However, HAMSA significantly increased butyrate in non-stunted children ($p = 0.01$) but had no effect on stunted children. None of the children reported any adverse effects. Minor changes in the fecal microbiome were noted between non-stunted and stunted children.

Discussion/Conclusion: In young children from Southern India, the type-2 RS HAMS was more effective at changing parameters of colonic fermentation than the type-4 RS HAMSA, particularly in stunted children, and without major shifts in composition of the fecal microbiome.
Organic alternative to in feed antibiotics in agriculture

Benjamin W. Bauer¹, Joshua R. McIntyre¹, Kerry B. Walsh², Robert J. Moore³, Dragana Stanley¹

¹Central Queensland University, School of Medical and Applied Sciences, Rockhampton, QLD, Australia
²Institute for Future Farming Systems, CQ University, Rockhampton, QLD, Australia
³RMIT University, School of Science, Bundoora, VIC, Australia

Introduction: Antibiotic growth-promoters (APGs) that are currently used in agriculture to control pathogen populations. There is a fear that their overuse may lead to microbial resistance and a development of a zoonotic super bug. There is also heightened consumer demand on banning the use of antibiotics in animal feed. This can be observed in the increased volume of free range and organic agricultural products. Natural products are needed to control pathogen populations.

Methods: 16S rRNA gene sequencing was done on cloacal swabs taken from trailed animals.

Results: We investigated anti-pathogenic effects of biochar, bentonite and zeolite as well as a number of herbs and spices. Both biochar and bentonite significantly reduced phylum Proteobacteria, in particularly genus Campylobacter and specifically Campylobacter jejuni. These are of clinically importance as Campylobacter jejuni is a human pathogen carried by chickens. We proceeded to test biochar in farming production system with n > 5000 birds per shed on control and biochar supplemented feed. During the sudden outbreak of Spotty Liver Disease, related mortality was as high as 20% in a control flock and did not occur in biochar treated birds; thus 2% biochar in feed protected the birds from Campylobacter hepaticus borne disease in agreement to our previous results. Zeolite could almost completely remove the entire family of Enterobacteriaceae. Promising results were obtained with number of spices.

Discussion/Conclusion: It is possible to replace AGPs in agricultural feed with carefully designed natural feed additives.
Antibiotic treatment reduces oral and colonic expression of sweet taste receptor TAS1R2

E.L. Beckett¹, G. Burns², M. Lucock³, M. Veysey¹,⁴, S. Keely²
¹School of Medicine and Public Health; ²School of Biomedical Sciences and Pharmacy; ³School of Environmental and Life Sciences, the University of Newcastle, Australia; ⁴Hull York Medical School, London, UK

Introduction: Antibiotic treatment is associated with weight gain and an increased risk of obesity. The mechanisms driving this association are not yet fully clear; however, dysbiosis has been identified as a potential causative factor in weight gain and obesity. Procedures that impact diet, weight, and the gastrointestinal microbiota, such as gastric banding surgery, have been linked to modified expression of TAS1R taste receptors on the tongue. Because TAS1Rs are also expressed on enteroendocrine cells in the gastrointestinal tract, they may be involved in detection of sweet compounds and appetite control. Therefore, we assessed how antibiotic induced dysbiosis influenced expression of TAS1R2 and TAS1R3, the two components of the heterodimeric sweet taste receptor, on the tongue and in the colon.

Methods: Female Balb/c (n = 8/group) mice received a 5 day course of amoxicillin and clavulanate, or control treatment. 3 days after completion of antibiotic treatment, mice were sacrificed and colons and tongues collected. RNA was isolated (TRIZOL) and expression of TAS1R2 and TAS1R3 assessed by qPCR, normalised to GAPDH.

Results: Expression of TAS1R2 was decreased in colon (1.0 ± 0.28 vs. 0.37 ± 0.07 relative expression units, p = 0.048) and tongue (1.0 ± 0.34 vs. 0.14 ± 0.03 relative expression units, p = 0.026) tissue of mice treated with antibiotics. However, TAS1R3 expression did not significantly differ in either tissue following antibiotic treatment (Colon: 1.0 ± 0.28 vs. 1.04 ± 0.36 relative expression units, p = 0.81; Tongue: 1.0 ± 0.34 vs. 0.14 ± 0.03 relative expression units, p = 0.92).

Discussion/Conclusions: Reduced expression of TAS1R2 on the tongue following antibiotic treatment may reduce sensation of sweet dietary compounds on the tongue, increasing consumption. Reduced expression in the colon may lead to reduced enteroendocrine signalling of sweet compounds detected in the colon, altering appetite control. Future studies are required to assess the duration of these effects, the direct influence on dietary intake and weight, and how altered mRNA expression translates into changed protein expression levels.
Stress-mediated activation of NLRP6 drives immune dysfunction in functional gastrointestinal disorders (FGIDs)

Jessica Bruce, Ian Grainge, Karla Mettrick, Nicholas J. Talley, Marjorie M. Walker, Simon Keely
Priority Research Centre for Digestive Health and Neurogastroenterology, University of Newcastle, Callaghan, NSW 2308, Australia

Introduction: FGIDs are highly prevalent, affecting between 19–26% of the population worldwide. Currently, FGIDs are characterised by changes in gastrointestinal motility and function in the absence of structural abnormalities identifiable by routine diagnostic practice. FGIDs are considered to be brain-gut axis diseases whereby altered stress responses result in alterations in GI motility and visceral sensitivity. More recent investigations have also uncovered alterations in the microbiota and immune function in patients with FGIDs.

Aims: We hypothesised that stress hormones may influence host-microbiota interactions and aimed to examine the role of corticotrophin-releasing hormone (CRH), the principle stress hormone, on epithelial microbe-sensing in vitro and ex vivo.

Methods: The goblet cell-like HT29-MTX-E12 (E12) cell line was seeded and differentiated over 21 days on permeable membranes to form polarised monolayers. Monolayers were treated basolaterally with 5.0 mM CRH for 18 hours and challenged with *E. coli* at an MOI of 100. Supernatant, washes and monolayer fractions were plated and counted for CFUs. Monolayers were taken for mRNA and protein analysis. Endoscopic biopsies were taken from the duodenum of patients with functional dyspepsia (n = 5) and healthy volunteers (n = 5). The expression of epithelial NLRP6, mast cell tryptase and CD117 was examined by immunocytochemistry and sections scored for cytoplasmic staining by image analysis.

Results: CRH treatment led to a 2-fold increase in NLRP6 protein in E12 monolayers, concurrent with a 40 ± 8% reduction in bacterial adherence (n = 3, p < 0.01). FD patient biopsies showed a similar increase in epithelial NLRP6, compared to healthy volunteers, which coincided with increased expression of mast cell tryptase and the mast cell growth factor receptor, CD117.

Discussion/Conclusion: Together, these initial studies may indicate a role for CRH-mediated regulation of NLRP6 in the pathology of FD and suggests that NLRP6 may play a role in recruiting mucosal mast cells in FGID patients, providing a link between stress-signalling and mucosal-immune homeostasis.
Regulation of lung macrophage metabolism and gene expression via the gut-microbiota-lung axis and SCFAs protects against cigarette smoke-induced lung pathology

Kurtis F. Budden¹, S.L. Gellatly¹, D.L.A. Wood², M. Morrison³, M.A. Cooper⁴, P. Dennis⁵, P. Hugenholtz⁶, P.M. Hansbro¹
¹Priority Research Centre for Healthy Lungs, University of Newcastle and Hunter Medical Research Institute, Newcastle, NSW, Australia; ²Australian Centre for Ecogenomics, The University of Queensland, Brisbane, QLD, Australia; ³Diamantina Institute, University of Queensland, Brisbane, QLD, Australia; ⁴Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia; ⁵School of Agriculture and Food Sciences, University of Queensland, Brisbane, QLD, Australia; ⁶Australian Centre for Ecogenomics, The University of Queensland, Brisbane, QLD, Australia

Introduction: Cigarette smoke exposure is the major risk factor in the development of chronic obstructive pulmonary disease (COPD). Primarily a respiratory disease mediated by aberrant macrophage and neutrophil responses, COPD patients frequently present with gastrointestinal pathology and microbial dysbiosis. Disruption of the gut-microbiota-lung axis, including production of the microbial metabolites short chain fatty acids (SCFAs), contributes to the development of a number of lung diseases. However, the precise mechanisms remain unclear particularly in diseases dominated by innate immune system dysfunction such as COPD.

Methods: Mice receiving a diet supplemented with readily fermentable resistant starch, SCFAs (acetate, propionate, butyrate) in drinking water, or naïve controls were exposed to cigarette smoke for 8 weeks. Lung inflammation and pathology were assessed by bronchoalveolar lavage and histological analysis, and caecal SCFA levels quantified by gas chromatography. Functional metabolic outputs of isolated lung macrophages were assessed using a Seahorse XFe96 Analyser and gene expression was profiled by qPCR.

Results: Cigarette smoke exposure reduced caecal SCFA concentration, which was restored by resistant starch supplementation. Both resistant starch and SCFAs alleviated cigarette smoke-induced pathology, with acetate the most effective SCFA. Cigarette smoke exposure increased maximal respiration, Krebs cycle activity, and non-mitochondrial respiration, with corresponding changes in the expression of genes involved in glycolysis (Glut1, Ldha, Pkm1/2), mitochondrial function (Ucp2, Pgc-1b), NADPH oxidase activity (Nox1-4) and inflammatory phenotype (Tnfa, Il-1b, Ym-1, Fizz-1). These changes in macrophage phenotype, which help to compensate for chronic damage from cigarette smoke, were enhanced by acetate. In particular, acetate further increased maximal respiration, Krebs cycle activity and non-mitochondrial respiration.

Discussion/Conclusion: Resistant starch and acetate supplementation alleviated cigarette smoke-induced lung pathology and were associated with altered macrophage metabolism and gene expression. Manipulation of innate immunity in chronic lung disease via the gut-microbiota-lung axis and SCFAs may present a novel therapeutic strategy.
Introduction: Depression affects 1 in 6 people over the course of their lifetime and is a major cause of suicide. A link has been established between the gut microbiome and the brain, implicating the microbiome as one possible influence on our mental health. This study aims to characterize the gut microbiome in a clinically depressed population before and after probiotic administration, and to correlate changes in the gut microbiome with changes in mental health.

Methods: A triple blind placebo controlled clinical trial. 50 participants take either a probiotic or placebo (Winclow Probiotics) twice daily for 8 weeks. Standardized measures of mental health (e.g. Beck Depression Inventory) are taken pre-test, post-test and at 1 month follow up. Fecal samples are collected pre and post-test. An additional 50 fecal samples will be collected from a non-depressed group. Fecal samples are processed for DNA, and subject to 16S rRNA gene sequencing of the V4 region. The gut microbiome pre and post-test, and in depressed and non-depressed groups will be compared and correlated to measures of mental health.

Results: 50 adults are currently enrolled in the clinical trial and have provided pre and post-test fecal samples. Microbiome analysis will be carried out in early 2017. Participant allocation has yet to be unblinded, hence between-group differences for measures of mental health are currently unknown.

Discussion/Conclusion: This study will determine whether probiotics are a useful treatment for mild to moderate depression, and whether gut microbiome changes are observed in a depressed population with probiotic administration. Furthermore, changes to the gut microbiome may be correlated to improved mental health, with implications for the design of custom probiotics in the future.
Functional and genomic analyses of the *Faecalibacterium prausnitzii* Mam protein

S. Burman\(^1\), J.M. Chatel\(^2\), J. Daly\(^3\), G. Tyson\(^3\), P. Langella\(^2\), P. Ó Cuív\(^1\), M. Morrison\(^1\)

\(^1\)The University of Queensland, Faculty of Medicine, The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, QLD, 4102; \(^2\)MICALIS Institute, INRA, Jouy-en-Josas, France; \(^3\)The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Centre for Ecogenomics, Brisbane, Saint Lucia, QLD 4067, Australia

*Faecalibacterium prausnitzii* is a vital gut commensal bacterium and a dominant member of the gut microbiota in healthy adults. It produces a protein (Mam for Microbial Anti-inflammatory Molecule) that is cleaved and the resulting peptides are known to possess immunomodulatory capacities. However, our understanding of the mechanisms involved in the regulation and production of these peptides is very limited. Moreover, it is not known if the peptides are well conserved across the *F. prausnitzii* group and if they vary in their immunomodulatory capacities. Here, we report the presence of an ABC type transporter gene (cpdA) immediately upstream of *mam* in *F. prausnitzii* strain A2-165. The two genes were confirmed to be co-transcribed using RT-PCR and gene sequencing methods, indicating that both genes are coordinately expressed, and can be involved in Mam protein processing and secretion. Additionally, Mam protein homology and sequence conservation from ~70 *F. prausnitzii* population genomes were analysed using bioinformatics tools. While *mam* genes are present in all the genomes analysed, the Mam protein sequences are quite divergent in those regions from which the putative immunomodulatory peptides are derived; and some genomes were also shown to possess more than one copy of the *mam* gene. These analyses have significantly expanded our understanding of Mam protein diversity across the *F. prausnitzii* group. This will facilitate the stratification of Mam proteins for a systematic evaluation of their immunomodulatory properties.
The induction of dysbiosis in the small intestine promotes allergic sensitisation

G. Burns¹, B. Goggins¹, K. Minahan¹, M.M. Walker², N.J. Talley², P. Foster¹, J. Horvat¹, S. Keely¹
¹School of Biomedical Sciences and Pharmacy, University of Newcastle; ²School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

Introduction: Food allergy is characterised by a T helper type-2 immune response against a food antigen, manifesting as symptoms including nausea, diarrhoea, vomiting or anaphylactic events. It is estimated that 10% of the Australian population have a food allergy, and common allergens include cow’s milk, shellfish and peanuts. Epidemiological studies have identified antibiotics as a significant risk factor for food allergy in infants. We examined how the broad spectrum antibiotic amoxicillin influenced mucosal immune responses to peanut proteins and the development of peanut allergy in mice.

Methods: Balb/C mice were treated daily with 5 mg/kg amoxicillin or PBS for 5 days (days 0–4). On days 5 and 6 animals received 0.2 mg peanut extract or PBS vehicle by oral gavage. Animals were rechallenged with peanut or vehicle by oral gavage on days 11 and 13 and sacrificed on day 16 and immune responses to peanut challenge in blood and intestinal tissues were assessed by protein, mRNA and histological analysis.

Results: The proportion of circulating eosinophils was increased in the blood of mice treated with both antibiotics and peanut. Histological examination revealed an increase in small intestinal eosinophils, predominantly at the villous tips, indicating recruitment to the mucosa. RNA and protein analysis revealed an increase in IL-5 associated with increased Nod-Like Receptor Protein 3 (NLRP3) inflammasome activation.

Discussion/Conclusion: These studies demonstrate that antibiotic treatment prior to food antigen challenge can lead to altered mucosal immune homeostasis, facilitating IL-5-mediated eosinophil recruitment, characteristic of allergic responses. Importantly, we have demonstrated an adjuvant-free model of food sensitisation and small intestinal eosinophilia. These findings contribute to a better understanding of how mucosal disruption by antibiotics contributes to the development of allergic sensitisation and reaction.
Statin therapy causes microbiome alteration and gut dysbiosis in mice

Jose A. Caparros-Martín1,2,3, Ricky R. Lareu2,4, Josh Ramsay2,3, Jörg Peplies5, F. Jerry Reen6, Philip Newsholme2,3, Jeff Hughes4 and Fergal O’Gara1,2,3,6

1Western Australia Human Microbiome Centre (WAHMC), Curtin University, Perth, WA, Australia; 2Curtin Health Innovation Research Institute, Curtin University, Perth, WA, Australia; 3School of Biomedical Sciences, Curtin University, Perth, WA, Australia; 4School of Pharmacy, Curtin University, Perth, WA, Australia; 5Ribocon, Bremen, Germany; 6BIOMERIT Research Centre, School of Microbiology, National of Ireland, Cork, Ireland

Statins are a group of highly prescribed therapeutics used as first-line agents to regulate serum cholesterol in patients at high risk of coronary vascular events. In this presentation, we describe for the first time profound changes in the composition of the commensal gut microbiota in mice receiving statins. This remodelling affected the diversity of the gut community, which became dominated by the family of Gram-negative bacteria Bacteroidales S24-7. Compared to the controls, the statin-associated intestinal microbiota was defined by a higher capacity to produce energy and by the depletion of genes encoding motility-related proteins. As it has been observed in patients, statin-treated mice trended to gain more weight than the control cohort and showed higher levels of blood glucose. Furthermore, statin treatment altered the size and composition of the bile acid pool in the intestine, which may explain the observed gut dysbiosis. Our study suggests that statin therapy affects the intestinal microflora by deregulating the bile acid metabolism and thus unbalancing the gut-liver axis. Since the demonstrated importance of the gut microbiota in host well-being, our work expands on the knowledge of the physiological consequences of taking statins and provides a new perspective to prevent their unintended metabolic effects.

Introduction: Statins are powerful lipid-lowering therapeutics commonly prescribed to prevent cholesterol-related cardiovascular diseases in patients at high risk. However, their consumption is associated with a number of secondary side effects that have raised concerns about their safety (1). This has made statins a controversial therapeutic option, and questions have been raised as to whether the long-term benefits of statins can outweigh the risks. One of the most common events associated with statin intolerance is an increased risk of developing type-2-diabetes mellitus (T2DM) (2). Up to now, the aetiology of these unintended effects are not well understood and it has been hypothesised that the physiological state of the patients plays an important role in their development (3). However, despite the well-known role of the gut microbiota in the pathogenesis of T2DM and the importance of a healthy gut flora in human health and wellness, it has not been investigated as to whether statins could modulate this microbial community.

Methods: Age-matched female C57BL/6J mice were co-housed (5–6 per cage) and randomly distributed into 3 treatment groups: no-statin control (vehicle), pravastatin or atorvastatin. A 10 mg/kg solution/suspension of pravastatin or
atorvastatin was prepared fresh and administered daily via gastric gavage for 12 weeks. After completion of the treatment, animals were euthanized and faecal material collected from the cecum. DNA was isolated using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, 51604). 16S libraries were constructed by amplifying the V3–V4 region of the 16S rRNA gene and sequenced using the MiSeq platform (Illumina). Data was processed using the SILVA pipeline and closed reference Operational Taxonomic Units (OTUs) with 90% similarity were picked against the SILVA SSU Ref dataset (release 123). Analysis of the microbial community was carried out using R (version 3.2.4).

