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Review Article

Role of the Gut Microbiome in Human Health: A Report of the ILSI South East Asia Region Gut Microbiome Conference Series

SIM Shao Zhe Edric[1], CONWAY Patricia Lynne[1,2]*, SMITH Geoffry[3] and YEONG Boon Yee[3]

[1]*Singapore Centre for Life Sciences Engineering, Nanyang Technological University, Singapore*

[2]*The University of New South Wales, School of Biological, Earth and Environmental Sciences, Sydney, Australia*

[3]*International Life Sciences Institute (ILSI) South East Asia Region, Singapore*

***Corresponding author:** CONWAY Patricia Lynne, Singapore Centre for Life Sciences Engineering, Nanyang Technological University, Singapore

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Introduction

The human gut microbiome is a complex microbial community consisting of bacteria, viruses, fungi and protozoa. The gut microbiome plays a critical role in human health, the immune system, and the development of diseases [1]. Advances in technology and analytical tools have made it possible to study the gut microbiome in greater detail. Sequencing of the 16s rRNA and shot-gun metagenomic sequencing have allowed for the identification, characterization of previously unculturable microbes, and prediction of functional capabilities [2]. A myriad of factors such as the environment, host genetics, lifestyle, and diet contribute to changes in the composition of gut microbiome. These changes can be detrimental as a reduced microbial diversity of the microbial community has been associated with health challenges [3]. Functional decline of the gut microbiome is associated with an increased incidence of infections and an increased risk of chronic diseases. Therefore, elucidating the mechanisms underpinning the structure and function of the microbiome, as well as strategies for modulating the microbiome will be essential for disease prevention and personalized medicine.

The aim of the ILSI SEA Region Gut Microbiome Conference Series was to highlight recent scientific advances made in understanding the gut microbiome. The program included speakers from

a. Australia: Mark Morrison (University of Queensland Diamantina Institute), Andrew Holmes (University of Sydney), Emma Halmos (Alfred Hospital, Melbourne), Anthony Hannan (University of Melbourne), Laurence Macia (University of Sydney), Caitlin Cowan (University of Sydney), and Emad M El-Omar (University of New South Wales, Sydney);

b. New Zealand: Nicole Roy (University of Otago);

c. Singapore: Lee Yuan Kun (National University of Singapore);

d. USA: Janice Rueda (Archer Daniels Midland), Lauren Brink (Reckitt Benckiser)

The conference programme addressed several themes, describing the infant gut microbiome, the role of the gut microbiome in human health, the immune system, metabolic health as well as cognitive and mental health. In each of these themes, the effects of diet on the gut microbiome were discussed. Dietary approaches to treat dysbiosis of the gut microbiome implicated in diseased states were examined. Scientific data presented were based on published scientific articles, and these were made available online and as such, ethics approval was not required for this paper.

Infant Gut Microbiome Insights

The infant gut microbiome begins as a dynamic ecosystem of microorganisms that colonizes the gastrointestinal (GI) tract largely following birth [4]. Metagenomic approaches revealed that several key phyla such as Proteobacteria and Actinobacteria dominate initially [5]. Subsequently, the gut microbiome develops successively to become more diverse as Bacteroidetes and Firmicutes begin to dominate [6]. The infant gut microbiome is unstable and vulnerable to alterations during the first three years. Selective forces result in drastic changes to the gut microbiota in terms of diversity and taxonomic composition. The forces that affect the development of the infant gut microbiome includes maternal and neonatal exposures such as mode of delivery, mode of feeding, environment, infant diet and antibiotic use [7]. Therefore, the first three years of life represent a critical developmental window for the infant gut microbiome and its alteration during this window will lead to considerable variations in the gut microbiota composition which have the potential to significantly affect infant health and development through adolescences. The following sub-section explores the effects of infant diet on the gut microbiome.

Influence of Diet on Infant Gut Microbiome

In his presentation, Mark Morrison emphasized the importance of dietary choices during a crucial period of infant development. He detailed experimental studies of different infant diets in children aged one to six years from Europe and rural Africa [8]. Children in rural Africa had a representative diet low in fat and animal protein while rich in starch, fiber, and plant polysaccharides. On the other hand, children from Europe had a representative diet high in animal protein, fat, sugar and starch, while low in fiber. Children from Europe had higher Firmicutes to Bacteroidetes (F/B) ratio compared to children from rural Africa, possibly driven by their high-calorie diet intake. In addition, *Prevotella, Xylanibacter* (Bacteroidetes), and *Treponema* (Spirochaetes) were uniquely found in the fecal microbiota of children from rural Africa. These genera are known to produce high levels of short-chain fatty acids (SCFAs) which is consistent with the finding that children from rural Africa had the highest levels of SCFAs, in particular propanoic acid and butyric acid, compared to children from Europe. These findings highlighted the importance of diet choices as they play a major role in modulating the composition and function of the gut microbiota.

Morrison further suggested that early life alterations to the gut microbiome may have long-lasting consequences through life. Experimental studies in a mouse model, using low-dose penicillin (LDP) administration in early life, showed that early life alterations disrupt the microbiota and host-microbe metabolic interactions [9]. LDP administration in early life suppressed the growth of several taxa, including *Lactobacillus, Candidatus Arthromitus* (segmented filamentous bacteria, SFB), and *Allobaculum*. In addition, intestinal barrier function and immunity were compromised, which has been implicated in the development of metabolic syndromes such as obesity. The inclusion of a high-fat diet (HFD) in mice administered with LDP in early life showed increased abdominal fat and increased expression of genes involved in fatty acid metabolism and lipid synthesis. This suggests an increased sensitivity to excess dietary intake and a predisposition to diet-induced obesity. These findings were also observed in naïve germ-free mice transplanted with an altered stool microbiota from LDP treated mice, further proving that host-microbe metabolic interactions are dictated by an altered microbiota during early life only. Taken together, Morrison emphasized that early life represents a critical window of development for the host and that metabolic consequences arising from an altered microbiota during early life could induce metabolic syndromes such as obesity when associated with excess dietary intake. It is, therefore, of considerable interest to target the critical developmental window that is the most dynamic and malleable.

Influence of Diet on Infant Gut-Immune Axis and Development of the Immune System

The World Health Organization (WHO) recommends that, if possible, babies should be exclusively breast fed for the first six months of life. Infant formula represents an alternative source of early nutrition for infants and extensive studies with breast-fed babies (BF) versus formula-fed babies (FF) have shown that different early life diet influences the gut-immune axis and development of the infant immune system. In the following sections, insights into the role of human breast milk bioactives in the development of infant immune system are discussed.

Role of Human Milk Bioactives

Lauren Brink provided insights into the role of important human milk bioactives in the development of the infant immune system. Human breast milk has been recommended as the first early life nutrition as it contains the necessary nutrients for holistic infant development. She highlighted a variety of bioactive molecules linked to modulation and development of the immune system. These molecules include lactoferrin, components of the milk fat globule membrane (MFGM), human milk oligosaccharides (HMOs), docosahexaenoic acid (DHA), and arachidonic acid (ARA).

Lactoferrin is a bioactive glycoprotein that lines the epithelia and mucosa surfaces, improves intestinal structure and strengthens the physical barrier against invading pathogens [10]. Lactoferrin has antimicrobial properties due to its high affinity for ferric iron. It deprives bacterial pathogens of ferric iron, which is necessary for growth and attached to target host cell surface ligands [11]. In addition, lactoferrin is capable of inducing local and systemic changes in the expression of signalling molecules that stimulate innate and adaptive immune cells, and produce anti-inflammatory effects [12]. Clinical studies involving infants fed with infant formula supplemented with lactoferrin reported a lower risk of respiratory tract infections (RTIs) [13], less diarrhoea and coughing as compared to infants fed with non-supplemented infant formula [14]. Lactoferrin is an important key bioactive molecule capable of modulating the immune response through several mechanisms, and thereby reducing the risk of RTIs in infants.

The milk fat globule (MFG), specifically the MFG membrane (MFGM), is functionally important as it accounts for up to 4% of

the total protein content of human milk [15]. It surrounds the lipid droplets that were derived from mammary gland epithelial cells. Components of MFGM have been shown to contribute to the maturation and modulation of the infant immune system, and neurodevelopment [16]. MFGM modulates antibody production by increasing the production of IgA antibody from B cells and reducing specific IgG antibodies. In addition, proteins within MFGM regulate T cell differentiation into Treg cells which releases cytokines, playing an important role in determining the level of immune response. Glycans in MFGM have been shown to induce antimicrobial effects by preventing pathogens from binding to mucosa surfaces. The lysozyme within MFGM is capable of breaking down the cell walls of bacteria, ultimately destroying potentially harmful bacteria. Specific glycoproteins in MFGM have been shown to elicit prebiotic benefits, supporting the growth of beneficial bacteria in the gut [16,17]. Clearly, MFGM and its many components represent an important bioactive molecule in the development of the infant immune system.