Results:
Statin therapy drives gut dysbiosis in mice by modulating the size and composition of the bile acid pool in the gut.

Figure 1. The host physiology response to statins. Effect of statin therapy on body weight (A) and glucose metabolism (B) of mice. As described in patients, a faster body weight gain trend and a higher blood glucose concentration at week 12 were observed in statin-treated compared to control mice, these changes being more remarkable after atorvastatin treatment. * P < 0.05 by one-way ANOVA and Tukey post hoc test.

Figure 2. Statin therapy disturbs mouse gut microbiota. A. Principal component analysis suggests differences in the composition of the gut microbiota in response to statins. B. Statin treatment resulted in a decrease in community diversity as is shown by both Shannon’s and Simpson’s indices. Interestingly, these variations did not affect the richness but the evenness of the species distribution, suggesting that the gut microbiota became dominated by a few species. * P < 0.05 by one-way ANOVA and Tukey post hoc test.

Figure 3. Characterization of the statin-associated gut microbiota. A-B. Distinctive gut microbiota composition associated to statin consumption revealed by Linear Discriminant Analysis (LDA). C. Relative levels of the indicated Families of aerobic Bacteria after statin treatment.

Figure 4. Statins modulate the size and composition of the bile acid pool in the gut. A. Relative levels of bile acids in the gut. B-C. mRNA levels of bile and synthesis-related genes (B) and of PPAR target genes (C) in the liver. *** P < 0.001, ** P < 0.01, * P < 0.05 by one-way ANOVA and Tukey post hoc test.
Activation of Pxr1 by statins triggers gut dysbiosis and metabolic alterations in mice.

Discussion/Conclusion: We have described for the first time profound changes in the composition of the commensal gut microbiota in mice receiving statins. This remodelling affected the diversity of the gut community, which became dominated by the family of Gram-negative bacteria Bacteroidales S24-7. Furthermore, statin treatment altered the size and composition of the bile acid pool in the intestine by activating Pxr1, which may explain the observed gut dysbiosis.
Oral delivery of pancreatitis-associated protein (PAP) by Lactococcus lactis displays protective effects in mouse acute colitis model through Treg induction

Natalia Breyner1,2, Rodrigo D. de Carvalho2, Tatiana Rochat3, Priscilla Bagano Vilas Boas1,2, Daniela Pontes1, Florian Chain1, Harry Sokol1, Marcela Azevedo2, Anderson Myioshi2, Vasco A. Azevedo2, Philippe Langella1, Luis G. Bermúdez-Humarán1 and Jean-Marc Chatel1
1Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
2Federal University of Minas Gerais (UFMG-ICB), Belo Horizonte, MG, Brazil
3VIM, INRA, 78350 Jouy-en-Josas, France

Introduction: Antimicrobial peptides secreted by intestinal immune and epithelial cells are important effectors of innate immunity. They play an essential role in the maintenance of intestinal homeostasis by limiting microbial epithelium interactions and preventing unnecessary microbe-driven inflammation. Pancreatitis-associated protein (PAP) is a member of the Regenerating islet-derived III (RegIII) proteins family, which binds selectively to specific carbohydrate structure of bacteria. The use of living genetically engineered strains of the food-grade bacterium Lactococcus lactis delivering therapeutic molecules in situ has been promising to treat different human diseases. We hypothesized that exogenous PAP delivered by recombinant L. lactis-PAP might increase its concentration at mucosal level and thus act against inflammatory process.

Methods: PAP cDNA was inserted in lactococci plasmids, in order to produce PAP secreted (pSEC-PAP) or cytoplasmic (pCYT-PAP). Then pSEC-PAP and pCYT PAP were introduced in strain NZ9000 (LL-PAP). The ability of LL-PAP to produce and secrete human PAP was then tested by ELISA. In vivo, we used a well-established DiNitro-BenzeneSulfonic-acid (DNBS)-induced colitis model. Briefly mice were orally administered with LL or LL-PAP during 7 days before and 4 days after intra-rectal injection of DNBS. Mice were sacrificed 4 days after DNBS injection. Weight was monitored daily. Macroscopic and microscopic scores were established after sacrifice. Cytokines secreted by Mesenteric Lymph Node (MLN) were monitored by ELISA.

Results: We showed a decrease in colitis severity in mice treated with LL-PAP compared to those treated with the control L. lactis strain characterized by: i) protection against weight loss; ii) lower macroscopical and histological scores; iv) down-regulation of pro-inflammatory cytokines secreted by lymphocytes in MLN; and v) up-regulation of TGF-β and Treg cells.

Discussion/Conclusion: Based on these findings, we hypothesize that a treatment with LL-PAP is able to reduce colon inflammation and enhance barrier integrity in acute colitis model through Treg cells induction.
A proposal for a randomized study of allogeneic vs. autologous fecal microbiome transplant (FMT) in patients with non-alcoholic steatohepatitis (NASH)

Hari Conjeevaram, M.D.; Elif Oral, M.D.; Hellan Kwon, M.D.; Merritt Gilliland, Ph.D.
University of Michigan, Ann Arbor, MI, USA

Introduction: Non-alcoholic liver disease (NAFLD) including NASH is a common form of chronic liver disease and NASH patients are at risk of progression to cirrhosis, hepatocellular carcinoma and premature death. Several reports suggest that intestinal bacterial are critical modulators of body weight, body fat composition, and insulin resistance (IR) and specific composition of the intestinal bacterial community might be essential for NAFL/NASH development with portal entry of pro-inflammatory cytokines and bacterially derived products. Recently, transfer of intestinal microbiota from lean donors into obese individuals with metabolic syndrome was shown to result in significant improvement in peripheral insulin sensitivity, a trend towards improved hepatic insulin sensitivity and significantly increased diversity of gut microbial composition. We hypothesize that fecal microbiota transplantation (FMT) from lean individuals without NAFLD will alter composition of the gut microbiome, improve insulin sensitivity and histologic features in patients with NASH.

Methods: Phase 1: A pilot study of open-labeled FMT from lean donors without NAFLD in 10 patients with NASH will be starting soon. Phase 2: 24 patients with NASH will be randomized to receive allogeneic (from lean donor without NAFLD) vs. autologous FMT. The primary endpoint is improvement in histologic features of NASH (defined as improvement in steatosis and > 2-point decrease in NASH Activity Score) 24 weeks after FMT. Secondary outcomes will be changes in insulin sensitivity along with changes in gut microbiome diversity.

Results: We expect this study will provide a unique opportunity to study the role of gut microbiome and its relation to clinical (metabolic features and biochemical profiles) and histologic features of NASH.

Discussion/Conclusion: Gut Microbiome plays an important role in development and progression of NASH. Our ultimate goal is to find a novel treatment strategy for its management and gain further knowledge of underlying pathophysiologic mechanisms of NASH severity by studying the gut microbiome before and after FMT.
Iron supplementation does not alter the gut microbiome in early pregnancy

Luisa F. Gomez-Arango1,2, David M. Frazer3, Helen L. Barrett1,2,4, Leonie K. Callaway1,2,4, Gregory J. Anderson3,5, Marloes Dekker Nitert1,5
1UQ Centre for Clinical Research, The University of Queensland, Brisbane Australia
2School of Medicine, The University of Queensland, Brisbane Australia
3QIMR Berghofer Medical Research Institute, Brisbane Australia
4Obstetric Medicine, Royal Brisbane and Women’s Hospital, Brisbane Australia
5School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane; Australia

Introduction: The use of multivitamin and mineral supplements is very common in pregnancy. Many of these supplements also contain variable amounts of iron. Iron is an essential nutrient for many bacteria. Some, but not all, studies have shown that iron supplementation alters gut microbiome composition, which could have adverse consequences for the host, e.g. higher rates of inflammation. The aim of this study was to analyse whether iron supplementation affects gut microbiome composition in early pregnancy.

Methods: Faecal microbiome samples were obtained from overweight and obese women participating in the SPRING (Study of Probiotics IN Gestational diabetes) RCT at baseline. Faecal microbiome composition was assessed by 16S rRNA sequencing and analysed using the QIIME software suite. Dietary intake of iron was assessed by food frequency questionnaire at 16 weeks gestation. Multivitamin and iron supplement use was recorded and the women were grouped as receiving either low (< 60 mg/day; n = 94) or high (≥ 60 mg/day; n = 65) supplementary iron.

Results: The mean supplementary iron intake in the low group was 7.5 ± 5.6 mg/day versus 62.2 ± 8.4 mg/day in the high group. Dietary iron intake did not differ between the groups (11.1 ± 4.0 vs. 11.2 ± 5.6 mg/day). Iron supplementation did not significantly affect the composition of the faecal microbiome at the phylum, class, order or family level. At the genus level, the abundance of Oscillospira was higher in the low supplementary iron intake group. Moreover, supplementary iron intake explained only 3.7% of the variation between the low and high intake groups (p = 0.031).

Discussion/Conclusion: Despite the 8-fold higher mean iron intake from supplements, the faecal microbiome composition of overweight and obese pregnant women at 16 weeks gestation was not substantially altered. The lower abundance of Oscillospira in women with a higher iron intake could have implications for inflammation since Oscillospira abundance is reduced in patients with Crohn’s disease.
Iron deficiency anaemia: Effects on small intestine T-lymphocyte and immune function

Anh Do¹, Yuwen Li¹, Ayesha Shah², Pegah Ghasemi², Erin Shanahan², Teressa Hansen², Natasha Koloski³, Nicholas Talley³, Gerald Holtmann³
¹The University of Queensland, Brisbane, QLD, Australia
²Queensland Health, Brisbane, QLD, Australia
³University of Newcastle, Newcastle, NSW, Australia

Introduction: Iron deficiency anaemia (IDA) is a frequent finding in young females with IBS-like symptoms. However, the majority of patients have normal endoscopic findings. IDA can be both caused by, and alter, inflammatory processes. It is known that low level inflammation can drive symptoms in the gut, however the precise relationships between IDA, gastrointestinal immune processes and symptoms have not been assessed. This study aimed to characterise T-lymphocytes in peripheral blood and duodenal lamina propria in IDA patients, and assess their association with GI symptoms.

Methods: 23 IDA patients with normal endoscopy and colonoscopy and 8 healthy controls (HCs) were enrolled. Full blood count and serum ferritin level were obtained. Mononuclear cells (PBMCs) were isolated from peripheral blood and lamina propria (LP) cells from duodenal mucosal biopsies. Assessment of T-cells in PBMCs and the LP was conducted utilising anti-human antibodies CD3, CD4, CD8, CCR4, CXCR3, CCR6, α4 and β7, and analysis using flow cytometry. GI symptoms were recorded via SAGIS (Structured Assessment of Gastrointestinal Symptoms Instrument). Statistical significance was assessed using T-test or Spearman correlation.

Results: In IDA patients, CD4/CD8 ratio was significantly lower than HCs (p < 0.05, 1.39 ± 0.28 vs. 2.1 ± 0.1). CD4⁺CD8⁺ cells and gut-homing T-cells (CD4⁺α4⁺β7⁺CCR9⁺) in peripheral blood were also significantly higher (all p < 0.05) in IDA patients, compared to HCs (0.54% and 2.25% vs. 0.24% and 0.69% respectively). In IDA patients with mild to moderate GI symptoms, a positive correlation between α4β7 expression on T-cell in the duodenal LP and bloating score (SAGIS) (r = 0.61, p = 0.02), and a negative correlation between a4b7 and the mean corpuscular volume (r = -0.65, p = 0.02), was observed.

Discussion/Conclusion: Our data suggested a significant immune activation in patients with IDA, including increased gut-homing T-cells. This immune activation in IDA represents a potential contributor to GI symptoms, with implications in particular for those presenting with GI disorders in combination with IDA.
Alterations of T-lymphocyte subpopulations in association with bowel symptoms in functional dyspepsia and functional dyspepsia-irritable bowel syndrome overlap

Anh Do1, Yuwen Li1, Erin Shanahan2, Teressa Hansen2, Natasha Koloski3, Simon Keely3, Marjorie M. Walker3, Nicholas Talley3, Gerald Holtmann2
1The University of Queensland, Brisbane, QLD, Australia
2Queensland Health, Brisbane, QLD, Australia
3University of New Castle, New Castle, NSW, Australia

Introduction: Functional dyspepsia (FD) and irritable bowel syndrome (IBS) are the two most prevalent functional gastrointestinal disorders (FGIDs). FD and IBS affect the upper and lower intestine, respectively, but both have similar pathophysiology including abnormal GI function and immune activation and frequently overlap. Here, we aimed to investigate the alteration of T-cells subpopulations and their association with symptoms in FD and FD-IBS patients.

Methods: 43 patients undergoing endoscopy with FD (n = 20), FD-IBS (n = 23) and healthy controls (HCs, n = 9) were recruited. GI symptoms were assessed and standardised nutrient challenge and gastric emptying test were performed. Mononuclear cells (PBMCs) were isolated from peripheral blood using Ficoll density gradient. Lamina propria (LP) cells were isolated from duodenal biopsies using collagenase. T-cell subpopulations in PBMCs and LP cells were analysed by flow cytometry using anti-human antibodies CD3, CD4, CD8, CCR4, CXCR3, CCR6, CCR9, α4, β7. Statistical significance was assessed using T-test or Spearman correlation.

Results: PBMCs analysis showed an increase in CD4:CD8 ratio in FD patients, but no change in FD-IBS compared to HCs. Gut-homing T-cells (CD4+α4β7+CCR9+) were observed to be higher in both FD (1.9% of CD4+ population) and FD-IBS patients (7.9%) compared to HCs (0.49%, p < 0.05). This increase was positively correlated to retrosternal burning (r = 0.4, p = 0.04). Larger population of CD4+CD8+ lymphocytes was observed in patients with FD-IBS but not patients with FD only. FD-IBS, but not FD patients showed increase in both CCR4 and CCR6 on circulating CD4+ T-cells and CXCR3 on LP CD4+ T-cells. The increases in CXCR3 and CCR6 negatively correlated with upper GI symptoms including fullness, and postprandial pain.

Discussion/Conclusion: T-cell profiles in the duodenal mucosa and peripheral blood are altered in patients with FGIDs. There are distinct differences between FD and FD-IBS overlap in relation to specific T-cell subpopulations, consistent with a more severe manifestation of immune activation and GI symptoms in patients with FD-IBS overlap.
Early manipulation of intestinal microbiota

Erin Donaldson¹, Joshua McIntyre¹, Steven Baldwin¹, Robert Hughes², Robert Moore³, Dragana Stanley¹
¹School of Medical and Applied Sciences, Central Queensland University, Rockhampton, QLD, Australia
²South Australian Research and Development Institute, Pig and Poultry Institute, Roseworthy, SA, Australia
³RMIT University, School of Science, Bundoora, VIC, Australia

Introduction: Mature intestinal microbiota can often recover from disruptions caused by limited antibiotic use. Probiotic treatments have no lasting effects on mature microbiota in humans. In one case, it was demonstrated that probiotic was no longer detected in stool 8 days after the ingestion of probiotic was withdrawn. The initial inoculum post birth shapes the gut microbiota for life. The first bacteria to settle in the intestine are able to adhere to epithelial cells with no competition, to rapidly establish, proliferate and set the intestinal environment. Germ-free mouse models for microbiota colonisation requires birth by c-section and antibiotic administration and show high litter effect. Chickens are an easy model for microbiota studies: hundreds of eggs collected over time from the same parental stock can be stored, incubated and hatched together. Provided eggs and the equipment are kept sterile, chicks do not require antibiotic administration nor parental presence (i.e. no breastfeeding). We investigated at-hatch probiotic administration and the possibility of designing the intestinal microbiota.

Methods: We administered probiotics (commercial probiotics and strains we isolated) to chicks hatched in germ-free and normal conditions and compared the intestinal microbiota across all gut sections to classic probiotic administration and PBS inoculated controls.

Results: The best preforming birds (heath and weight-wise) across multiple trials were those given freshly cultured probiotics. The worst preforming birds were those given the same probiotic at hatch, as a commercial powder. Only freshly grown probiotics administered at hatch were able to permanently colonise the gut.

Discussion/Conclusion: Our data suggest rethinking of classic probiotic administration and a need for research into the ways probiotic strains can be delivered to achieve permanent colonisation without resort to continuous dosing.
Influence of selenium nanoparticles on intestinal health and *Faecalibacterium prausnitzii* abundance

Sheeana Gangadoo¹, James Chapman², Robert J. Hughes³, Thi Thu Hao Van⁴, Robert J. Moore⁴, Dragana Stanley²
¹Institute for Future Farming, Rockhampton, QLD, Australia
²School of Health, Medical and Applied Sciences, Rockhampton, QLD, Australia
³South Australian Research and Development Institute, Pig and Poultry Production Institute, Roseworthy, SA, Australia
⁴RMIT University, School of Sciences, Bundoora, VIC, Australia

**Introduction:** Nanoparticles are finding their way into human and animal feed despite the fact that not much is yet known about their influence on host health and intestinal microbiota. In agriculture, nanoparticles (e.g. silver) were used as an alternative to in-feed antibiotics due to their ability to control pathogens and improve growth performance. These nanoparticles were soon abandoned due to toxicity effects experienced on the host. We therefore, investigated the delivery of essential metals in the form of a nanoparticle, focusing on selenium. Selenium is added to all poultry feed formulations as correct, bioavailable amounts are essential for good performance and optimal health to reduce toxicity.

**Methods:** We synthesised selenium nanoparticles “de novo” and added to chicken feed at 0.3, 0.9 and 1.5 ppm nanoSe. We then investigated the effects on gut microbiota using histology and classic toxicology studies to evaluate host toxicity.

**Results:** We found that the faecal and caecal microbiota communities had very different responses to nanoSe and that 0.3 ppm nanoSe supplemented birds were the worst growth performers, followed by 1.5 ppm nanoSe. The 1.5 ppm nanoSe group showed high levels of gut and liver damage and high tissue toxicity, plus extreme modifications of microbiota, compared with low and moderate NanoSe concentrations. *Faecalibacterium prausnitzii* was strongly correlated with concentration of nanoSe in the feed (p = 2E⁻¹⁴ r = 0.63) and not present in the control groups. We used this finding to develop *F. prausnitzii* faecal enrichment methodology using a gut simulator chemostat culture system.

**Discussion/Conclusion:** Levels of toxicity detected in our study suggest that a careful and optimised approach to nanoparticles in feed is needed.
Metaproteomics: Predicting risk of developing type 1 diabetes

Patrick Gavin¹, Jane Mullaney¹, Danny Zipris² and Emma Hamilton-Williams¹
¹University of Queensland Diamantina Institute, Brisbane, Australia
²Barbara Davis Center for Childhood Diabetes, University of Colorado, Denver, Aurora, CO, USA

The prevalence of type 1 diabetes (T1D) is rising rapidly, most likely caused by changing environmental factors such as microbial exposure. Alterations in the intestinal microbiota are believed to be involved in the mechanisms triggering T1D. Our recent work has demonstrated that individuals with T1D or at the highest risk of progressing to T1D have alterations in their gut bacteria compared with first-degree relatives (FDR) at low risk of disease and healthy controls. To link changes in the microbiota with functional changes in the intestine and pancreas, we profiled human proteins present in stool using proteomics. To date, attempts to characterise the link between the microbiota and T1D have used sequencing to catalogue the microbiota present before and after the onset of disease using stool samples. Proteomic analysis of stool samples is a relatively new approach that has been used as a non-invasive means to identify inflammatory disease signatures in Crohn’s disease, inflammatory bowel disease, colon cancer and cystic fibrosis. Moreover, stool contains gut bacteria and their proteins as well as host proteins derived from the pancreas, intestinal cell wall and the immune system. We developed a method for protein extraction in stool which enriched for human proteins and also compiled a combination microbial and human protein database to identify proteins which could provide potentially unique signatures of T1D and FDRs. We applied bioinformatics to the proteomic information obtained in order to visualise networks, interactions and associations between the microbiome and host.
Role of anti-inflammatory gut bioactives in the modulation of immune response in Crohn’s disease

R. Giri¹, ⁵, P. Ó Cuív², ⁵, J. Begun³, ⁴, ⁵
¹School of Biomedical Science, The University of Queensland, Brisbane, Australia
²The University of Queensland Diamantina Institute, Brisbane, Australia
³Mater Research Institute-The University of Queensland, Brisbane, Australia
⁴School of Medicine, The University of Queensland, Brisbane, Australia
⁵Translational Research Institute, Brisbane, Australia

Crohn's disease (CD) is a chronic inflammatory condition of the gastrointestinal tract. Although, the pathogenesis of CD is not fully understood, it is believed to result from the interaction between various genetic and environmental factors, including the microbiome, resulting in inappropriate gut inflammation. Under homeostatic conditions the gut immune system exists in a tolerogenic state with the microbiota. However, dysbiosis in the gastrointestinal microbiota and an imbalance of pro and anti-inflammatory cytokines in the gut characterise CD.

In this study, the ability of gut bacteria cultured from healthy faecal samples to produce bioactives that suppress NF-κB activity within intestinal epithelial cells and human derived organoids was investigated. Caco-2 and LS174T cells were transduced with a NF-κB Luciferase reporter to investigate NF-κB suppressive effects of cell-free culture supernatants (CS) from 20 different isolates as well as Faecalibaceterium prausnitizii A2-165, a bacterium known to produce NF-κB suppressive peptides.

LS174T and Caco-2 reporter cells stimulated with TNFα and IL-1β respectively results in NF-κB reporter stimulation and we demonstrated this could be suppressed by CS prepared from F. prausnitizii and isolate AHG0090. Furthermore, CS prepared from AHG0090 and passed through a 3 kDa retained its NF-κB suppressive effect on both reporter cell lines. The CS also suppressed the expression of the NF-κB dependent cytokine IL-8 in CD human derived gut organoids. Future studies will investigate the effect of gut microbiota derived NF-κB suppressive bioactives on healthy and CD ex vivo gut epithelial cell models, and preclinical animal models to expedite the development of new therapeutics applicable to CD.
Hypoxia inducible factor (HIF)-1 accelerates epithelial wound healing through integrin regulation

Bridie Jane Goggins¹,², Kyra Minahan¹,², Noah Outteridge¹,², Darryl Knight¹,², Jay Horvat¹,², Simon Keely¹,²
¹School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, Australia
²Hunter Medical Research Institute, New Lambton Heights, Australia

Introduction: The characteristic inflammation associated with IBD contributes to repeated cycles of epithelial wounding and repair in the intestine. The epithelium functions as a selective barrier, critical for mucosal protection. During intestinal inflammation, damage to the vasculature leads to reduced oxygen availability (hypoxia) at the mucosa. Epithelial wound healing processes occur in this hypoxic environment and are critical to restore barrier integrity and gut homeostasis. A key factor in the coordination of mucosal wound healing is the transcription factor hypoxia inducible factor (HIF)-1. HIF-1 mediates an array of protective mechanisms for cell survival and repair. Previous work has shown that pharmacological stabilisation of HIF-1α by prolyl hydroxylase inhibitors (PHDi) is protective in murine models of colitis. Importantly, our work has identified HIF-1-mediated induction of integrin-β1 at the mucosa as critical to this process. We examined the functional role and post-translational activity of epithelial α-integrin subunits dimerizing with integrin-β1 to promote HIF-1-mediated wound healing by PHDi.

Methods: Cell migration and inhibition scratch assays were performed on T84 monolayers (~1% O₂ and/or PHDi (AKB-4924), wound closure was monitored over 24 hours. Monolayers were stained by immunofluorescence for α-integrin subunits at 12 hours post-wound. Novel biopsy-wound models were used to extend the study of PHDi-mediated wound healing in vivo.

Results: PHDi treatment significantly accelerated wound closure in T84 monolayers, which was inhibited by antibodies functionally targeting integrin-α3. Immunofluorescent imaging of cell monolayers showed both hypoxia and PHDi treatments increased expression of integrin-α3, localized at the leading edge of the wound. PHDi treatment also significantly accelerated mucosal wound closure in the colons of mice following biopsy wounds.

Discussion/Conclusion: These data suggest that PHDi-mediated HIF-1 stabilisation promotes mucosal healing through regulation of epithelial integrins, in particular α3β1. Integrin-α3β1 is a key regulator of epithelial cytoskeletal organization therefore PHDi compounds may enhance wound closure through integrin-α3β1-mediated reorganisation of the cytoskeleton.
A fibre-deprived diet influences *Collinsella* abundance in the overweight and obese pregnant microbiome and alters maternal metabolic risk

Luisa F. Gomez-Arango1,2, Helen L. Barrett1,2,3, Shelley Wilkinson2, Leonie K. Callaway1,2,3, H. David McIntyre2, Mark Morrison2,4, Marloes Dekker Nitert1,5

1QCLC Centre for Clinical Research, The University of Queensland, Brisbane, Australia
2School of Medicine, The University of Queensland, Brisbane, Australia
3Obstetric Medicine, Royal Brisbane and Women’s Hospital, Brisbane, Australia
4Diamantina Institute, The University of Queensland, Brisbane, Australia
5School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia

**Introduction**: Overweight and obese women are at higher risk of developing gestational diabetes mellitus. We have reported a positive association between the genus *Collinsella* and circulating insulin in early pregnancy. However, the link between *Collinsella* abundance and its impact on glucose metabolism is unknown. The aim of this study was to validate the observed microbial-hormonal interaction and to elucidate the functional interaction between dietary fibre intake, *Collinsella* and insulin levels at 16 weeks gestation.

**Methods**: Study validation was performed in 57 overweight and 73 obese SPRING (Study of Probiotics in the Prevention of Gestational Diabetes Mellitus) participants. Gut microbiome profiles were assessed by 16S rRNA sequencing and analysed within QIIME. 16S *Collinsella* abundance was validated by qPCR. Insulin levels were correlated to *Collinsella* abundance with bootstrapped Spearman rank correlation tests. Detailed dietary fibre intake was calculated from food frequency questionnaires from the start of pregnancy until 16 weeks gestation. Women were grouped based on fibre consumption (low 13.8 [IQR 12.1–16.1] g/day; high 21.6 [19.4–26.2] g/day). Dietary fibre intake was correlated with *Collinsella* abundance and insulin levels.

**Results**: Abundance of the genus *Collinsella* was positively associated with circulating fasting insulin levels (rho = 0.30, p = 0.0006). 16S *Collinsella* abundance was positively correlated with targeted-qPCR *Collinsella* (rho = 0.63, p < 0.0001). *Collinsella* abundance was increased in women with low fibre intake (low: 0.012 [0.002–0.027] vs. high: 0.005 [0–0.017], p = 0.0144). The relative increase in *Collinsella* was negatively associated with dietary fibre intake (rho = -0.19, p = 0.025).

**Discussion/Conclusion**: This study shows the connections between dietary fibre, *Collinsella* abundance and maternal metabolic risk. Low dietary fibre may promote *Collinsella* overgrowth, which may affect intestinal barrier function influencing metabolism in pregnancy.
Resistant starch as a novel prebiotic in infants

Geetha Gopalsamy\textsuperscript{a}, Claus T. Christophersen\textsuperscript{b}, Elissa Mortimer\textsuperscript{a}, Anthony Bird\textsuperscript{c}, Graeme Young\textsuperscript{a}

\textsuperscript{a}Flinders University of South Australia, Bedford Park, Australia; \textsuperscript{b}Edith Cowan University, Perth, WA, Australia; \textsuperscript{c}Commonwealth Scientific and Industrial Research Organisation, Adelaide, SA, Australia

\textbf{Introduction}: Disruption of the infant’s emerging gut microbiota is linked to aberrant host immune development. In adults, fermentation of high amylose maize starch (HAMS), a resistant starch, has a prebiotic effect. It is unknown whether the infant microbiota can ferment HAMS. Were such a capacity to exist, early intake of HAMS might programme the gut microbiota during a critical developmental period. This study will determine if faecal inocula of infants possess the capacity to ferment HAMS and characterise the changes to microbial composition following any such fermentation.

\textbf{Methods}: Faecal samples were collected from 16 infants pre and post commencement of solids. Using \textit{in vitro} batch fermentation methodology, fermentation of HAMS and a modified HAMS (mHAMS) by infant faecal inocula was assessed. Only weaning infants were assessed for their capacity to ferment mHAMS. At 24 hours of incubation, values for pH and SCFA production were compared to parallel incubations with no added substrate, negative control. DNA was extracted from ferments at 0 and 24 hours. Illumina MiSeq sequencing and qPCR was performed on extracted DNA.

\textbf{Results}: Pre-weaning infants had some capacity to ferment HAMS, as evidenced by a significant reduction in pH (p < 0.05) and a significant increase in the production of total SCFAs (p < 0.05) at 24 hours when compared to parallel negative controls. Fermentation of HAMS significantly increased after commencement of solids. Only in weaning infants did fermentation of the RS increase diversity (Shannon p < 0.05) and have positive effects on microbial profile, increasing the abundance of Bifidobacteria and Bacteroides and increasing the ratio of Bacteroidetes to Firmicutes.

\textbf{Discussion/Conclusion}: HAMS is fermented by pre-weaning infant faecal inocula; however, this capacity is markedly increased after solids are commenced. Microbial changes following incubation of weaning infant faecal inocula with HAMS, suggest that these starches may function as a novel infant prebiotic. In vivo studies are now required.
Reduced GI symptoms and premotor cortex activation in Crohn’s disease following anti-TNFα treatment

Marcus Gray1,2, Che-yung Chao1, Nicholas J. Talley4, Natasha A. Koloski1,3,5, Gerald J. Holtmann1,3

1Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia
2Translational Research Institute, Brisbane, QLD, Australia
3School of Medicine, University of Queensland, Brisbane, QLD, Australia
4Faculty of Health & Medicine, University of Newcastle, Callaghan, NSW, Australia

Introduction: Background: Inflammatory Bowel Disease (IBD) is associated with a significant burden of disease which is centred around the subjective symptoms of gastrointestinal (GI) distress. In a significant proportion of patients, subjective symptoms persist after the healing of GI mucosal lesions, potentially reflecting dysregulation in the gut-brain axis. We employed an interoceptive awareness task to engage brain circuitry associated with sensing bodily state, and examined the effects of anti TNF-a therapy on GI symptoms in patients with Crohn’s Disease.

Methods: 10 patients with Crohn’s disease (30.0 ± 13.0 years, 5 female, 6 ileocolonic, 2 colonic and 2 ileal disease) participated. All were in stable clinical remission (CDAI < 150) following chronic anti-TNFα therapy (6 adalimumab/4 infliximab). Patients were examined at peak and trough anti-TNFα levels (before and after anti-inflammatory treatment). We assessed visceral sensory function via a standardised nutrient challenge, and brain function via functional MRI scanning during an interoceptive awareness task requiring patients to focus attention on their body state (estimating their heartbeat) or on external stimuli (estimating presentations of a heart picture on a computer screen). During sensory testing 600 ml of enteral feeding solution was consumed over 15 minutes while the intensity of GI symptoms was quantitated.

Results: Following anti-TNF-α, unpleasant visceral sensation during nutrient challenge (subjective fullness) was significantly reduced (22% reduction, p < 0.031). Symptoms of nausea varied across patients, and overall there was no significant reduction in nausea following anti-TNFα. Increased attention to body state strongly recruited core interoceptive circuits including bilateral insula and anterior cingulate cortices (Figure 1a). Anti-TNFα did not significantly alter the activation of this circuit during interoceptive awareness task. Interestingly however, changes in nausea ratings following anti-TNFα correlated with changes in premotor cortex responses representing the trunk and stomach (figure 1b).
Anti-TNFα treatment in Crohn's disease reduces GI symptoms and alters neuro-circuitry underlying motivation

Marcus Gray1,2, Che-yung Chao1, Nicholas J. Talley4, Natasha A. Koloski1,3,5, Gerald J. Holtmann1,3
1Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; 2Translational Research Institute, Brisbane, QLD, Australia; 3School of Medicine, University of Queensland, Brisbane, QLD, Australia; 4Faculty of Health & Medicine, University of Newcastle, Callaghan, NSW, Australia

Introduction: Inflammatory Bowel Disease (IBD) confers an increased risk for symptoms of depression. Immune activation in IBD is closely linked with the intensity of gastrointestinal symptoms and extra-intestinal comorbidities such as depression or anxiety. Motivational change (decreased reward and increased loss sensitivity) is a core feature of depressive symptoms. We employed a reinforcement learning task to engage core motivational brain circuitry and examined the effects of anti-TNFα therapy on motivational neuro-circuitry in patients with Crohn's disease.

Methods: 10 patients with Crohn’s disease in remission (30.0 ± 13.0 years, 5 female, 6 ileocolonic, 2 colonic and 2 ileal disease, CDAI < 150) participated. All received chronic anti-TNFα therapy (6 adalimumab/4 infliximab). Patients were examined at peak and trough anti-TNFα levels (before and after anti-inflammatory treatment). We assessed visceral sensory function via a standardised nutrient challenge, and brain function via functional MRI scanning during a reinforcement learning task. During this task, participants had to learn to distinguish rewarding stimuli from losing stimuli (gaining/losing fake $10 notes). During sensory testing 600 ml of enteral feeding solution was consumed over 15 minutes while the intensity of GI symptoms was quantitated.

Results: Anti-TNF-α reduced visceral sensation during nutrient challenge (subjective fullness). Motivational circuits were strongly activated by rewards and losses (Figure 1a), including within the nucleus accumbens and primary interoceptive cortex (insula). Anti-inflammatory therapy altered activity the brains motivational circuits during reinforcement learning, including within the nucleus accumbens (Figure 1b) and insula cortex (Figure 1c). Across patients, depressive symptoms correlated with drug induced changes to reward in the right insula cortex.

Discussion/Conclusion: Immune modulation of the gastrointestinal tract with anti-TNFα agents is associated with significant reduction in symptoms during a standardised nutrient challenge, and alterations in core motivational circuitry within the brain. Insula response reflect depressive symptoms before anti-TNFα, suggesting that reducing TNF reduced visceral hypersensitivity and improved motivational functioning.
Anti-TNFα in IBD improves positive attribution bias and gastrointestinal symptoms

Marcus Gray1,2, Che-yung Chao1, Nicholas J. Talley4, Natasha A. Koloski1,3,5, Gerald J. Holtmann1,3
1Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; 2Translational Research Institute, Brisbane, QLD, Australia; 3School of Medicine, University of Queensland, Brisbane, QLD, Australia; 4Faculty of Health & Medicine, University of Newcastle, Callaghan, NSW, Australia

Introduction: Inflammatory Bowel Disease is associated with immune activation, unpleasant GI symptoms, and an increased incidence of anxiety and depression. Depressive symptoms appear linked to adaptive motivational changes following inflammation. Depression is characterised by attributional biases about the self, world and future. Anti TNFα treatment is effective in interrupting the inflammatory cascade and promoting mucosal healing in IBD. The impact on cognitive attribution biases has not been examined.

Methods: 9 Crohn’s disease patients (26.1 ± 10.6 years, 5 female, 5 ileocolonic, 2 colonic and 2 ileal disease) in stable clinical remission with anti-TNFα therapy (all CDAI < 150, 5 adalimumab / 4 infliximab) underwent visceral sensory testing (standardised nutrient challenge) and functional brain scanning at peak and trough anti-TNFα levels (72 hours before and after biologics).

During sensory testing 600 ml of enteral feeding solution was consumed over 15 minutes while the intensity of GI symptoms was quantitated. During brain scanning implicit attribution biases were assessed by sequentially presenting positive-negative health words or self-other referential words. During repeated 45s blocks, patients classified (y/n) individual words as either “self or positive”, “self or negative” “other or positive” or “other or negative”. Faster identification of “self or positive” versus “other or positive” category reflects positive attribution bias.

Results: Anti-TNFα unpleasant visceral sensation during nutrient challenge (subjective fullness) was significantly reduced (Figure 1a, 22% reduction, p < 0.031). Visceral sensory change strongly correlated with improvements in implicit health beliefs (Pearson’s r = -0.669, p < 0.05). Anti-TNFα significantly increased positive attributions of health (p < 0.001). Altered amygdala response reflected improved attribution biases.