Other bioactive molecules are the human milk oligosaccharides (HMOs). HMOs predominantly exists as 2'-fucosyllactose (2FL) in approximately 80% of women [18]. HMOs act as prebiotics by altering intestinal metabolic activity, and promote the growth of healthy bifidobacteria and hence intestinal microbial fermentation that releases SCFAs. These SCFAs lower the gut luminal pH which suppresses the growth of harmful bacteria. SCFAs also support immune function within the gut by triggering the development of immature T cells into regulatory T (Treg) cells and thereby indirectly altering the production of cytokines by increasing anti-inflammatory cytokine production while decreasing inflammatory cytokine production. Hence, HMOs are seen as important immunomodulatory bioactives often found in human breast milk.

The DHA and ARA are long-chain polyunsaturated fatty acids (PUFA) that play an intrinsic role in infant growth and development. DHA has been shown to modulate the immune system by being incorporated into immune cell membranes, affecting cell signaling pathways, and altering the release of anti-inflammatory mediators such as resolvins. Clinical studies with infant fed infant formula supplemented with DHA and ARA reported fewer incidences of RTIs and bronchitis as compared to infants fed with a standard formula [19-21].

Collectively, human breast milk contains many bioactive molecules that are critical for the development and growth of infants, and this has inspired the promising use of such molecules in infant formula, resulting in clinically relevant reductions in illness.

Gut Microbiome and Human Health

The gut microbiome exists in a dynamic state of equilibrium and plays an important role in mediating biochemical transformations and host physiology processes that impact host health and disease progression. An imbalanced gut microbiota, often termed gut dysbiosis, may cause several diseases such as functional gut disorders (FGD) [22]. FGD is a widespread and common disease that affects the whole gastrointestinal system, potentially impacting the lifestyle of the host and society. However, the exact cause of FGD remains largely unknown and appears to be multifactorial and varied between patients. Over the years, a growing body of evidence suggests that the gut microbiome may play an important role in the pathology, persistence, and modulation of these diseases [23]. Therefore, studies are needed to study interactions between the gut microbiome, host and lifestyle factors e.g. diet, to better elucidate the causes of FGD.

Nicole Roy focused on elucidating the etiology of one of the most common FGD, irritable bowel syndrome (IBS). IBS is often characterized by the presence of abdominal pain associated with bowel movement. These symptoms, however, exist on a spectrum and are categorised in different subtypes: constipation, diarrhoea, and mixed (IBS-C, IBS-D, and IBS-M, respectively) [24]. She highlighted that experimental studies that attempt to study the causes of FGD are often lacking, focusing on only one or two aspects of pathogenesis. In recent years, Nicole Roy and several scientists attempted to address the multifactorial etiology of IBS through a Christchurch IBS cohort to investigate mechanisms for gut relief and improved transit (COMFORT) [25]. The study was designed to incorporate a wide range of pathophysiological data from patients with IBS, and included clinical, dietary and biological samples. Therefore, the COMFORT cohort study could represent a novel resource that uses a systems biology approach to tackle the multifactorial aetiology of FGD. She highlighted that the diagnosis and treatment of FGD such as IBS remains a challenge as there are no reliable biomarkers to define the disease [26].

A metagenomic study involving biological samples from patients with IBS attempted to identify biomarkers of IBS [27]. Urine and fecal metabolomes of patients with IBS revealed 89 unique urine metabolites and 77 unique fecal metabolites which were significantly depleted in these patients compared to healthy individuals. Amino acids, fatty acids, adenosine, inosine, and purine were found to be important metabolites for the discrimination of patients with IBS and healthy individuals. Despite recent advances, Roy stressed that there has not been a unique metabolite capable of distinguishing between IBS subtypes. She described recent research that has shown that bile acid metabolism may be associated with IBS; suggesting that bile acids could represent a unique biological signature for patients with IBS [28]. Upon further investigation in the COMFORT study, fecal samples collected from IBS patients were analyzed for their fecal bile acid concentrations [29]. Patients with IBS of different subtypes were found to have different concentrations of bile acid. Four bile acid metabolites, namely cholic acid (CA), chenodeoxycholic acid (CDCA), glycol-cholic acid (GCA) and taurine, were significantly different between IBS-C and IBS-D, with higher concentrations of these bile acid metabolites reported in patients with IBS-D. Although the current study revealed differences in bile acid concentrations within IBS subtypes, inherent variability between patients with IBS meant that current diagnostic and research-based tools could not reliably and objectively differentiate subtypes within IBS patients. Roy emphasized the increasing need to characterize unique biological

signatures of FGD or within FGD subtypes that are objectively measured and scientifically validated.