Discussion/Conclusion: Immune modulation with anti-TNFα agents reduced symptoms during a standardised nutrient challenge, and improved self-attribution bias of health. This suggests systemic reductions of circulating TNF reduces visceral hypersensitivity and translates into improved implicit beliefs about one’s health via alterations in limbic (amygdala) function.
The prebiotic impact of dietary FODMAPs on intestinal microbiota

E.P. Halmos1,2, C.T. Christophersen3,4, A.R. Bird3, J.G. Muir1, P.R. Gibson1

1Monash University, Melbourne; 2Walter & Eliza Hall Institute, Melbourne; 3CSIRO, Adelaide; 4Edith Cowan University, Perth, Australia

Introduction: A low FODMAP diet (LFD) is mainstream treatment for managing irritable bowel syndrome (IBS). However, there are concerns that restricting FODMAPs, particularly oligosaccharides, leads to a loss of prebiotic effects and an ‘at-risk’ bacterial profile. Conversely, FODMAPs are prebiotic, but trials have only investigated oligosaccharide supplements, providing supra-physiological doses where background FODMAP intake has not been considered. This study aimed to re-address the impact of changing dietary FODMAPs on microbiota compared to a habitual diet using findings from a blinded cross-over study (Gut. 2015;64:93–100).

Methods: Twenty-seven IBS and six healthy subjects underwent evaluation of their habitual diet followed by randomisation to 21 days of provided low or typical amounts of FODMAPs (‘Australian diet’) and matched for other nutrients. Five-day faecal samples were collected at the end of each dietary period, pooled, and analysed for bacterial abundance.

Results: Mean daily oligosaccharide intakes for the habitual diet was 3.8 g, compared with LFD 1.6 g and Australian diet 5.5 g. In relation to the habitual diet, the LFD was associated with decreased total bacteria and absolute but not relative abundance of butyrate-producing Clostridium cluster XIVa and mucus-associated Akkermansia muciniphila. In contrast, the Australian diet was associated with five- and seven-fold increases in those bacteria relative to total, respectively (see Table).

<table>
<thead>
<tr>
<th>Abundance</th>
<th>Bacteria</th>
<th>Australian diet</th>
<th>Low FODMAP diet</th>
<th>Habitual diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Faecalbacterium prausnitzii</td>
<td>1.11 [0.82–1.40]</td>
<td>0.95 [0.69–1.22]</td>
<td>1.29 [0.92–1.66]</td>
</tr>
<tr>
<td></td>
<td>Bilidobacteria spp.</td>
<td>1.33 [0.74–1.92]</td>
<td>0.87 [0.47–1.27]</td>
<td>1.48 [0.79–2.18]</td>
</tr>
<tr>
<td></td>
<td>Akkermansia muciniphila</td>
<td>0.10 [0.03–0.16]</td>
<td>0.02 [0.01–0.03]</td>
<td>0.01 [0–0.02]</td>
</tr>
<tr>
<td></td>
<td>Ruminococcus torques</td>
<td>0.04 [0.02–0.06]</td>
<td>0.06 [0.04–0.09]</td>
<td>0.05 [0.02–0.08]</td>
</tr>
</tbody>
</table>

Values: means [95% CI] for 33 observations (n = 30 for A. muciniphila)

Discussion/Conclusion: A LFD reduces total bacteria non-selectively, but an increase of dietary oligosaccharide intake of only 2.2 g is associated with a strong prebiotic effect. Small increases in dietary FODMAPs are enough to selectively increase beneficial bacteria.
Postoperative Crohn’s disease recurrence is associated with specific changes in the faecal microbiome – Potential pathogenic and protective roles

Amy L. Hamilton¹, Michael A. Kamm¹,², Shu-Mei Teo³, Peter De Cruz¹, Emily K. Wright¹, Hai Feng⁴,⁵, Kathryn J. Ritchie¹, Joseph J.Y. Sung⁶, Carl D. Kirkwood⁴, Michael Inouye³

¹St. Vincent’s Hospital and University of Melbourne, Melbourne, Australia; ²Imperial College, London, UK, ³Centre for Systems Genomics, University of Melbourne, Melbourne, Australia; ⁴Murdoch Children’s Research Institute, Melbourne, Australia, ⁵Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Introduction: Crohn’s disease usually recurs after “curative” resection. This may relate to specific microbial populations playing a pathogenic role.

Methods: Faecal samples were obtained peri-operatively (baseline), 6, 12 and 18 months after surgery from 130 patients in the POCER study. Endoscopic recurrence was assessed (Rutgeerts Score ≥ 12) at 18 months. The V2 region of the 16S rRNA gene was sequenced using Illumina MiSeq. Data were processed using the QIIME pipeline and analysed for diversity and differential abundance at genus level using R. Hierarchical cluster analysis was performed on relative abundance at family level; each cluster was assessed for outcome at 18 months.

Results: Alpha diversity increased significantly after surgery (all patients, baseline vs 18 months (p = 0.048). At six months, diversity was greater for patients who remained in remission vs. recurrence (p = 0.04). Overall bacterial composition (β diversity) differed between recurrence and remission at 18 months (p = 0.008), as well as over time (all patients and all samples: baseline, six, 12 and 18 months; p = 0.001). Nine genera (four from the order Clostridiales, two from Lactobacillales and Bacteroidales) were differentially abundant between disease recurrence compared to remission. Comparison of endoscopic outcomes at 18 months based on cluster analysis resulted in six distinct groups based on predominance of certain bacterial families (Table 1).

<table>
<thead>
<tr>
<th>Cluster</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lachnospiraceae</td>
<td>Bacteroidaceae</td>
<td>Veillonellaceae</td>
<td>Enterobacteriaceae</td>
<td>Prevotellaceae</td>
<td>Other</td>
</tr>
<tr>
<td>n samples per group</td>
<td>148</td>
<td>35</td>
<td>28</td>
<td>15</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>n remission at 18M</td>
<td>99</td>
<td>18</td>
<td>16</td>
<td>2</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>n recurrence at 18M</td>
<td>49</td>
<td>17</td>
<td>12</td>
<td>13</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Unadjusted OR (95% CI)</td>
<td>0.45 (0.27-0.75)</td>
<td>1.3 (0.66-2.78)</td>
<td>1.07 (0.48-2.37)</td>
<td>8.70 (1.82-41.7)</td>
<td>0.92 (0.21-4.0)</td>
<td>2.05 (0.80-5.26)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.003</td>
<td>0.414</td>
<td>0.873</td>
<td>0.007</td>
<td>0.920</td>
<td>0.136</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>0.47 (0.27-0.82)</td>
<td>1.69 (0.77-3.73)</td>
<td>0.83 (0.35-1.98)</td>
<td>6.35 (1.24-32.44)</td>
<td>0.76 (0.16-3.70)</td>
<td>1.90 (0.69-5.26)</td>
</tr>
<tr>
<td>Adjusted P value</td>
<td>0.007</td>
<td>0.190</td>
<td>0.677</td>
<td>0.026</td>
<td>0.731</td>
<td>0.220</td>
</tr>
</tbody>
</table>

Table 1: Odds ratio for recurrence in the six identified clusters based on hierarchical cluster analysis.
Discussion/Conclusion: In faecal samples from postoperative patients, dominance of the bacterial family Lachnospiraceae (group one) is associated with lower rates of recurrence at 18 months, whereas dominance of Enterobacteriaceae (group four) is associated with disease recurrence. This may be as a result of higher relative populations of enteric pathogens such as *Proteus, Serratia and Escherichia* in samples within this cluster. The specific bacterial genera associated with disease recurrence mirror the results of the cluster analysis, including decreases within the orders Clostridiales and Lactobacillales, and increased abundance of Bacteroidales.
Intrafamilial spread of Helicobacter pylori infection in North Jakarta

R. Herardi¹, A.F. Syam¹, M. Simadibrata¹, S. Setiati¹, N. Damindro², O.D. Asmara¹
¹Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia
²Cilincing District General Hospital, North Jakarta, Indonesia

Introduction: Helicobacter pylori infection is higher in close communities and in members of family groups than in the general population. This may be due to relapses or reinfections between members of the same family. The objective of this study was to determine the importance of test and treat families of patients infected Helicobacter pylori infection.

Methods: Twenty-one patients who lives with Helicobacter pylori-infected family, without previous helicobacter pylori test and eradication treatment, underwent test using Headway ¹⁴C-urea breath test (UBT) in Cilincing District General Hospital from October to December 2016.

Results: Twenty-one subjects were included, consist 13 (61.9%) males. Mean age was 34.9 years old. Subgroup study was done and no significant difference was found. Nine of 21 (42.9%) patients are infected Helicobacter pylori. The proportion is higher than the prevalence of helicobacter pylori infection in this area (15.0%).

Discussion/Conclusion: The proportion of Helicobacter pylori is significantly higher among families of infected patients. It suggest to test and treat of Helicobacter pylori infection to families of the infected patients.
Acquired and innate immune responses are inhibited in IBS-D in symptom flare vs. symptom free

Patrick Hughes\(^1,2\), Chris Mavrangelos\(^1\), Melissa Campaniello\(^1\), Peter Bampton\(^3\), Jane Andrews\(^2,4\)
\(^1\)Centre for Nutrition and GI Diseases, Department Medicine, University of Adelaide & South Australian Health and Medical Research Institute (SAHMRI)
\(^2\)Faculty of Health Science, University of Adelaide
\(^3\)Department of Gastroenterology and Surgery, Flinders Medical Centre, Adelaide
\(^4\)Department of Gastroenterology and Hepatology, The Royal Adelaide Hospital, Adelaide, Australia

**Introduction:** Altered immune function correlates with symptoms in irritable bowel syndrome but the type of immune response involved remains controversial (Hughes et al. Am J Gastroenterol. 2013). This due to 2 main reasons; grouping of all IBS patients together rather than stratifying by bowel habit, and an over-reliance on cross-sectional rather than longitudinal studies. We aimed to compare the circulating immune profile of diarrhoea-predominant IBS patients (IBS-D) in symptom flare compared to symptom free.

**Methods:** 5 IBS-D patients (ROME II) completed HADS questionnaire and provided a venous blood sample when in self-reported symptom flare and when symptom free. Peripheral blood mononuclear Cell (PBMC) were isolated from blood and frozen. Thawed PBMC were stained with antibodies against TH (CD4), TC (CD8), T\(_{\text{CENTRAL MEMORY}}\) (CD45RA\(^{-}\), CD197\(^{+}\)), T\(_{\text{REG}}\) (CD127\(^{-}\) CD25\(^{+}\)) with homing markers (CD49d, \(\beta7\)) and monocyte subsets (CD3\(^{-}\), HLADR\(^{+}\) CD14, CD16, CD11c) or stained with CellTracker (Thermo) and stimulated with CD3/CD28 beads for 4 days before flow cytometry analysis. Cytokine concentrations in supernatants from PBMC stimulated with LPS (1 ng/ml overnight) or stained with CD3/CD28 for 4 days were determined by multiplex or ELISA.

**Results:** HADS scores were significantly higher in IBS patients in flare compared to symptom free. LPS stimulated IL-1\(\beta\), IL-6 and TNF-\(\alpha\) concentrations and CD3/CD28 stimulated IFN-\(\gamma\) and TH proliferation were significantly decreased in symptom flare compared to symptom free. The proportion of gut homing CD4 and CD8 T\(_{\text{CM}}\) were decreased in symptom flare compared to symptom free. The proportions of T\(_{\text{REG}}\) or monocyte subsets did not differ between symptom flare and symptom free.

**Discussion/Conclusion:** The acquired and innate immune system are inhibited in IBS-D patients when they are in symptom-flare relative to symptom-free. The mechanisms underlying this inhibition remain to be determined and warrant further investigation.
Reducing the maternal dietary intake of indigestible and slowly absorbed short-chain carbohydrates is associated with improved infantile colic: A proof-of-concept study

M. Iacovou, E.C. Mulcahy, H. Truby, J.S. Barrett, P.R. Gibson, J.G. Muir
1Department of Gastroenterology, Monash University, Melbourne, VIC, Australia
2Department of Nutrition and Dietetics, Monash University, Melbourne, VIC, Australia

Introduction: Infantile colic is a common complaint for which parents seek professional advice. In breastfed infants, mothers are often advised to avoid intestinal-gas-producing foods (e.g., onions and legumes). Anecdotal relief of infantile colic when the mother reduced dietary FODMAPs (Fermentable, Oligo-, Di-, Mono-saccharides and Polylols), prompted assessment of the concept that maternal low FODAMP diet might be efficacious for infantile colic.

Methods: Exclusively breastfeeding mothers and their colicky, typically-developing, healthy infants who met the Wessel Criteria for infantile colic were recruited from the community. After assessment of habitual maternal diet, mothers were provided a 7-day low FODMAP diet. Using the validated Barr diary, crying, fussing, sleeping, feeding and awake-and-content durations were captured at baseline and during the dietary intervention. Analysis was corrected for infant's age. At baseline and at the end of the dietary intervention, breast milk was analysed for FODMAP and microbiota content and infant faecal samples for changes in pH and microbiota.

Results: Eighteen breastfeeding mothers (aged 27-40 years) adhered to the diet that reduced FODMAP intake by about 75%. Infants were of gestational age 37–40 weeks and aged 2–17 weeks. At entry, crying durations were a mean [95% CI] of 142 [106–61] min and fell by 52 [178–120] min ($p = 0.005$; ANCOVA). Combined crying-fussing durations fell by 73 [301–223] min ($n = 13; p = 0.007$), as did crying episodes ($p = 0.01$) and fussing durations ($p = 0.011$). Infant sleeping, feeding, and awake-and-content durations did not change. Infant faecal pH did not change. Breast milk lactose content was stable and other known FODMAPs were not detected. Abundance and diversity of breast milk and infant faecal microbiota changed.

Discussion/Conclusion: Maternal low FODMAP intake may be associated with a reduction in infantile colic symptoms. Crying-fussing durations reduced greater than the clinical significance of $> 25\%$. A controlled evaluation is needed to assess if microbiota change was an effect of diet.
Post-OP Crohn’s disease maintenance: Is AZA a better option – An Indian study

B.P.N. Kaushik, Das Kshaunish, Sarkar Rajib, Dhali Gopal Krishna
Department of Gastroenterology, School of Digestive and Liver Disease, Institute of Postgraduate Medical Education and Research, Kolkata, India

Introduction: Postoperative clinical recurrence of Crohn’s disease occurs in about one third of patients within one year of surgery and increases gradually. As there is no data from India regarding the response of AZA in postoperative prophylaxis, present study was conducted.

Methods: A retrospective and prospective analysis of Crohn’s disease patients on AZA was done. AZA was either given to maintain medically induced remission or as a postoperative prophylactic agent.

Results: Total of 164 Crohn’s disease patients on AZA for median duration of 42.5 months (range: 6–192 months) included in study. Fisher’s-exact-Test, Paired-t-test and Mann-Whitney-U-test used. Data represented in table 1, table 2.

Discussion/Conclusion: In our study azathioprine (AZA) did not show any benefit in maintaining remission in postoperative Crohn’s patients compared to medically induced remission group.

Table 1: AZA given to maintain medically-induced remission (n = 137)

<table>
<thead>
<tr>
<th></th>
<th>After diagnosis and prior to AZA therapy</th>
<th>Post AZA therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flares, no (Mean ± SD)</td>
<td>1.1 ± 1.6</td>
<td>0.7 ± 2.2</td>
<td>p = 0.027</td>
</tr>
<tr>
<td>Steroid courses, no (Mean ± SD)</td>
<td>1.0 ± 1.3</td>
<td>0.6 ± 2.0</td>
<td>p = 0.031</td>
</tr>
<tr>
<td>Surgery (%)</td>
<td>2.9</td>
<td>2.2</td>
<td>p = NS</td>
</tr>
<tr>
<td>Chronic disabling disease (%)</td>
<td>17.5</td>
<td>10.2</td>
<td>p = NS</td>
</tr>
<tr>
<td>Hospitalization, no (Mean ± SD)</td>
<td>0.2 ± 0.6</td>
<td>0.9 ± 6.1</td>
<td>p = NS</td>
</tr>
<tr>
<td>Blood transfusion units, no (Mean ± SD)</td>
<td>1.2 ± 8.9</td>
<td>2.3 ± 13.6</td>
<td>p = 0.052</td>
</tr>
</tbody>
</table>

Table 2: AZA given as postoperative prophylaxis (n = 27)

<table>
<thead>
<tr>
<th></th>
<th>After diagnosis and prior to AZA therapy</th>
<th>Post AZA therapy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flares, no (Mean ± SD)</td>
<td>0.1 ± 0.3</td>
<td>0.3 ± 0.7</td>
<td>p = NS</td>
</tr>
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<td>Steroid courses, no (Mean ± SD)</td>
<td>0.1 ± 0.3</td>
<td>0.3 ± 0.7</td>
<td>p = NS</td>
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<td>Surgery (%)</td>
<td>3.7</td>
<td>0</td>
<td>p = NS</td>
</tr>
<tr>
<td>Chronic disabling disease (%)</td>
<td>7.4</td>
<td>11.1</td>
<td>p = NS</td>
</tr>
<tr>
<td>Hospitalization, no (Mean ± SD)</td>
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<td>0.4 ± 0.8</td>
<td>p = 0.059</td>
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<tr>
<td>Blood transfusion units, no (Mean ± SD)</td>
<td>0</td>
<td>0.6 ± 1.8</td>
<td>p = NS</td>
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</tbody>
</table>
The role of small intestinal bacterial overgrowth in pathogenesis of lactase deficiency

O. Knyazev, N. Fadeeva, I. Ruchkina, A.I. Parfenov, P. Shcherbakov
Moscow Clinical Research Center, Moscow, Russia

Introduction: Human microflora is a stable genetically determined system. The concentration of microorganisms in the small intestine fluctuates from $10^1$ to $10^4$ CFU/ml of the intestinal content. Secondary lactase deficiency (SLD) is inability to digest lactose, the predominant sugar of milk. This inability results from decrease of lactase enzyme activity, which is produced in the small intestine.

Purpose: To define the influence of SIBO in patients with SLD in adult patients.

Aims and methods: In this study, 386 patients (the mean age – 33.9 ± 9.09; F/M 249/137) with postinfectious irritable bowel syndrome (IBS) were analyzed concerning lactase deficiency. All patients underwent intestinal endoscopy with biopsies from the mucosa of the descending duodenum in order to determine lactase deficiency. The biopsies were taken in order to determine lactase deficiency (normal, mild and severe) by means of lactose quick test (LQT). To diagnose small intestinal bacterial overgrowth (SIBO) all patients underwent lactulose breath test during 2 hours.

Results: SLD was detected in 36.5% of patients with postinfectious IBS. Mild SLD was determined in 25.6% of patients, and severe SLD – in 10.9% of patients. The specific clinical symptoms of mild SLD were moderate flatulence with abdominal pain (80.7%); the majority of patients (73.7 %) had normal stool consistency, one time a day; the other patients had semi-liquid faeces, 2–3 times a day (26.3%). The clinical symptoms of severe SLD were diarrhea (stool 44 times a day) in 85.7% of patients, abdominal pain and flatulence (90.5%). SLD in all cases was accompanied by SIBO (the average level of lactulose breath test was 80.3 ± 28.3 ppm, N ≤ 20 ppm). It turned out that the degree of lactase deficiency depends on the severity of SIBO in the lumen of the small intestine. Thus, when mild SLD average value SIBO was 72.4 ± 25.1 ppm, whereas severe SLD average indicators of SIBO achieved higher values, 99.3 ± 26.9 ppm (N ≤ 20ppm). To establish the degree of dependence of SIBO in the small intestine and the degree of deficiency of lactase in the small intestine biopsies performed a statistical analysis of the results by calculating the Spearman rank correlation coefficient to study a statistically significant link between the various phenomena. In this study, an inverse correlation between the degree of lactase deficiency in patients with the SLD and the severity of SIBO in the small intestine, i.e. the higher the hydrogen concentration in the exhaled air, the less activity of the enzyme lactase in the small intestine biopsy specimens ($r = -0.49$, $p < 0.001$).

Conclusion: SIBO in all cases was accompanied by SLD. Thus, the high frequency of the SLD associated with SIBO in the small intestine in patients postinfectious IBS can be explained by the growth of pathogenic microflora in the small intestine.

Disclosure of Interest: None declared.
Antibiotic resistance genes in the gut microbiome of pregnant women

Miharu Kobayashi¹,², Luisa F. Gomez-Arango²,³, William Carey-Foster²,³, Leonie K. Callaway²,³,⁴, Mark Morrison⁵, Helen L. Barrett²,³,⁴, Marloes Dekker Nitert¹,²
¹School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia
²UQ Centre for Clinical Research, The University of Queensland, Brisbane, Australia
³School of Medicine, The University of Queensland, Brisbane, Australia
⁴Obstetric Medicine, Royal Brisbane and Women’s Hospital, Brisbane Australia
⁵Diamantina Institute, Faculty of Medicine and Biomedical Sciences, The University of Queensland, Brisbane Australia

Introduction: Antibiotics is the most commonly prescribed medication during pregnancy. Treatment with antibiotics can result in the disruption of gut microbiome composition and enrichment of antibiotic resistant organisms expressing antibiotic resistance genes (ARG). Currently, there is limited data on the presence of ARGs in the pregnant gut microbiome and whether it affects maternal microbiome, metabolism and pregnancy outcomes. Therefore, this study aims to determine the prevalence of ARGs in pregnant women and its association with gut microbiome composition and maternal metabolism.

Methods: Faecal microbiota profiles at 16 weeks gestation of 215 overweight or obese women recruited in the Study of Probiotic IN Gestational diabetes (SPRING) study were assessed by 16S rRNA sequencing. Data were processed with QIIME (Quantitative Insights Into Microbial Ecology). Gene counts of 9 ARGs (Shv-1, Vim-1, Imp, Cmy-2, Oxa-1, Cata-1, Dfra-17, BacA and TolC) were analysed by semi-quantitative real time PCR.

Results: ARG counts are variable in the gut microbiome of pregnant women at 16 weeks gestation with a median of 3 ARGs (range 0 to 9) present. BacA and TolC were commonly detected. BacA gene density correlated positively with Enterobacteriaceae. Furthermore, BacA gene counts correlated positively with fasting insulin levels at 16 weeks gestation and was higher in women with later onset of gestational diabetes mellitus (GDM). Alpha diversity was increased in women with Vim-1 and Imp present in their gut microbiome and in women with a high number of ARGs. Vim-1 correlated negatively with Ruminococcaceae whereas Imp expression was positively correlated with fasting glucose level.

Discussion/Conclusion: ARGs are commonly detected in the gut microbiome during early pregnancy and is not associated with reduced bacterial richness. BacA is associated with higher abundance of Enterobacteriaceae, a family known to produce endotoxin that can increase insulin resistance thereby potentially contributing to the pathogenesis of GDM.
Fecal microbiota transplantation: Should we form European cooperative network?

P. Kohout, J. Vejmelka
Department of Internal Medicine, Thomayer Hospital, Prague, Czech Republic

Fecal microbiota transplantation (FMT) represents very effective and quick treatment of recurrent Clostridium difficile infection. There is awaiting broad use of this method in other indications – not only gastroenterological. To ensure the safety and efficacy of this procedure it is inevitable to form Cooperative network within the FMT-engaged departments.

Czech model represents FMT – network gathering the doctors and departments that either provide FMT or are interested in this method. Anonymous FMT - data are collected, processed, monitored and presented in annual Czech symposium Clostridium difficile Day. Czech FMT – coordination working group comprises: data collection with safety and efficacy vigilance, FMT – guidelines process, regulation authorities negotiations and educational activities. According to current Czech FMT – data analysis FMT represents safe and effective treatment of complicated CDAD (Clostridium difficile associated diseases).

The number of FMT – centers raised from 1 (2010) to 23 (2016) across the Czech Republic, 260 FMT were performed in patients with complicated CDAD (more than 1 recurrence, mostly from relatives, in 47% of them via gastroscope working channel, 22% via nasojejunal tube, 4% via nasogastric tube, in 18% using enema and in 9% using colonoscope. Efficacy in CDAD was 85%, in our department 90% from 20 applications.

European cooperation FMT – group could enforce the development of FMT and improve the FMT safety in the treatment of CDAD and prospectively in other diseases.
Responses of circulating granulocytes in functional dyspepsia patients

Yuwen Li¹,², Anh Do², Erin Shanahan², Natasha Koloski², Teressa Hansen², Ayesha Shah², Simon Keely³, Nicholas Talley³, Gerald Holtmann²
¹Department of Gastroenterology, First affiliated Hospital, Sun Yat-sen University, Guangzhou, GuangDong Province, P.R. China 510080
²Department of Gastroenterology & Hepatology Princess Alexandra Hospital, 199 Ipswich Road, Woolloongabba, QLD 4102, Australia
³Hunter Medical Research Institute, University of Newcastle, Kookaburra Circuit, New Lambton, NSW 2295, Australia

Introduction: The most prevalent functional gastrointestinal disorders (FGID) affecting upper gastro tract is functional dyspepsia (FD). Some studies have shown increased mast cells and eosinophils in the mucosa and CD4+ T-cells in peripheral blood, which might explain some abnormal gastrointestinal (GI) functions. In this study, we aimed to investigate that the inflammatory responses of peripheral blood granulocytes, correlating with GI symptoms observed in FD patients.

Methods: Granulocytes were isolated from 46 FD patients and 14 healthy controls using density gradient centrifugation and dextran sedimentation and cultured for 6 h with and without LPS (1 µg/ml). Cytokine production (IL-6, TNF, IL-1β & IL-8) was measured using ELISA. GI symptoms were assessed utilizing the SAGIS (Structured Assessment of Gastrointestinal Symptoms Instrument), standard nutrient challenge test (NCT) and gastric emptying test (GET) were also performed in all patients. Eosinophil population in peripheral blood was examined using flow cytometry with anti-human CD11b, CD16, Siglec 8, CD66b conjugated antibodies. Data were presented as mean ± SEM. Statistical significance was assessed using Spearman correlation.

Results: The increase of cytokine production including IL-6, TNF, IL-1β and IL-8 associated with delayed gastric emptying. In the patients with moderate to severe fullness, the increase of eosinophils linked to worse fullness symptom (r = 0.6410, p = 0.02). In the patients with severe early satiety, the percentage of eosinophils is also positively correlated with fullness symptoms (r = 0.79, p = 0.02), and negatively correlated with reported quality of life (r = -0.94, p = 0.0048).

Discussion/Conclusion: Our data suggest that granulocyte responses including inflammatory cytokine production and increase in number of circulating eosinophils were linked to altered gut functions and symptom generations.
Keeping your MAITs in line; interrogating MAIT cell activation in an animal model of inflammatory bowel disease

Rink-Jan Lohman, Jeffrey Y.W. Mak, Ligong Liu, Anh Do, David Fairlie
Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

Introduction: Mucosal-Associated Invariant T cells (MAITs) are specialised MHC-class Iβ lymphocytes that express canonical semi-invariant T-cell receptors (TCR). MAITs are activated by MR1 on APCs, crosslinking MAIT TCR after binding vitamin B2-related ligands produced by commensal bacteria. They likely have roles in mucosal immunity where bacterial contact is common. Our laboratory has produced stable MR1-ligands that were used to interrogate the function of MAITs in a mouse model of inflammatory bowel disease (IBD).

Methods: Mice (C57B/6J, female) were administered 2.5% DSS via drinking water for 5 days, followed by 2 days regular water. Synthetic MAIT-activating Vitamin B2 metabolite and MR1 ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) was intracolonically administered daily (10 mg/kg) for 7 days. Mice were monitored for IBD symptoms (weight change, diarrhea, rectal bleeding, faecal blood). Post-mortem colon length, oedema, myeloperoxidase and (MPO) activity and histopathological changes were measured.

Results: The MAIT activator 5-OPRU caused significant worsening of IBD-related diarrhea and rectal bleeding over the 7-day protocol. There was also a significant reduction in colon length, worsened oedema, histopathological colon wall thickening and crypt damage compared to DSS controls. MPO activity was exceptionally high in animals treated with 5-OPRU. Non-DSS-5OPRU treated control animals also showed significant IBD symptoms such as diarrhea and weight loss, however, colons appeared normal upon histopathological and MPO inspection.

Discussion/Conclusion: The study suggests that MR1 ligand 5-OPRU significantly exacerbates existing IBD, possibly even contributing to intestinal pathology in the absence of underlying disease. Even though currently speculative, we suggest the MR1 ligand may mimic a bacterial overload in the gut that stimulates MAIT cells to mount an immune response involving MPO-producing neutrophils, possibly to rid the colon of ‘invading bacteria’. In the process, an immune-cell driven exacerbation of local mucosal injury occurs, leading to worsened IBD pathology. The data suggest that compounds which act to quell MAIT cell activity may be useful in treating IBD-like disorders without upsetting the colonic microbiome.
Gut microbiota composition and behaviour problems in early childhood

Amy Loughman¹, Martin O’Hely², Fiona Collier², Michael Conlon³, Christos Symeonides⁴, Anne-Louise Ponsonby⁴ and Peter Vuillermin²,⁴ on behalf of the Barwon Infant Study Investigator Group
¹RMIT University, Melbourne, VIC, Australia
²Deakin University, Geelong, VIC, Australia
³CSIRO, Adelaide, SA, Australia
⁴Murdoch Childrens Research Institute, Parkville, VIC, Australia

Introduction: The gut microbiome has been demonstrated to have an association with brain development and function, predominantly in animal models. It is considered likely that the functional properties of the gut microbiome mediate the relationship between diet quality and mental health in both adults and children. There is a dearth of human studies regarding associations between the gut microbiota and child behaviour.

This study aimed to explore predictive relationships between infant faecal microbiota composition at 12 months and behavioural outcomes at 2 years of age in a longitudinal cohort study in the Barwon region of Victoria, Australia.

Methods: Faecal samples were collected from infants at 12 months of age. 16S sequencing was conducted using the Illumina MiSeq platform. When children were aged 2 years, parents completed the Child Behavior Checklist (CBCL), a well-validated 99-item questionnaire of problem items from which subscales of Internalising, Externalising and Total Problems are generated. Analysis was conducted using Calypso and R.

Results: CBCL scores were classified as ‘normal range’ or ‘elevated’ on the basis of borderline-clinical cut-off scores on any of the three subscales. Only 22 of the 217 participants for whom microbiome and behavioural data were available were classified as having ‘elevated’ behavioural problems (Internalising, Externalising or Total Problems). The microbiota diversity was significantly higher in participants with ‘normal range’ versus ‘elevated’ CBCL scores (Shannon Index). Random forest analysis revealed evidence that the two groups could be distinguished on the basis of the abundance of 22 separate OTUs.

Discussion/Conclusion: These results are compatible with a prospective association between the variation in infant gut microbiota composition at 12 months of age and subsequent child behavioural problems. Further investigation will be conducted to determine bacterial taxonomic associations, possible confounding and mediating relationships of other environmental and biological predictors, and mechanisms underlying this relationship.
Platelet-activating factor signalling drives pulmonary inflammation in animal models of colitis

Andrea Mathe, Sean Mateer, Jessica Bruce, Paul Foster, Jay Horvat, Philip Hansbro, Simon Keely
Gastrointestinal Research Group, Viruses, Infection/Immunity, Vaccines and Asthma Program, and Priority Research Centre for Asthma and Respiratory Disease, Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia; and School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, New South Wales, Australia

Introduction: Inflammatory bowel disease (IBD) is associated with a number of immune-mediated pathologies in peripheral tissues termed extra-intestinal manifestations (EIM). The organs affected by EIM include the lung, liver, skin and eyes. Approximately 54% of IBD patients have some form of respiratory pathology, which range from subclinical respiratory inflammation to active respiratory disease. The mechanism by which IBD can induce respiratory pathologies is unknown, but associated with increased intestinal permeability and systemic inflammation. Given that pathologies are characteristic of many animal models of colitis, we hypothesized that subclinical pulmonary pathology would occur in murine models of colitis and thus provide a model to investigate the pathogenesis of colitis-induced respiratory disease.

Methods: Animals were subjected to DSS and TNBS models of colitis and compared to appropriate controls. Intestinal and pulmonary pathology was assessed by histological evaluation. Blood, lung and intestinal tissues were examined by PCR, Immunoblot and ELISA.

Results: Pulmonary inflammation was observed in both colitis models, and characterised by neutrophil and monocyte recruitment to the lung. Systemic IL-6 levels were elevated in the DSS colitis model and found to recruit neutrophils from the bone marrow to the lung. Endotoxin levels in the lung were increased with colitis and associated with increases in platelet activating factor receptor (PAFR) along with IL-1β and CCL2 expression. PAF signalling in the lung induced the expression of IL-1β and the recruitment of PAFR positive neutrophils, but did not induce CCL2. Intratracheal delivery of PAFR antagonists prevented pulmonary inflammation and in vitro treatment of alveolar macrophages with PAFR antagonists prevented induction of IL-1β and CCL2 by DSS animal serum.

Discussion/Conclusion: The results from this study identify a number of potential pathogenic factors that may be involved in the development of IBD-induced extra-intestinal pathologies.
Microbial colonisation of the duodenal-jejunal bypass sleeve used for treatment of obesity: Results of a pilot study

Jessica McMaster1,2, Erin Shanahan1,3, Graeme Rich1,3, Veronique Chachay2,3, Mark Morrison4, Gerald Holtmann1,3
1Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Queensland, Australia
2School of Human Movement and Nutrition Sciences, University of Queensland, Brisbane, Queensland, Australia
3PAH Southside Clinical Unit, School of Clinical Medicine, University of Queensland, Brisbane, Queensland, Australia
4University of Queensland Diamantina Institute, University of Queensland, Brisbane, Queensland Australia

Introduction: Alterations in the gastrointestinal microbiota have been observed in obesity, following weight loss, and more specifically following bariatric surgery. The endoscopically-placed duodenal-jejunal bypass sleeve (Endobarrier®) induces clinically significant weight loss; however, the impact of this device on the microbiota, or microbial colonisation of the sleeve itself, has not been reported. The aim of this pilot investigation was to assess any microbial biofilm on the sleeve, and links between patient outcomes and bacterial colonisation of the sleeve.

Methods: Four morbidly obese female patients with type 2 diabetes mellitus (median age 54.5 years; median baseline BMI 45.1 kg/m²) underwent 48 weeks of Endobarrier implantation in conjunction with dietary counselling. Upon device explant, samples representing both the loosely adherent and biofilm-associated microbiota were obtained, and DNA extraction performed. DNA was amplified using barcoded primer sets that target the gene encoding 16S ribosomal RNA. The resultant libraries were sequenced using the MiSeq platform and bioinformatics analysis performed using QIIME.

Results: The median total body weight loss achieved was 22.9%. Assessment of the sleeve device revealed a dense biofilm on the mucosa-adjacent surface, with less colonisation on the luminal surface. There was a trend linking magnitude of weight loss achieved with increased microbial diversity (r = 0.919; p = 0.08). The microbiota was dominated by the genus Lactobacillus, and members of the Enterobacteriaceae family (75% patients) or the genus Collinsella (1 patient), with lower levels of more typical members of the duodenal community. The strongest driver of the sleeve microbiota was each patient’s unique profile, with inter-individual differences greater than those observed between different device surfaces.

Discussion/Conclusion: This is the first study to characterise the microbiota dwelling on the sleeve device used for the treatment of morbid obesity. A larger cohort is being recruited to further elucidate the impact of the gastrointestinal microbiota on device tolerance and effectiveness.
Alteration of the microbiome effects neuroprotective mechanisms in an animal model of glaucoma

Zachary E. McPherson¹,², Mark McEvoy¹, Hae Ung Lee², Nick Talley¹, Ashish Agar³, Minas Coroneo³, Sven Pettersson²,⁴,⁵
¹The School of Medicine and Public Health, University of Newcastle, Callaghan, NSW, Australia
²The Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
³The Department of Ophthalmology, Prince of Wales Hospital, Sydney, NSW, Australia
⁴The Department of Microbiology, Cell and Tumor Biology, Karolinska Institutet, Stockholm, Sweden
⁵The SCELSE Microbiome Centre, NTU, Singapore

Introduction: Models of dysbiosis and use of germ-free mice (GF) have established microbiome mediated regulation of brain derived neurotrophic factor (BDNF) in the central nervous system. This protein plays an important role in tissue repair and neuroprotection. Glaucoma is a chronic blinding neurodegenerative disease that affects the neurons of the optic nerve. We recently demonstrated that irritable bowel syndrome is a risk factor for glaucoma. This suggested a link between dysbiosis and glaucoma. We assessed the neuroprotective potential of the microbiome, in the retina, with the use of an optic nerve crush model of glaucoma.