Gut Microbiome and Diet in FGD

The gut microbiome plays a crucial role in human health and disease prevention. Dysbiosis has been implicated in FGD, in particular inflammatory bowel disease (IBD). The prevalence of IBD has been increasing in modern society and this could be associated with a westernized dietary pattern that has become increasingly common [30]. A westernized dietary pattern such as fast food, which is often characterized by high fat, high sugar, and low fiber exposure, could be associated with the pathogenesis of IBD and other FGDs such as Crohn's disease (CD). To address the possible interrelationship between dietary patterns and incidence of IBD, Mark Morrison and several scientists conducted the Eastern IBD Gut Microbiota (ENIGMA) study which aimed to shed light on the microbiota and dietary differences in 87 Caucasian and Chinese patients with IBD and healthy individuals [31]. Based on the dietary recall of the participants, it was observed that IBD patients tended to include a westernized dietary pattern in their daily life and eat fast food more often than healthy individuals. The data suggested that access to protein and carbohydrate, and calorie intake is an important factor in mediating the composition of the gut microbiota. The nutrient profile that reflects a westernized diet favors a lower diversity and abundance of the microbiota, which is often associated with IBD [32,33]. Taken together, these results suggest that diet is one of the many factors that plays an important role in FGD such as IBD. However, the mechanisms associating diet and its role in the pathogenesis of IBD were not elucidated in the ENIGMA study. Therefore, Morrison and several scientists embarked on on-going ENIGMA II study to address the influence of dietary factors on the etiology of IBD by functionally characterizing the microbiota of Asian and Western populations.

The importance of diet in patients with FGD was further elaborated by Emma Holmes. A diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) has been considered the first line therapy for treating patients with FGD such as irritable bowel syndrome (IBS), but there has been limited evidence of its therapeutic efficacy [34]. Her team conducted a dietary intervention study in IBS patients and healthy individuals to investigate the effects of a low FODMAP diet on IBS symptoms [35]. The study revealed that a low FODMAP diet reduced bloating, pain, and wind passage by more than 50% in patients with IBS compared to healthy individuals on a typical Australian diet. This suggests that a low FODMAP diet may generate therapeutic benefits by significantly reducing the symptoms of IBS. This efficacy over placebo was also observed in other studies from various countries, with 50-87% of patients with IBS reporting benefits [36-39]. Taken together, these findings led to the use of a low FODMAP diet as a first line dietary treatment for IBS in many guidelines such as the National Institute for Health and Care Excellence, the British Society of Gastroenterology, and the American College of Gastroenterology.

Emma Holmes further clarified the low FODMAP diet as a first line dietary treatment. The recommendation to adopt the FODMAP diet follows a structured protocol consisting of three steps:

a. restriction of FODMAP, which reduces the individual intake of FODMAP while assessing the response to restriction;

b. reintroduction of FODMAP, which identifies specific types and doses of FODMAP in individual responses;

c. personalization of FODMAP, which aims to expand the diet and reintroduce other foods for a long-term diet [40].

She also highlighted the importance in distinguishing dietary interventions for therapy and for health. The former aims to treat established diseases and targets a specific population, such as patients with obesity, FGD, and kidney failure. In contrast, the latter aims to promote nutritional adequacy and disease prevention. She emphasized that dietary interventions for health should ideally include carbohydrates which are not absorbed and reach the large bowel for fermentation by colonic bacteria. The colonic fermentation of carbohydrate produces SCFAs and gas, and promotes fecal bulking. SCFAs reduce pH in the luminal environment of the gut, discouraging the growth of pH-sensitive pathogenic bacteria while promoting butyrate-producing bacteria [41]. Hence, carbohydrates in the diet promote an anti-inflammatory environment that is associated with health and disease prevention. On the other hand, dietary interventions for therapy typically include a low FODMAP diet characterized by poorly absorbed short-chain carbohydrates. Although dietary interventions for therapy could treat symptoms in patients with FGD, it may not necessarily translate into a healthy diet for these patients. This begs the question: Does a low FODMAP diet cause dysbiosis?