Methods: Optic nerve crush, an injury-based model of glaucoma, was performed in 8–12 week old GF, specific pathogen free (SPF) mice and GF mice conventionalized with SPF microbiome (CON). The optic nerves were crushed in the different mouse groups. Mice were sacrificed for histological and RNA analysis of their retina at various time points. Retinal ganglion cells were stained with Anti-RBPMS. The cellular density of the retinae was calculated and compared over time after crush. RNA was prepared from retinae and RT-qPCR was performed to assess the BDNF gene’s expression.

Results: SPF mice demonstrated 23.9% (p = 0.002) and 54.3% (p = 0.005) more retinal ganglion cell survival at days 7 and 30, respectively, compared to GF mice. BDNF mRNA at baseline was not significantly different between GF and SPF mice. By day 3 after optic nerve crush, BDNF mRNA levels rose to 4.69 fold baseline levels in SPF mice and were 2.8 fold higher than GF levels at the same time point (p < 0.001). By day 7, both SPF and GF BDNF levels had returned to normal. CON mice demonstrated similar results to SPF mice.

Discussion/Conclusion: The normal SPF microbiome influences tissue repair mechanisms through elevation of BDNF in an injury based model of neurodegeneration of the optic nerve.
Hierarchical structure of mucin within the gastrointestinal tract and its interaction with dietary components

Oliver Meldrum1,2, Gleb E. Yakubov1,3, Michael A. McGuckin4, Michael J. Gidley1,2
1ARC Centre of Excellence in Plant Cell Walls, The University of Queensland, Brisbane, QLD 4072, Australia
2Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD 4072, Australia
3School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia
4Immunity, Infection and Inflammation Program, Mater Medical Research Institute and The University of Queensland School of Medicine, South Brisbane, QLD 4101, Australia

Introduction: The adherent mucus layer is a vital component of the body’s epithelial surfaces. Mucus assembly is characterised by the presence of multiple levels of mucin structure at different length scales. This gives mucus its unique set of rheological and barrier properties that enable it to establish an effective physical and selective diffusion barrier as well as to hydrate the underlying epithelium. The rheological and structural characteristics of intestinal (MUC2) mucin, the functional component of the mucus layer and their interaction with cell wall components that are liked with a number of health benefits are investigated in this study. Biochemically well characterised porcine intestinal mucin was utilised as a model for human mucins to characterise their viscoelasticity, structure and dynamics as a function of concentration, pH and Ca2+. The mesoscopic forces that mediate the integrity of the mucin network were investigated using reducing (dithiothreitol), chaotropic (guanidinium chloride) and chelating agent (ethylenediaminetetraacetic acid) agents.

Methods: The rheological and structural characteristics of mucin were investigated using a combination of particle tracking microrheology, narrow gap oscillatory shear and high shear rheometry, and confocal microscopy.

Results: In this work, the complex rheological properties of the gelling mucus preparation are in a striking contrast with that of extensively purified mucin. The role and importance of non-mucin components have been elaborated, and the contribution to such interactions as hydrogen bonding, Ca2+-mediated links, and disulfide bonds has been evaluated. The micro rheological moduli are shown to be substantially smaller than the bulk values as determined by conventional shear rheology. These bulk viscoelastic values are dominated by the elastic moduli, while the microrheological response is less dominant. Additionally, we compare the ability of soluble dietary fibers and plant cell walls to alter the rheological and diffusion properties of purified intestinal mucin.

Discussion/Conclusion: Altering the organisation of the mucus layer as a result of interactions with food components such as plant cell walls and soluble dietary fiber will provide new insights into the ways in which these nutritional components affect the barrier properties of mucus, and provide a possible underpinning mechanism contributing to their health promoting properties.
A type 1 diabetes associated core microbiome driven by interleukin 2 pathway and MHC genetic variation

J.A. Mullaney, J.E. Stephens, C. Fong, B.E. Geeling, E.E. Hamilton-Williams
The University of Queensland Diamantina Institute, Brisbane, QLD, Australia

Changes in the gut microbiota has been implicated in the pathogenesis of many autoimmune conditions including type 1 diabetes (T1D). We performed an analysis of associations between the gut microbiota and T1D genetic risk using a mouse model of T1D. We identified a core microbiome associated with the T1D susceptible NOD strain and we demonstrate that disease protective alleles at the Idd3/5 (IL2, Ctl4, Slc11a1 and Acadl) loci, all of which mediate profound protection from T1D are associated with shifts in the microbiome. Comparison of the intestine of type 1 diabetes susceptible NOD mice with protected strains revealed subclinical pathology in the NOD intestine including increased immune cell infiltrates, reduced goblet cell mucous production and reduced Paneth cell anti-microbial peptide production, which were partly corrected by protective alleles of the MHC and Idd3/5. Immunotherapeutic administration of interleukin-2, mimicking the effects of the protective Idd3 allele, was able to reduce gut inflammation in NOD mice and shift the microbiota. These findings demonstrate for the first time that T1D associated genetic variants that restore immune tolerance to islet antigens also result in functional changes in the gut immune system and resultant changes in the microbiota.
Fecal transplant special way: The results of jejunal delivery in our practice

B. Nagy, M.D.; Zs. Csapó, M.D.
Flór Ferenc Hospital of Pest County, Kistarcsa, Hungary

Background: In line with the international trends in our hospital increasing occurrence of Clostridium difficile infection is observed following antibiotic treatment. According to previous prosperous results of different treatment tools we launched the fecal transplantation. Among possible ways of methods the jejunal route was chosen in 2012. In our retrospective study we present the results of the last five years.

Patients and methods: Among patients with confirmed and recurrent Clostridium difficile infections and completed treatment with metronidazole and vancomycin we offered the opportunity of fecal transplantation. Among the several transplant methods we chose jejunal route, and repeated it 3 times in a row. The donor stool we used was not collected from relatives, but from healthy volunteers after receiving appropriate screening tests. Treatment was considered successful if the diarrhea disappeared and inflammatory parameters improved.

Results: In the past 5 years, 82 patients were involved in this transplant program. Among the patients there were 61 females and 21 males with the average age of 74 years. Treatment was successful in 80 patients however the the mortality within 30 days post treatment was 28 patients.

Discussion: The jejunal stool transplantation seems to be an efficient and effective method for the treatment of Clostridium difficile caused colitis. Due to our results we recommend to improve the methods of informed consent for patients and relatives to enhance the willingness to take part in a procedure where the patient receives stool. Patients and relatives should know about the existence of this method to have the chance to treat them in an earlier stage. Based on our results, however the infection was treated, frequent early death occurred, and the main reason was delayed treatment. Autopsies found the bowel infection cured in all cases, but many other complications as organ diseases caused by the prolonged state of infection were detected.
Esophageal dysbiosis in Barrett’s esophagus and esophageal adenocarcinoma

Thi-My-Tam Nguyen1, Erin Shanahan1,2,3, Lutz Krause1, David C. Whiteman4, Bradley J. Kendall2,3,4, Luke F. Hourigan2,5, Andrew P. Barbour3, Gerald Holtmann2,3,6, Mark Morrison1 and Michelle Hill1

1The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Woolloongabba QLD 4102 Australia; 2Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Woolloongabba QLD 4102 Australia; 3School of Medicine, The University of Queensland, Translational Research Institute, Woolloongabba QLD 4102 Australia; 4QIMR Berghofer Medical Research Institute, Brisbane City QLD 4006 Australia; 5Gallipoli Medical Research Institute, School of Medicine, The University of Queensland, Greenslopes Private Hospital, Brisbane QLD 4120 Australia; 6Faculty of Health and Behavioral Sciences, The University of Queensland, St Lucia QLD 4072 Australia

Introduction: Before the mid-1970s, esophageal adenocarcinoma had represented less than 5% of all esophageal cancer cases. Now esophageal adenocarcinoma represents almost half of all cases; making it one of the most rapidly increasing cancers among the Western populations. The current risk factors for esophageal adenocarcinoma and the premalignant condition Barrett’s esophagus cannot explain why an individual progresses from Barrett’s esophagus or the cause for such a rapid shift in esophageal adenocarcinoma incidences. One possible, yet understudied risk factor for disease progression may be the microbiome. Here we investigate the correlation between the microbiome of esophageal adenocarcinoma and the premalignant conditions gastroesophageal reflux disease and Barrett’s esophagus.

Methods: 50 biopsy tissue samples were collected from 30 individuals with confirmed gastroesophageal reflux disease (n = 10), Barrett’s esophagus (n = 10), and esophageal adenocarcinoma (n = 10). Samples taken from Barrett’s esophagus and esophageal adenocarcinoma patients were collected from different sites of the esophagus; termed matched squamous and columnar for Barrett’s esophagus, and matched stomach and tumor for esophageal adenocarcinoma. The total DNA was extracted from the samples and used to produce PCR amplicon libraries of the V6-V8 hypervariable regions of Bacterial 16S rRNA genes, which were subjected to Illumina-based sequencing. Data analysis was performed using QIIME, Graphpad Prism and Calypso.

Results: Comparison between the study groups revealed esophageal disease was associated with altered microbial community composition but not alpha diversity. Higher relative abundance in Veillonella-affiliated sequences was observed in esophageal adenocarcinoma samples, with a commensurate lower abundance in the Streptococcus OTUs, correlating with severity in reflux disease. In addition, we observed a higher abundance of Prevotella in gastroesophageal reflux disease samples compared to both tissue types from esophageal adenocarcinoma patients, and columnar tissue with a trend in squamous tissue from patients with Barrett’s esophagus. For matched normal and disease tissue from the same participant, microbial compositional differences were found for Barrett’s esophagus in an undetermined genus from the family Streptococcaceae.
Discussion/Conclusion: Our findings reveal microbial differences are manifest between Barrett’s esophagus and esophageal adenocarcinoma tissue. As studies looking into the microbiome in esophageal disease is limited, increasing our understanding of Barrett’s esophagus and esophageal adenocarcinoma is vital. Currently it is not possible to predict which patients with Barrett’s esophagus are likely to progress to esophageal adenocarcinoma; understanding the etiology and microbial pathogenesis of esophageal reflux disease may potentially contribute to improvements in treatment and/or management of Barrett’s esophagus patients.
Tight junction dysfunction not present in a chronic fatigue syndrome/myalgic encephalomyelitis cohort

Rachel J. Passmore¹,² (corresponding author), Dr. Samantha C. Johnston¹,², Dr. Donald R. Staines², Prof. Sonya M. Marshall-Gradisnik¹,²
¹School of Medical Science, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia
²National Centre for Neuroimmunology and Emerging Diseases, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

Introduction: Gastrointestinal symptoms are prevalent in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), and a significant proportion of patients are also diagnosed with irritable bowel syndrome. Altered microbial composition has been observed in CFS/ME patients, this could lead to dysfunction in intestinal tight junctions, which may contribute to gastrointestinal symptoms. Previously, only lipopolysaccharide (LPS) and fatty-acid binding protein (FABP2) have been examined as tight junction indicators, and these have shown contradictory results.

The aim of this study was to investigate markers of tight junction dysfunction, namely zonulin, in addition to LPS and FABP2, in CFS/ME compared with healthy controls.

Methods: Serum concentrations of zonulin, LPS and FABP2 were analysed using sandwich Enzyme Linked Immunosorbent Assays (ELISA) in CFS/ME patients who satisfied Fukuda diagnostic criteria, and healthy controls. Data was analysed using either an unpaired parametric t-test or Mann-Whitney U test where appropriate, with a significance of p < 0.05.

Results: No significant difference was observed between the CFS/ME participants and healthy controls for concentrations of zonulin (p = 0.186), FABP2 (p = 0.691), or LPS (p = 0.245).

Discussion/Conclusion: Our findings suggest that tight junction dysfunction is not present in CFS/ME patients despite reports of gastrointestinal disturbance. This study was the first to investigate the intestinal permeability marker zonulin in CFS/ME patients; results indicated zonulin concentrations were not significantly different between CFS/ME patients and healthy controls. One previous study investigated FABP2 in CFS/ME patients, and in accordance with our FABP2 results the function of tight junctions appeared to be normal. LPS concentrations have shown varied results in CFS/ME patients between studies. This may indicate systemic inflammation is present in some CFS/ME cohorts, though when taken into consideration with FABP2 and zonulin measurements, it suggests that dysfunctional tight junctions and the subsequent microbiota translocation from the lumen is not the source of this inflammation.
Clostridium difficile isolation and characterization from hospitalized patients – A pilot study from gastroenterological ward of a tertiary institution

Špela Pintar¹, Maja Rupnik¹,², Pavel Skok¹,³
¹University of Maribor, Faculty of Medicine, ²National Laboratory for Health, Environment and Food, ³University Medical Centre, Maribor, Slovenia

Introduction: Clostridium difficile is the most common cause of nosocomial infectious diarrhoea. Inflammatory bowel diseases (IBD), ulcerative colitis, and Crohn’s disease are chronic diseases causing a prolonged inflammation of gastrointestinal tract. These patients are often colonized with C. difficile but its role is inconclusive.

Objectives: To compare C. difficile colonization and selected inflammatory parameters in patients with IBD and without IBD.

Patients and methods: We obtained fecal samples from 161 randomly selected patients, hospitalized at Department of Gastroenterology during the period between 1/12/2015–1/5/2016. Total DNA was isolated from feces and C. difficile was detected using the real time PCR amplification of specific 16S RNA gene and toxigenic strains were confirmed by the amplification of tcdB gene. After collecting patient’s basic information, inflammatory parameters (neutrophil granulocytes, leukocytes, CRP, erythrocyte sedimentation, albumins, ferritin, and iron) and therapy (pre-and hospital antibiotics, corticosteroids, biological therapy) we divided isolates in two groups: IBD group and control group.

Results: The final analysis included 151 samples, (male 75, female 76), 48 (31.8%) from IBD patients and 103 (68.2%) from control group. In IBD group 23/48 samples (47.9%) were positive for C. difficile; 7 of which (7/48, 14.6%) were TcdB+. In the control group 42/103 (40.8%) were positive for C. difficile, 11 of which (11/103, 10.7%) were TcdB+. Between the two groups, significant differences were confirmed only in the use of corticosteroids and biological therapy prior to hospitalization, p < 0.001. Regarding the use of antibiotics prior to hospitalization, differences between the two groups were not confirmed, p = 0.72. There were no significant differences observed in inflammatory parameters within IBD patients (CD positive compared to CD negative).

Conclusion: The results of our study suggest that IBD patients and patients from the control group were colonized with C. difficile in comparable proportions. The majority of strains were nontoxigenic, which will not cause C. difficile infections, but could be regarded as a marker for disturbed gut microbiota.
Mucosal washings, a new technique for sampling the mucosal microbial community

C. Poulton¹, D.A. Lemberg², A.S. Day³, E. Wine⁴, S.T. Leach¹
¹School of Women’s and Children’s Health, UNSW, Sydney, Australia
²Sydney Children’s Hospital, Sydney, Randwick, Australia
Crohn’s and Colitis Australia

Introduction: It is accepted the gut microbiome contributes to health and disease. However, identifying specific characteristics within the microbiome that contribute to disease such as Inflammatory Bowel Disease (IBD) has been elusive. One key aspect to studying microbial communities is sampling and the gut microbiome has generally been studied through faeces. Yet, faeces may be a sub-optimal sample to investigate the pathogenic potential of the microbiome. The aim of this study was to develop a novel sampling technique that involves washing the intestinal gut mucosa and assess if this new technique will assist in the deconvolution of the gut microbiome.

Methods: A method of sampling the mucosal microbial community during colonoscopy was developed and used in children with and without known mucosal inflammation. These isolated microbial communities were compared to the microbial communities in faeces and biopsies and assessed for their richness, diversity and composition using 16S rDNA analysis.

Results: Mucosal washings yielded comparable qualities of sequences to faecal samples and significantly more sequences than biopsy samples. Diversity and community structure of mucosal washings differed from both faeces and biopsy samples.

Discussion/Conclusion: The gut microbiome is not homogenous and differs with reference to location along the gastrointestinal tract and proximity to the mucosal epithelium. The combination of mucosal washings, biopsies and faeces, provide more insight into the gut microbiome than any one sample alone. Investigations that include multiple sampling types should greatly assist with identification of the pathogenic elements that may contribute to IBD within the intestinal microbiome.
Reduced abundance of *Faecalibacterium prausnitzii* in the terminal ileum mucosa-associated microbiome correlates with increased small intestinal permeability in chronic liver disease

Ashok S. Raj¹,⁵, Erin R. Shanahan¹,², Cuong D. Tran³, Purnima Bhat⁴, Linda M. Fletcher¹,⁵, Mark Morrison², Gerald Holtmann¹,⁵, Graeme A. Macdonald¹,⁵
¹Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia; ²University of Queensland Diamantina Institute, Translational Research Institute, University of Queensland, Brisbane, Australia; ³Health and Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia; ⁴Australian National University School of Medicine, Australian National University, Canberra, Australia, ⁵School of Medicine, Translational Research Institute, University of Queensland, Brisbane, Australia

**Introduction**: Chronic liver disease (CLD) is associated with dysbiosis of the stool microbiota, but little is known about the mucosal microbiota of the terminal ileum, some of which may be beneficial for mucosal integrity. Our aim was to evaluate for dysbiosis of the terminal ileum mucosal microbiota and relationships with small intestinal permeability and disease severity in subjects with CLD.