Holmes described a study conducted by Staudacher *et al.,* which evaluated the gut microbiota of 19 patients with IBS on a low FODMAP diet and compared with 22 healthy individuals on their usual diet [42]. The data suggest a reduced absolute abundance of bifidobacteria. Interestingly, a low FODMAP diet that reduced IBS symptoms also led to a reduction in bifidobacteria. Similarly, a single-blinded parallel study with 40 IBS patients was carried out with subjects being were given either a low or high FODMAP diet [37]. The study found that a low FODMAP diet reduced symptoms of IBS, and also reduced the relative abundance of bifidobacteria. This was also observed in a separate double-blinded cross-over study involving 20 patients with IBS [43]. They received a low FODMAP diet for three weeks and were then supplemented with FOS or placebo for ten days. The data suggest that a low FODMAP diet is associated with a decrease in the absolute abundance of bifidobacteria and actinobacteria. In a separate study conducted by Holmes and her team, the effects of a low FODMAP diet for the treatment of IBS on the luminal microenvironment of the gut were investigated in 27 patients with IBS and 6 healthy individuals [44]. Participants were randomly given a low FODMAP diet or a regular FODMAP diet for 21 days, after which they entered a washout period in which they resumed their regular diet and switched to an alternate diet. Data suggested that a low FODMAP diet was

associated with a 37% decrease in the absolute abundance of total bacteria. A seven-fold and five-fold decrease in relative abundances of *Clostridium* cluster XIVa and *Akkermansia muciniphila* were also observed, respectively. Holmes concluded that although a low FODMAP diet has good efficacy in reducing the symptoms of IBS, the gut microbiome will lose potentially healthy bacteria. The long-term consequences of an altered microbiome due to low FODMAP diets remain largely unknown. She emphasized that there is an increasing need to elucidate the mechanistic underpinnings of low FODMAP diets on the gut microbiome, and to develop a reliable microbiota biomarker to manage patients with IBS on a low FODMAP diet recommendation.

Holmes presented a recent prospective single centre case control study of 56 pairs of IBS patients and household individuals. The study employed shotgun metagenomic sequencing of the fecal microbiome to assess the effects of low FODMAP diets on the gut microbiome of the participants [45]. The fecal microbiomes of patients with IBS had less bacterial diversity, lower levels of *Bacteroides cutis* and *Bacteroides stercorirosoris*, and higher levels of Firmicutes. Genes associated with carbohydrate metabolism pathways were enriched. These findings suggest that a dysbiotic microbiome environment is associated with IBS. Participants received a low FODMAP diet for four weeks and had a normal FODMAP diet reintroduced for the next eight weeks. The study reported that a low FODMAP diet led to significant improvements in 75% of IBS cases. The microbiomes of IBS patients had a higher degree of response to a low FODMAP diet as compared with the microbiomes of healthy household individuals. The intervention of a low FODMAP diet normalized *Bacteroides* levels and pathobionts (including *Clostridium difficile, Streptococcus parasanguinis, Paeniclostridium sordellii*) levels towards levels observed in microbiomes of healthy household individuals. Functional profiling suggested that a low FODMAP diet reduced genes and pathways related to carbohydrate metabolism, specifically a decrease in the degradation of disaccharide trehalose and glycolysis, to levels observed in the microbiomes of healthy household individuals. Upon reintroduction of a normal FODMAP diet, the microbiomes of IBS patients were maintained and the symptoms of IBS were controlled. Collectively, Holmes concluded that the structure of the fecal microbiome may predict the response of an individual to the FODMAP diet. In addition, a low FODMAP diet may correct IBS-associated dysbiosis, metabolic pathways, and such a correction was maintained during the reintroduction of a normal FODMAP diet.

Gut Microbiome and Immune System

The immune system, which is comprised of innate and adaptive immune components, engages in complex and dynamic interactions with the gut microbiome. Such intricate interplays, also known as gut-immune axis, have been shown to play an important role in host defense and in maintaining intestinal homeostasis. For instance, certain species of the gut bacteria, such as *Clostridium* clusters IV, XIV & XVIII, are able to reinforce the intestinal epithelial barrier and induce Treg cells in the colon, thus improving host immunity [46]. Therefore, alterations to the intricate balance between the gut microbiome and the immune system may trigger many chronic, inflammatory, and endocrine associated diseases. Here, the following section introduces recent advancements in understanding the gut-immune axis, and how diet may modulate the gut microbiome and immune function of the host.

Influence of Diet on Gut Microbiome and Immune Function

Laurence Macia discussed the increase in incidence of many noncommunicable diseases such as Type I diabetes and Crohn's disease and acknowledged that the host immune system and systemic chronic inflammation (SCI) are often involved in these diseases. SCI is characterized by activation of the immune system that differs from an acute immune response. A multitude of factors including social, biological, psychological, and environmental factors are able to affect the immune system, leading to SCI.

Macia elaborated that diet and its capacity to modulate the gut-immune axis could represent a basis for certain non-communicable diseases due to the production of several key metabolites by the gut microbial community. Under conditions of gut symbiosis, key metabolites such as SCFAs play an important role in regulating immune differentiation and function, ultimately promoting homeostasis essential for health and disease prevention. However, under conditions of gut dysbiosis, abnormal levels of key metabolites are produced, contributing to immune dysregulation and inflammation. Interestingly, non-communicable diseases such as Type I diabetes, asthma, IBD, and food allergies have been associated with a dysbiosis condition in the gut, subsequent alteration of the immune system, and inflammation. Evidence from a recent study has shown that key bacterial metabolites can directly impact the immune cell metabolic profile, dictating the outcome of an immune response as an inflammatory or anti-inflammatory phenotype. Taken together, the modulation of the gut-immune axis through diet and the changes in the production of key bacterial metabolites may have a profound impact on the immune system.

The gut microbiota is increasingly becoming a promising target for the treatment of non-communicable diseases, and several studies have attempted to use dietary fiber as a dietary intervention targeting the gut microbiota. Macia shed light on how dietary fiber induces regulatory immune cells to promote an anti-inflammatory response to treat non-communicable diseases. In an experimental house-dust mite (HDM) model of allergic airway disease (AAD), a high fiber diet protected the mice against development of AAD [47]. The high fiber diet modulated the gut microbiota composition and improved SCFA production. The increase in acetate production induced Treg cells, suppressing the development and progression of AAD. Similar results were observed in another study involving specialized diets of acetate and butyrate. These diets have shown to enhance the number and function of Treg cells, conferring protection against Type 1 diabetes [48]. She described two main mechanisms of induction of Treg cells by dietary metabolites acetate and butyrate derived from bacterial fermentation of dietary fiber:

a. direct cell signaling through metabolite-sensing G-protein coupled receptors (GPCRs); and

b. inhibition of HDAC [49].

The activation of GPCRs leads to the activation of downstream signaling pathways such as MAP kinases, or β-arrestin 2 which results in the inhibition of inflammatory NF-κB function, promoting an anti-inflammatory immune response. Dietary metabolites such as butyrate are able to inhibit histone deacetylase, allowing lysine residues within histones to be acetylated, hence promoting transcription of key Treg cell genes such as FoxP3. In a separate study, the role of dietary fiber and vitamin A on the gut microbiota and immune system in the prevention of food allergies were investigated [50]. The data showed that dietary fiber and vitamin A increased SCFA production, and maintained a tolerogenic environment within the gut, enhancing tolerogenic CD103+ dendritic cell (DC) functions. The data also reported that CD103+ DC induced Treg cells development which are necessary for conferring protection against food allergies. Insights from these studies indicate that dietary fiber may suppress the development of non-communicable diseases such AAD, food allergies, and Type 1 diabetes, through Treg cells development either directly, or indirectly through CD103+ DC in mice.

Macia believes that although the development of Treg cells is important in promoting an anti-inflammatory environment and maintaining immune tolerance, other types of immune cells could also contribute to immune homeostasis. Her team set out to study regulatory B (B10) cells due to their involvement in immune suppression via the production of the anti-inflammatory cytokine IL-10. It has been shown that IL-10 can inhibit the differentiation of pro-inflammatory Th1 and Th17 T cell subsets, and also promotes Treg cell differentiation, which are important for the prevention of non-communicable diseases. Macia and her team, therefore, questioned if dietary metabolites derived from bacterial fermentation of dietary fiber could enhance B10 cells in the same way as Treg cells to promote an anti-inflammatory response and disease prevention? Her study showed that acetate directly induced mouse B10 cell development and differentiation from mouse B1a precursors, and this was independent of proliferation and survival of existing B10 cells [51]. Data from this study suggests that acetate induced B10 cell differentiation through GPR43 activation. However, the production of IL-10 was found to be independent of GPR43 activation and protein acetylation in IL-10 promotor region. Liquid chromatography-coupled mass spectrometry (LC-MS) revealed that acetate was utilized by B1a precursor cells and incorporated into acetyl-CoA, enhancing the production of citrate levels in TCA cycle. The study also found that acetate increased the respiratory capacity and metabolic activity of B1a precursor cells. Interestingly, acetate was able to induce B10 cell differentiation from B1a precursor cells in the absence of energy in the form of adenosine triphosphate (ATP), suggesting that an alternative mechanism unrelated to TCA cycle was involved.