**Methods**: Subjects with and without CLD, undergoing routine colonoscopy were prospectively recruited. Those with mucosal inflammation or functional bowel disease were excluded. Bacterial DNA was sequenced from mucosal biopsies taken from the terminal ileum, using Illumina® Miseq. Small intestinal permeability was measured by the plasma ratio of lactulose:rhamnose (L:R), and hepatic stiffness by Transient Elastography. The presence of the metabolic syndrome was assessed by the IDF/AHA/NHLBI 2009 consensus criteria. Statistical analysis was performed by SPSS v22 and Calypso version 5.2

**Results**: 21 subjects with CLD (male:female 15:12; age 40–76 years) and 25 controls (M:F 13:12; age 36–73 years) were assessed. In CLD subjects, there was a strong inverse correlation between small intestinal permeability and the relative abundance of *Faecalibacterium prausnitzii* (r = -0.79, p = 0.015, corrected for multiple comparisons, Fig. 1). As a community, the microbial composition of CLD was similar to controls, with no significant separation on redundancy analysis (p = 0.71), and similar microbial diversity (Shannon index, p = 0.68). There was no effect of hepatic stiffness or the metabolic syndrome on the terminal ileum microbiota in CLD subjects (p > 0.05).
**Small intestinal permeability and Faecalibacterium prausnitzii**

![Graph showing the correlation between small intestinal permeability and the relative abundance of Faecalibacterium prausnitzii](image)

**Figure 1**: The correlation between small intestinal permeability and the relative abundance of *Faecalibacterium prausnitzii* in the terminal ileum of CLD subjects. (Abbreviations: L:R, Lactulose:Rhamnose ratio)

**Discussion/Conclusion**: In CLD, reduced abundance of *Faecalibacterium prausnitzii* in the terminal ileum mucosa may be implicated in the pathogenesis of increased small intestinal permeability.
Treatment of ZAP-70 mutant SKG mice with anti-IL-23 antibody alters fecal microbiota composition and prevents outgrowth of bacteria associated with susceptibility to spondyloarthritis and ileitis

L.M. Rehaume¹, N. Matigian¹, K. Ormerod², A. Kang¹, R. Linedale¹, O. Zbarskaya¹, Kristine Kikly³, J. Daly², N. Lachner², P. Hugenholtz², M. Morrison¹, K.A. Lê Cao¹, R. Thomas¹

¹The University of Queensland Diamantina Institute, Brisbane, QLD, Australia
²Australian Centre for Ecogenomics, The University of Queensland, Brisbane, QLD, Australia
³Eli Lilly, Biotechnology Discovery Research, Indianapolis, Indiana, USA

Introduction: Identification of disease-associated or protective bacteria may elucidate biomarkers or probiotics for people suffering from or at-risk of developing spondyloarthritis (SpA). The colitogenic Prevotella copri was associated with new-onset rheumatoid arthritis, and ankylosing spondylitis patients have increases in several bacterial families including Porphyromonadaceae. It is unclear how the microbial community differs due to genetic susceptibility to SpA, or the impact of additional triggers. We hypothesized that IL-23, a key driver of SpA, modifies the gut microbiota and response to pro-inflammatory triggers.

Methods: BALB/c ZAP-70W163C-mutant (SKG) mice housed under specific pathogen-free (SPF) conditions treated with microbial β-1,3-glucan (curdlan) develop IL-23- and microbiota-dependent SpA-like arthritis and ileitis. Altered Schaedler flora (ASF)-colonized SKG and BALB/c mice were treated with curdlan, and then weekly with anti-IL-23 p19-specific mAb or isotype control mAb, or with curdlan or control. SPF-SKG mice were treated for 3 weeks with anti-IL-23 or isotype mAb, then with curdlan or control. The fecal microbiota profiles were analyzed longitudinally by RT-PCR and next-generation sequencing. Arthritis, spondylitis and ileitis were assessed histologically.

Results: After colonization of germ-free mice with ASF, 4/8 bacterial strains were detected: Clostridium sp., Lactobacillus murinus, Mucispirillum schaedleri and Parabacteroides sp. After curdlan, the relative abundance of L. murinus was increased in mice treated with curdlan and anti-IL-23 mAb compared with curdlan alone. In SPF-SKG mice treated with anti-IL-23 mAb or anti-IL-23 mAb then curdlan, the abundance of multiple Prevotellaceae and Porphyromonadaceae spp. decreased relative to SPF-SKG mice treated with isotype then curdlan.

Discussion/Conclusion: Interaction of the microbiota with the immune system of SKG mice alters the composition of both a simplified consortium and an unrestricted bacterial community. Treatment of SPF-SKG mice with anti-IL-23 mAb not only suppresses SpA development but shifts the fecal microbiota composition and prevents the usual outgrowth of bacteria associated with arthritis and inflammatory bowel disease in response to curdlan.
A novel approach to quantify small intestinal bacterial load: A pilot study in Crohn’s disease, functional gastrointestinal disorders and patients with iron deficiency

Ayesha Shah¹, Ariya Nair², Daniel Burger¹, Anh Do², Marjorie Walker³, Linda Fletcher¹, Nicholas J. Talley³, Mark Morrison⁴, Erin R. Shanahan⁵, Gerald Holtmann¹
¹University of Queensland, Faculty of Medicine and Biomedical Sciences, Translational Research Institute, Department of Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; ²Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia; ³University of Newcastle, Faculty of Health & Medicine, Newcastle, NSW, Australia; ⁴University of Queensland, Diamantina Institute, Microbial Biology and Metagenomics, Brisbane, QLD, Australia; ⁵Princess Alexandra Hospital/University of Queensland, Brisbane, QLD, Australia

Introduction: The density of bacteria colonising the small intestine may be relevant for the understanding of a variety of GI disorders. However, existing technologies have limitations and the widely used breath tests lack specificity and sensitivity. We therefore sought to use a molecular based method to determine and compare bacterial density in the second part of the duodenum in consecutive patients with Functional Gastrointestinal Disorders (FGIDs), Crohn’s disease and iron deficiency anaemia (IDA).

Methods: We recruited 12 patients with FGIDs (5 with functional dyspepsia, 7 with functional dyspepsia and Irritable bowel syndrome overlap), 12 patients with IDA and 12 patients with Crohn’s disease (Stricturing or fistulising Crohn’s disease or those with prior surgical resection). All patients underwent a standard glucose hydrogen breath test (GBT). To avoid bacterial cross contamination, biopsies were taken from the second part of the duodenum utilising the Brisbane Aseptic Biopsy Device (MTW, Germany) and total DNA extracted. Quantitative PCR analysis was used to determine total bacterial load, based on gene copy number ratio (bacterial 16S RNA: human β-actin).

Results: All patients had a negative GBT result. The ratio of bacterial 16S rRNA: human β-actin was significantly different for the three groups; FGID 0.024 ± 0.032; IBD 0.002 ± 0.001; IDA 0.013 ± 0.015 (mean ± SD; p < 0.05).

Discussion/Conclusion: Utilising a novel technique to characterize the small bowel microbiome, we observed significant differences between patients with FGID, Crohn’s disease and IDA. Contrary to what we expected, CD patients had the lowest and those with FGID had the highest bacterial density. This molecular approach may complement, or in future replace other clinical tests to quantitate gut bacterial colonization.
Is there a link between *H. pylori* and the epidemiology of Crohn’s disease?

Ayesha Shah¹, Nicholas J. Talley², Marjorie Walker², Natasha Koloski¹,², Mark Morrison³, Daniel Burger¹, Jane M. Andrews⁴, Michael McGuckin⁵, Mike Jones⁶, Gerald Holtmann¹

¹University of Queensland, Faculty of Medicine and Biomedical Sciences, Translational Research Institute, Department of Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; ²University of Newcastle, Faculty of Health & Medicine, Newcastle, NSW, Australia; ³University of Queensland, Diamantina Institute, Microbial Biology and Metagenomics, Brisbane, QLD, Australia; ⁴University of Adelaide & Royal Adelaide Hospital, Department of Gastroenterology & Hepatology, Adelaide, SA, Australia; ⁵University of Queensland, Mater Medical Research Institute, Translational Research Institute, Woolloongabba, QLD, Australia; ⁶Macquarie University, Department of Psychology, Sydney, NSW, Australia

**Introduction:** Case control studies suggest an inverse association between *Helicobacter pylori* and Crohn’s disease (CD). It is possible, this could be accounted for by confounders such as antibiotic therapy. Analysing the geographic distribution of *H. pylori* and the links with the incidence and prevalence of CD would be an alternative approach to circumvent these confounders.

**Methods:** The literature was searched for studies published between 1990 and 2016 that reported incidence or prevalence data for CD in random population samples in developed countries (GDP per capita > 20,000 USD/year). Corresponding prevalence studies for *H. pylori* in these same regions were then sought matched to the same time period (± 6 years). The association between the incidence and prevalence of CD and *H. pylori* prevalence rates were assessed before and after adjusting for GDP and life expectancy.

**Results:** 19 CD prevalence and 22 CD incidence studies from 10 European countries, Japan, USA and Australia with date matched *H. pylori* prevalence data were identified. The mean *H. pylori* prevalence rate was 43.4% (range 15.5–85%) and the mean rates for incidence and prevalence for CD were 6.9 and 91.0/100,000 respectively. The incidence ($r = -0.469$, $p < 0.03$) and prevalence ($r = -0.527$, $p = 0.02$) of CD was inversely and significantly associated with prevalence of *H. pylori* infection.

**Discussion/Conclusion:** Our data demonstrate a significant inverse association between geographic distribution of *H. pylori* and CD. Thus, it is highly unlikely that the findings of previous case control studies were simply due to confounding factors such as concomitant antibiotic use in CD patients.
Is there an association between enteric methane (CH4) production and symptoms in patients with unexplained GI symptoms?

Ayesha Shah¹, Linda Fletcher¹, Pegah Ghasemi², Teressa Hansen², Mark Morrison³, Gerald Holtmann¹
¹University of Queensland, Faculty of Medicine and Biomedical Sciences, Translational Research Institute, Department of Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; ²Department of Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; ³University of Queensland, Diamantina Institute, Microbial Biology and Metagenomics, Brisbane, QLD, Australia

Introduction: In humans, enteric methane (CH4) production is highly variable and related to the gastrointestinal microbiome and diet. Previous work suggests that CH4 production is more common in patients with ‘constipating’ conditions such as encopresis and diverticulosis. We aimed to explore the link between gastrointestinal symptoms breath CH4 exhalation in patients with unexplained GI symptoms.

Methods: Consecutive patients (n = 100) with unexplained GI symptoms underwent a combined H2/CH4 breath test after ingestion of 75 g of glucose. H2 and CH4 were measured by Breathtreacker microlyser (Quintron, USA). Gastrointestinal symptoms were assessed utilising the (Structured Assessment of Gastrointestinal Symptoms Instrument (SAGIS). The association between methane exhalation and symptoms during the 2 weeks prior the test were evaluated using non parametric test.

Results: 100 consecutive patients (55 f), aged 52.2 ± 15.7 years (mean ± SD) were included. Of these, 14 with positive GBT and 19 without SAGIS data were excluded, resulting in 67 data-sets available for analysis. Methane peak and methane baseline values were highly correlated (r = 0.96, p < 0.001). Methane peak (and baseline) were inversely correlated with the SAGIS diarrhoea score (-0.35, p < 0.01, Figure 1). Contrary to current opinion, CH4 exhalation was not associated with constipation (r < 0.1, p > 0.4). In addition, excessive belching and acid eructation were significantly associated with the baseline and peak CH4 exhalation (r all ≥ 0.3, p all < 0.04).

Discussion/Conclusion: There is an inverse association between CH4 exhalation and diarrhoea symptoms. At the same time, CH4 is associated with bloating and acid eructation. These data suggest that CH4 or metabolic products from CH4 producing microbes modulate human gut function.
Systematic review and meta-analysis: Prevalence of small intestinal bacterial overgrowth in chronic liver disease

Ayesha Shah1, Erin Shanahan1,2, Graeme Macdonald1, Linda Fletcher1, Mark Morrison2, Ashok Raj1, Mike Jones3, Gerald Holtmann1
1University of Queensland, Faculty of Medicine and Biomedical Sciences, Translational Research Institute, Department of Gastroenterology & Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; 2University of Queensland, Diamantina Institute, Microbial Biology and Metagenomics, Brisbane, QLD, Australia; 3Macquarie University, Department of Psychology, Sydney, NSW, Australia

Introduction: We aimed to assess and compare the prevalence of small intestinal bacterial overgrowth (SIBO) in patients (and controls) with chronic liver disease (CLD), liver cirrhosis and non-alcoholic fatty liver disease (NAFLD).

Methods: Using the search terms ‘small intestinal bacterial overgrowth (SIBO)’ and ‘chronic liver disease (CLD)’ or ‘small intestinal bacterial overgrowth (SIBO)’ and ‘cirrhosis’, and identified 19 case-control studies that met inclusion criteria. Data were extracted to calculate prevalence rates and 95% Confidence Intervals (CI).

Results: The final dataset included 1000 adult patients with CLD and 488 controls. 15 studies used breath test (nine Glucose breath test (GBT), four used Lactulose breath test (LBT), one Glycine -1-14C-labeled glycocholate and one utilized 14C-D xylose breath test), five utilised culture methods and one used quantitative PCR. Prevalence of SIBO in patients with CLD was 38.9% (95% CI: 36.90–42.00) and 9.8% (95% CI: 7.5–12.8) in controls. There is a 7-fold increase of SIBO in CLD (RR = 7.15, 95% CI: 4.912–10.41). In patients with cirrhosis the prevalence of SIBO was 40.1% (95% CI: 36.6–43.8) as compared to 7.3% (95% CI: 4.9-10.8) in the respective controls. In NAFLD the prevalence of SIBO was 33.5 % (95% CI: 27.4-40.2) as compared to 7.3% (95% CI: 4.9–10.8) in controls. Utilising breath tests, the prevalence of SiBO in CLD was 35.8% (95% CI: 32.6–39.1) as compared to 8.0% (95% CI: 5.7–11.0) in controls. Based upon culture techniques, prevalence of SIBO in CLD was 68.3% (95% CI: 59.62– 76.0) vs. 7.94% (95% CI: 3.44–12.73) in controls. There was no evidence that the association between SIBO and CLD was influenced by publication bias.

Discussion/Conclusion: Prevalence of SIBO is significantly increased in patients with CLD. However, there is no difference in the prevalence of SIBO between patients with cirrhosis or patients with NAFLD.
Life in the duodenum: Isolation of small intestinal mucosa-associated bacteria in functional dyspepsia patients

Erin Shanahan\textsuperscript{1,2}, Anh Do\textsuperscript{1}, Arya Sheela Nair\textsuperscript{1,3}, Abhilash Rameshkumar\textsuperscript{1,3}, Paraic O Cuiv\textsuperscript{2}, Mark Morrison\textsuperscript{2}, Gerald Holtmann\textsuperscript{1}

\textsuperscript{1}Department of Gastroenterology and Hepatology, Princess Alexandra Hospital and School of Clinical Medicine, Translational Research Institute, The University of Queensland, Brisbane, QLD, Australia
\textsuperscript{2}The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, QLD, Australia
\textsuperscript{3}School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

Introduction: The pathophysiology of functional dyspepsia (FD) is multi-factorial, however recent observations of duodenal mucosal permeability and inflammation suggest a role for immune activation and the microbiota. The duodenum has long been considered sterile, with bacteria only present due to cross contamination or luminal overgrowth. However, utilising the sheathed Brisbane Aseptic Biopsy forceps, we have previously overcome these issues and demonstrated the presence of a duodenal mucosal microbiota. It is essential however, to demonstrate this represents live organisms. Therefore, utilising the aseptic forceps to eliminate contamination, we aimed to obtain bacterial isolates representing the duodenal mucosa-associated microbiota.

Methods: Four patients with FD (Rome III) undergoing endoscopy were recruited with ethical approval. Biopsy samples were collected using the Brisbane Aseptic Biopsy Device\textsuperscript{4} and cultured under aerobic, microaerophilic and anaerobic conditions. Isolates were identified utilising 16S rRNA sequencing, and VITEK Mass Spectrometry. Further characterisation was undertaken to determine immuno-modulatory properties.

Results: Bacteria were cultured from mucosal biopsies of all four subjects. In all cases, \textit{Streptococcus} spp. dominated, representing on average 67\% of all colony forming units. Mixed \textit{Streptococcus- Veillonella} colonies highlighted the close metabolic interaction of these genera. A total of twelve unique isolates were obtained from the four patients, including \textit{Streptococcus salivarius}, \textit{Streptococcus gordonii}, \textit{Streptococcus infantarius}, \textit{Escherichia coli} and members of the genera \textit{Veillonella}, \textit{Actinomyces} and \textit{Neisseria}. All \textit{Streptococcus} isolates were able to survive a 2 h challenge at pH 4 and above, with no growth observed below pH 4. These strains did not induce a response from the epithelial cells but stimulated pro-inflammatory cytokine release from peripheral blood mononuclear cells.

Discussion/Conclusion: We have overcome issues of contamination during sampling and obtained mucosa-associated bacterial isolates from FD patients. This demonstrates the presence of live bacteria in the duodenal mucosal niche, with potential implications for a variety of GI disorders.
The effect of dietary fibre on gut microbiota composition in healthy adults: A systematic review and meta-analysis

D. So¹, K. Whelan², M. Rossi³, M. Morrison³, H. Staudacher⁴,⁵, K.L. Campbell¹,⁵
¹Faculty of Health Science & Medicine, Bond University, Gold Coast, Australia
²School of Medicine, Diabetes and Nutritional Sciences Division, King’s College, London, United Kingdom
³The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane, QLD, Australia
⁴Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia
⁵Department of Nutrition and Dietetics, Princess Alexandra Hospital, Brisbane, QLD, Australia

Introduction: Recent evidence has linked a number of disorders with disturbance of the composition of the gastrointestinal microbiota. The microbial community of the gastrointestinal tract may therefore be a modifiable risk factor in the aetiology of these conditions. Diet, particularly dietary fibre, is a major regulator of gastrointestinal microbiota composition. Certain types of dietary fibre can selectively stimulate beneficial bacteria (e.g. Bifidobacterium), leading to benefits including short chain fatty acid generation and immune modulation. However, the effect of dietary fibre on gastrointestinal microbiota composition is yet to be quantified. The aim of this systematic review was to establish the effect of dietary fibre on gastrointestinal microbiota composition in healthy adults.