The study found that the acetate, converted into acetyl-CoA mediated by enzyme ACSS member 2 (ACSS2), is an important substrate for protein acetylation by lysine acetyl transferase (KAT). This post-translational acetylation is necessary for IL-10 production in B10 cells. Taken together, Macia and her team showed that the dietary metabolite acetate altered the metabolic activity of B cells through the TCA cycle, and modulated the protein acetylation profile of enzymes important for B10 cell differentiation and IL-10 production from these cells. The impact of this finding was studied in a human in-vivo study involving short-term dietary fiber intervention to increase acetate levels. Dietary fiber intervention increased plasma acetate levels, and significantly increased B10 cells differentiation. Hence, dietary intervention using dietary fiber remains clinically relevant as dietary fiber was able to increase plasma acetate levels, and promotes B10 cell differentiation important for immune tolerance and homeostasis. It should be noted that dietary context is important as changes in micronutrient from dietary fiber to dietary carbohydrate resulted in a different immune function. A recent study showed that dietary carbohydrate promotes B cell production in the bone marrow of mice and enhances antigen-specific antibody production [52]. This suggests that micronutrient composition of the diet is critical in dictating immune function. Hence, the idea of an optimal diet in which dietary fiber or dietary carbohydrate should be added to achieve a synergistic and optimal immune outcome remains to be investigated.

Gut Microbiome and Metabolic Health

The gut microbiome is a potent modulator of metabolism, and studies have shown that gut microbiome may contribute to the metabolic health of the host. However, a dysbiosis in the gut microbiota may also contribute to the pathogenesis of many metabolic diseases such as obesity and Type II diabetes (T2D). The following section explores the gut microbiome in relation to metabolic diseases, and how microbiota-targeted interventions, such as diet, aims to treat and prevent such diseases.

Gut Microbiome and Diet in Obesity

Obesity is increasingly prevalent in both developing and developed countries and represents a major risk factor of many diseases such as metabolic syndrome (MS), and chronic diseases including Type 2 diabetes (T2D), cardiovascular diseases, and cancer. Obesity is characterized by an abnormal or excessive fat accumulation that may impair host health, leading to poorer mental health outcomes and reduced quality of life. Lee Yuan Kun provided insights into obesity that is often associated with high dietary fat intake and an altered gut microbiota. Obese individuals reported an increased dietary fat intake as compared to lean individuals [53]. In addition, it has been reported that obese individuals consistently have a reduction in microbial diversity and richness. Firmicutes to Bacteroidetes ratios were found to be significantly higher while the relative abundance of *Prevotella* was significantly lower in obese and overweight individuals.

Lee described a recent study that elucidated the mechanism linking gut microbiome, obesity and its related diseases [54]. The study showed that bacterial lipopolysaccharide (LPS) levels

in the intestinal lumen is highly correlated to an altered gut microbiome induced by high dietary fat intake. The altered gut microbiome loosens the epithelial layer tight junction, allowing the translocation of pathogens and LPS. The translocation of LPS was also observed in mice with genetically induced obesity as well as in mice with diet-induced obesity [55]. This phenomenon is referred to as "metabolic endotoxemia", and it has been demonstrated that high levels of LPS in metabolic endotoxemia triggers the onset of inflammation, and ultimately insulin resistance, leading to metabolic diseases such as T2D. These findings suggest that obesity and other metabolic diseases such as diabetes were associated with an altered microbiome induced by high dietary fat intake, and these observations were also confirmed in several human studies.

Lee observed that there are instances of overweight and obese individuals without metabolic abnormalities, suggesting an anti-metabolic disorder mechanism. Likewise, there are instances of lean individuals with high prevalence of metabolic abnormalities [56]. He recognized that although these findings were observed in communities with a Western life style, Asians could also suffer from these consequences given that obesity and other related metabolic disorders are increasing. The prevalence of diabetes in the context of obesity is slowing increasing and this could be associated to changes in dietary patterns that sensitise Asian people to the risk of diabetes. Lee presented a cross-sectional study conducted in Yogyakarta in Indonesia, which aimed to shed light on the association of gut microbiome in Indonesian adults and obesity and T2D under different dietary regimes [57]. The study revealed 3 distinct bacterial genera clusters, namely: *Bacteroides*, *Prevotella*, and *Romboutsia*. The gut microbiome of Indonesian adults who consumed a traditional diet consisting of Indica rice was dominated by *Prevotella*. In contrast, the gut microbiome of those who consumed a high carbohydrate and low-fat westernized diet was dominated by *Bacteroides*, while those who consumed a low carbohydrate and high fat westernized diet is dominated by *Romboutsia*.