Methods: A structured search of Medline, EMBASE, CINAHL and CENTRAL was conducted (to October 2016). Studies included randomised controlled trials (RCTs) evaluating the effect of dietary fibre on the gastrointestinal microbiota (diversity and abundances of specific beneficial species) in healthy adults. Two independent authors conducted the search and screened studies for inclusion.

Results: A total of 54 RCTs encompassing 1281 participants were eligible for inclusion. Studies evaluated microbiota composition using a range of techniques from culture through to metagenomic sequencing. Fibre supplementation trials accounted for the majority (42) of studies. Of these, 20 were prebiotic interventions, 16 used specific non-prebiotic fibres, and six administered a combination of fibres. Intervention doses ranged from 1.2 g/day–45.6 g/day. A meta-analysis is being performed to evaluate the difference in microbiota diversity and abundances of beneficial bacteria between fibre and control groups. Subgroup analyses will include exploring the effect of specific fibre types and dose-response.

Discussion/Conclusion: Our review revealed considerable variability in the fibre type, dose and methodologies used in RCTs investigating the impact of dietary fibre on gastrointestinal microbiota composition. These findings will help establish the magnitude to which dietary fibre modulates the gastrointestinal microbiota in healthy adults.
Stroke induces robust changes to intestinal mucosal microbiota

Dragana Stanley¹, Robert J. Moore²,³ and Connie H.Y. Wong⁴
¹School of Medical and Applied Sciences, Central Queensland University, Bruce Highway, Rockhampton, QLD 4702, Australia
²School of Science, RMIT University, Bundoora, VIC 3083, Australia
³Infection and Immunity Program, Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, VIC 3800, Australia
⁴Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences, Monash University, Clayton, VIC 3168, Australia

Introduction: Bacterial pneumonia is leading and often lethal complication after stroke, however no causative microorganism has been discovered to date. We have recently demonstrated that stroke triggers robust gut immune dysfunction and increased intestinal barrier permeability, both of which contributed to translocation and dissemination of intestinal bacteria to peripheral organs. Bacteria living on mucosal surfaces have been demonstrated to interact with the host and influence host gene expression and wound healing processes, thus we propose mucosal bacteria on intestinal epithelium play key roles in regulating bacteria translocation after stroke.

Methods: In the present study, we utilised an experimental model of stroke along with a number of bioinformatics software and algorithms to investigate the effect of stroke on mucosal microbial communities.

Results: We found that the microbial communities within the mucosa of gastrointestinal tract (GIT) were significantly different between sham-operated and post-stroke mice at 24 h following surgery. The differences in microbiota composition were substantial in all sections of the GIT and were significant even at the phylum level. In fact, the most abundant mucosal phylotypes were also profoundly affected by stroke. The main characteristics of the stroke-induced shift in mucosal microbiota composition were an increased abundance of Akkermansia muciniphila and an excessive abundance of clostridial species. Akkermansia changed its bacterial interactions in direction that could potentially help the host to control the translocation event. Furthermore, we analysed the predicted functional potential of the altered mucosal microbiota induced by stroke using PICRUSt and revealed significant increases in functions associated with infectious diseases, membrane transport, xenobiotic degradation, lipid metabolism and signalling related KEGG pathways.

Discussion/Conclusion: Taken together, our study uncovered stroke induces far-reaching and robust changes to the intestinal mucosal microbiota and a better understanding of the precise molecular events leading up to stroke-induced mucosal microbiota changes may represent novel therapy targets to improve patient outcomes.
High rates of *Clostridium difficile* infection in inflammatory bowel disease admissions to a tertiary referral centre

K.M. Taylor, B.J. Headon, R.P. Luber, M.P. Sparrow
Department of Gastroenterology, Alfred Health and Monash University, Melbourne, Australia

**Introduction:** Recent studies have shown a rising frequency of *Clostridium difficile* infection (CDI) in patients with both active and inactive inflammatory bowel disease (IBD). CDI is associated with increased hospitalisations, higher colectomy rates and increased mortality in IBD patients. ECCO guidelines recommend screening for CDI at every flare in patients with colonic disease.

**Aim:** To determine the rate, risk factors and outcomes of CDI in IBD inpatients admitted to the Alfred IBD Unit over one year.

**Methods:** A prospectively maintained database of IBD inpatients was searched for *C. difficile* tests performed from the beginning of February 2015 to the end of January 2016. CDI testing at our institution involves stool culture plus enzyme immunoassay for toxins A & B, but not toxigenic culture or molecular testing.

**Results:** There were 91 IBD inpatient admissions in the year examined. 20/91 admissions (22%) were non-diarrhoeal (e.g. penetrating disease or obstructive symptoms) and *C. difficile* testing was not performed. Diarrhoea was the predominant symptom in the remainder of admissions (71/91 [78%]) and *C. difficile* testing was performed in 93% of these patients. 15% of those tested were positive for *C. difficile* toxin and a further 5% were positive for *C. difficile* on culture alone; all were treated. 5% of those tested were initially negative for *C. difficile* toxin but became positive during their admission. There were no significant differences between IBD sub-type, frequency of colonic involvement or clinical outcomes overall in those who were positive or negative for CDI (see table 1). More patients in the CDI positive group had received corticosteroids plus at least one other immunosuppressant (thiopurine/methotrexate/anti-TNF) compared with the CDI negative group (62% vs. 19%, p = 0.004). 4/15 patients with acute severe ulcerative colitis (ASUC) had CDI. 3/4 of these patients required rescue therapy with infliximab or ciclosporin, and 2/4 proceeded to inpatient colectomy, compared with 6/11 requiring rescue therapy and 2/11 proceeding to colectomy in the CDI negative group (p = 0.6 for rescue therapy, p = 0.5 for colectomy).
CDI positive | CDI negative | p value
---|---|---
Mean age – years (SD) | 45 (16.3) | 40 (13.4) | 0.2
IBD subtype N (%) | | | |
UC | 6 (46) | 22 (41) | 0.39
CD | 5 (39) | 28 (53) | |
IBDU | 2 (15) | 3 (6) | |
Colonic involvement N (%) | 12/13 (92) | 45/53 (85) | 0.68
Medication use* N (%) | | | |
Corticosteroids | 8/13 (62) | 17/53 (32) | 0.06
Anti-TNF | 7/13 (54) | 15/53 (28) | 0.1
Thiopurine/Methotrexate | 5/13 (38) | 23/53 (44) | 1
Corticosteroids plus at least one other immunosuppressant | 8/13 (62) | 10/53 (19) | 0.004
Antibiotics | 4/15 (27) | 12/53 (23) | 0.71
Need for surgery N (%) | | | |
- all | 3 (23) | 3 (6) | 0.08
- colectomy | 2 (15.3) | 3 (6) | |
- defunctioning ileostomy | 1 (7.7) | | |
Death N (%) | 1 (8) | 0 (0) | 0.19
Mean length of stay – days (SD) | 10 (8.45) | 8 (7.44) | 0.41

Table 1: Differences between those testing positive or negative for CDI
*within 3 months prior to CDI

Discussion/Conclusion: CDI is common: in this cohort, 20% of IBD inpatients tested were positive, with an even higher rate (27%) in those with ASUC. In keeping with previous studies there was a greater prior use of multiple immunosuppressants in those positive for CDI, with a trend towards increased need for surgery. Although hospital acquisition of CDI may explain the subsequent development of a positive test, an initial false negative test should be considered. Repeat testing for CDI where there is a failure to respond to initial medical therapy, or the use of a more sensitive test at baseline (e.g. molecular testing), is warranted.
Oral α-galactosidase improves gastrointestinal tolerance to a diet high in galacto-oligosaccharides: Adjunct therapy to a low FODMAP diet in irritable bowel syndrome

C.J. Tuck¹, K.M. Taylor¹, J.S. Barrett¹, P.R. Gibson¹, J.G. Muir¹
¹Department Gastroenterology, Monash University and Alfred Health, Melbourne, VIC 3004, Australia

Introduction: Galacto-oligosaccharides (GOS) are indigestible short-chain carbohydrates (FODMAPs) with documented prebiotic properties, but are associated with triggering gastrointestinal symptoms in irritable bowel syndrome (IBS). This study aimed to assess whether oral α-galactosidase co-ingestion with foods high in GOS and low in other FODMAPs would reduce symptoms and breath hydrogen production in a double-blind, placebo-controlled, cross-over trial.

Methods: Patients meeting the Rome III criteria for IBS who produced > 10 ppm hydrogen on two consecutive breath samples following 10 g fructan were recruited. Participants were randomly assigned to full-dose enzyme (300 GALU α-galactosidase), half-dose (150 GALU α-galactosidase) and placebo (glucose). Following a 3-day low FODMAP run-in period, participants consumed provided diets high in GOS for a further 3-days. Gastrointestinal symptoms were measured daily using a 100 mm visual-analogue-scale. Breath samples were taken hourly on the second last day and analysed as area-under-the-curve, faecal samples were taken on the final day.

Results: Thirty-one patients with IBS (20 IBS-D, 4 IBS-C, 7 IBS-M) completed the study. The addition of high GOS foods resulted in a significant increase in overall symptoms with 21 patients exhibiting GOS-sensitivity (> 10 mm increase for overall symptoms). Of those, full-dose enzyme reduced overall symptoms (median 24.5 [IQR 17.5–35.8] mm vs. 5.5 [1.5–15.0] mm; p = 0.006) and bloating (20.5 [9.5–42.0] vs. 6.5 [2.0–15.8]; p = 0.017). Breath hydrogen production was minimal with no differences seen between placebo (mean 5248 ± SD 3339 ppm.12 h) and full-dose (5585 ± 3205; p = 0.597).

Discussion/Conclusion: An oral α-galactosidase supplement taken with high GOS foods provides a clinically significant reduction in symptoms in GOS-sensitive individuals with IBS. The lack of change in breath hydrogen suggests the mechanism may not be related to reduced gas and distention, rather suggesting a role of alterations to the microbiota. Future analysis of the faecal microbiota may provide insight. This strategy can be easily translated into practice to improve tolerance specifically to high GOS foods.
Faecal supernatants from patients with diarrhoea predominant IBS disrupt colonic epithelial barrier function and directly affect colorectal afferent nerves

Hannah Wardill1,2, Joanne Bowen2, Nicole Dmochowska1,2, Melissa Campaniello1, Chris Mavrangelos1, Jane Andrews2,3, Sam Costello4, Patrick Hughes1,2
1Centre for Nutrition and Gastrointestinal Disease, South Australian Health and Medical Research Institute (SAHMRI); 2Adelaide Medical School, University of Adelaide; 3IBD Service, Department Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia; 4Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, SA, Australia

Introduction: Irritable bowel syndrome (IBS) is a chronic debilitating disease of the gastrointestinal tract. Despite no over pathology, the luminal environmental is altered in IBS with evidence of microbial dysbiosis, inflammation and protease activity (Hughes et al. Am J Gastroenterol. 2013). However, it is unclear if these changes actively drive symptoms of altered bowel habit and abdominal pain, or simply reflect a consequence of disease. We aimed to characterise the effect of faecal supernatants (FSN) from patients with diarrhoea predominant IBS (IBS-D) on epithelial barrier function and pain sensing colonic pelvic afferent nerves.

Methods: FSNs were prepared from 10 patients with IBS-D (ROME III) and 8 sex/age-matched healthy controls (HC) at 0.3 g/ml ringers solution. Proteolytic and LPS activity were quantified in IBS(D)-FSN and HC-FSN. IBS(D)-FSN, HC-FSN or vehicle ± protease inhibitor cocktail (PIC) were applied apically and basolaterally to healthy mouse distal colon sections mounted into Ussing chambers. Resistance (RT) and conductance were measured for 2 h. IBS(D)-FSN and HC-FSN were applied to high-threshold putatively nociceptive pelvic colonic extrinsic afferents for 5 min and changes in mechanosensitivity determined.

Results: IBS(D)-FSN had increased proteolytic and LPS activity compared to HC-FSN. IBS(D)-FSN decreased RT and increased conductance compared to both HC-FSN and vehicle, indicative of epithelial barrier dysfunction. This effect was enhanced when FSN was applied basolaterally. Protease inhibition had a modest protective effect on IBS(D)-FSN-induced barrier dysfunction, but failed to completely prevent changes. IBS(D)-FSN but not HC-FSN directly activated high threshold pelvic colonic afferent endings and sensitised them to mechanical stimuli.

Discussion/Conclusion: Mediators present in IBS-D faecal samples impair epithelial barrier integrity and activate high-threshold putatively nociceptive colonic afferent nerves. Further characterisation of the composition of FSN is required to identify therapeutically relevant targets.
Translocation and dissemination of commensal bacteria in post-stroke infection

Dragana Stanley1, Linda J. Mason2, Kate E. Mackin3,4, Yogitha N. Srihanta3,4, Dena Lyras3,4, Monica D. Prakash5, Kulmira Nurgali5, Andres Venegas6, Michael D. Hill6, Robert J. Moore3,4,7, Connie H.Y. Wong8,*
*Note that the presenting author will be Connie H.Y. Wong

1School of Medical and Applied Sciences, Central Queensland University, Rockhampton, QLD, Australia; 2Department of Biochemistry, Monash University, Clayton, VIC, Australia; 3Infection and Immunity Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia; 4Department of Microbiology, Monash University, Clayton, VIC, Australia; 5Centre for Chronic Diseases, College of Health and Biomedicine, Victoria University, St. Albans, VIC, Australia; 6Stroke Unit, Department of Clinical Neurosciences and Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada; 7School of Science, Royal Melbourne Institute of Technology University, Bundoora, VIC, Australia; 8Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences, Monash University, Clayton, VIC, Australia

Introduction: Stroke is highly prevalent and is one of the leading contributors to morbidity and mortality worldwide. Despite the debilitating neurological deficits, the major cause of death after stroke is bacterial pneumonia. Negative bacterial cultures from suspected cases of pneumonia in stroke patients may reflect the presence of anaerobic bacteria that require special culturing techniques. Here, we tested the hypothesis that post-stroke infection is contributed by the bacteria from host gut microbiota.

Methods: We obtained microbiological data from clinical patients and also utilised an experimental mouse model of stroke in GF and SPF mice to assess the origin of bacteria in the lung post-stroke. To expand the study beyond culturable bacterial species and investigate the microbiota dynamics, we used high throughput 16S rRNA gene amplicon sequencing and relevant bioinformatics tools to analyse the microbiota of lungs from sham-operated and post-stroke mice.

Results: We demonstrated that stroke induces the activation of the sympathetic nervous system and triggers a series of events to rapidly increase gut permeability, and impair host antibacterial and intestinal barriers, to promote the translocation and dissemination of selective commensal bacteria to sites where they are not normally found and where they may play pathogenic roles. Using SourceTracker, we predicted that the bacteria seeding the post-stroke lung are originated from the small intestine. Data from inoculation experiments demonstrated detectable bacteria in the blood and all of the lymph nodes investigated, suggesting that gut bacteria can enter the post-stroke lung via both the bloodstream and lymphatic system.

Discussion/Conclusion: Take together, we provide evidence that post-stroke infection is caused by the translocation and dissemination of selective strains of bacteria that originate from the host gut microbiota and suggest that this is an important reason why antibiotic therapy may be ineffective.
Modulation of hydrogen sulfide production from faecal microbiota by diet and mesalazine: Utility of a novel in vitro gas-profiling technology

C.K. Yao¹, A.N. Rotbart², K. Kalantar-Zadeh², J.Z. Ou², J.G. Muir¹, P.R. Gibson¹
¹Department of Gastroenterology, Monash University and Alfred Health, Melbourne VIC, Australia; ²Centre for Advanced Electronics and Sensors (CADES), School of Engineering, RMIT University, Melbourne, VIC, Australia

Introduction: Excess colonic production of hydrogen sulphide (H₂S) have been implicated in the pathogenesis of ulcerative colitis. Dietary or pharmacological strategies that modulate the gut microbiota may alter H₂S production, but few studies have been performed. We recently developed a novel in vitro gas-profiling technology to characterise real-time H₂S production. The study aimed, therefore, to investigate the effects of (a) indigestible carbohydrates and (b) diet or pharmacological factors on faecal bacteria H₂S production using the gas-profiling technology.

Methods: Freshly-passed faeces were obtained from healthy subjects on stable diet and drug therapy. Faeces were prepared under anaerobic conditions, placed in separate fermentation chambers and spiked with the following substrates in two experimental sets: (a) effect of fibres: 1 g fructo-oligosaccharides (FOS), resistant starch (RS), psyllium or sterculia; and (b) effect of cysteine (5 mmol/l – a sulfur-rich amino acid), sodium sulfate (6.5 mmol/l), mesalazine (20 mmol/l – luminal concentrations reported with this efficacious drug) and FOS (1 g). H₂S release was sampled every 5 mins over 4 hours. Results are expressed as mean (SEM) percentage of suppression/stimulation relative to an unspiked control.

Results: At the end of 4 h, H₂S suppression was the greatest with resistant starch (-77 [8]%)%, followed by FOS (-52 [17]%)% (p < 0.001, two-way ANOVA). Sterculia and psyllium suppressed H₂S production by the same degree over time (-11 [11]% and -23 [24]% respectively). Cysteine markedly stimulated H₂S production by 2967 (1167)% in comparison to sulfate (20 [50]%; p < 0.001, two-way ANOVA). However, the concurrent presence of FOS effectively suppressed cysteine-stimulated H₂S production by (-90 [2]%). Mesalazine 20 mmol/l exerted minimal effects on H₂S production (-8 [29]%).

Discussion/Conclusion: H₂S production by faecal microbiota is readily modulable: (1) slowly-fermentable fibres passively adsorb it; (2) readily-fermentable fibres reduce active production with increased carbohydrate fermentation; (3) a sulfur-containing amino acid stimulated H₂S production, this being inhibited by carbohydrate fermentation; (4) inorganic sulfate and mesalazine had mild or minimal effects. These data indicate that a multi-pronged dietary approach might be required for the suppression of colonic H₂S production.
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<td>Simadibrata, M.</td>
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Symposium 207

Gut Microbiome and Mucosal or Systemic Dysfunction: Mechanisms, Clinical Manifestations and Interventions

May 19 – 20, 2017
Brisbane Convention & Exhibition Centre
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