Interestingly, individuals harbouring *Bacteroides*-driven microbiome were characterized with high fasting blood glucose levels and low BMI levels, while individuals harbouring *Prevotella*-driven microbiome were characterized with low fasting blood glucose levels and high BMI. This data suggests that although individuals harbouring *Bacteroides*-driven microbiome were lean, they suffered from T2D. Lee and a group of scientists also characterized the fecal bile acid (BA) profile in these individuals. The abundance of *Prevotella* was found to be positively associated with primary and conjugated BA, and secondary BA of ursodeoxycholic acid (UDCA), but negatively associated with secondary BA of deoxycholic acid (DCA) and lithocholic acid (LCA). Conversely, the abundance of *Bacteroides fragilis* and *Romboutsia* were negatively associated with primary, conjugated, and some secondary BAs. This indicates that BAs and the metabolism, which are induced by the consumption of dietary fats, are important in driving the growth and establishment of several bacterial genera in the gut microbiome. In lean individuals, unabsorbed conjugated BAs are deconjugated into primary BAs, while non-digested conjugated BAs bind antagonistically to the farnesoid X receptor (FXR), contributing to glucose homeostasis. Primary BAs are further metabolized by *Ruminococcaceae* into secondary BAs of DCA and LCA. These BAs act agonistically to TGR5 and FXR, further coordinating metabolic homeostasis. In obese individuals who do not suffer from T2D, the increase in abundance of *Romboutsia*, and decrease in abundance of *Ruminococcaceae* enhanced primary BAs levels which contributes to metabolic homeostasis. However, in T2D individuals, low levels of conjugated BAs were reported to be due to the increase in abundance of *B. fragilis* and decrease in abundance of *Prevotella*. Hence, the lack of antagonistic activity on FXR and TGR5 impairs glucose homeostasis, leading to T2D in these individuals. Taken together, the study provided evidence linking diets, the gut microbiome and host metabolism. *Ruminococcaceae* and *B. fragilis* were positively correlated with a westernized diet of high carbohydrate but negatively correlated with BMI. Conversely, *Romboutsia* was positively correlated with a westernized diet of high fats and BMI. However, *B. fragilis* may lead to high fasting blood glucose levels, resulting in T2D independent of obesity. *Prevotella* was dominant in the gut microbiome of individuals consuming traditional diet and negatively correlated with *B. fragilis* and *Ruminococcaceae*, hence preventing high fasting blood glucose and T2D.

The use of probiotics has been widely recognized as therapeutic treatment for metabolic diseases such as obesity and other related metabolic disorders. Lee discussed a recent study that utilized *Parabacteroides distasonis* to alleviate obesity and metabolic dysfunctions [58]. *P. distasonis* generated succinate in the gut, which activates intestinal gluconeogenesis, ultimately decreasing hyperglycaemia. *P. distasonis* also altered the BAs profile by enhancing UDCA and LCA levels, activating the FXR pathway and repairing gut barrier integrity, ultimately reducing hyperlipidaemia. In a separate study, three *Lactobacillus* strains derived from the human gut were investigated for their attenuation of metabolic diseases. These strains conferred protective effects against metabolic disorders by modulating the gut microbiota and enhancing acetate levels in the gut [59].

Since the gut microbiota is heavily influenced by diet, it is of considerable interest to tackle microbiome treatments with dietary approaches instead of probiotics, prebiotics, and drugs. Lee and a group of scientists studied the enterotypes of the gut microbiota and dietary habits across geographical regions revealed that diet high in meat, fat, vegetable, and digestible starch in the Western regions of US and Venezuela recruits *Bacteroides* enterotype [60]. Conversely, a diet low in meat, fat, high in vegetable, and resistant starches in South East Asia regions recruits *Prevotella* enterotype. The difference in diet suggests that meat and fat contents could be the drivers of different enterotypes. However, a diet comprising of high meat and fat, low in vegetable, and resistant starches in North Asia regions of Mongolia also recruits *Prevotella* enterotype. This suggests that presence of starches in staple food is responsible in determining the *Prevotella* enterotype. This observation is supported by the fact that people in North Asia regions consume barely, oat, and buckwheat which are high in resistant starch.

Likewise, people in South East Asia regions of Indonesia and Thailand consume carbohydrates high in resistant starches such as Indica rice. Compared to Western regions, people in the US consume carbohydrates low in resistant starches such as wheat flour and potato. Hence, it was hypothesized that the relative component of dietary fat and resistant carbohydrate could drive the recruitment of *Prevotella* enterotype, ultimately affecting the sensitivity towards metabolic diseases such as T2D. Lee hypothesized that individuals who consume a high fat diet, but low in resistant starch will have increased levels of free BAs in the colon, suppressing *Prevotella* and bile-sensitive bacterial species. This promotes the growth and survival of *B. fragilis*, which has been proven to increase fasting blood glucose levels, possibly leading to T2D. Individuals who consume a high fat and resistant starch diet will have decreased levels of free BAs in the colon, promoting the growth of *Prevotella* and bile-sensitive bacterial species. This will suppress *B. fragilis*, ultimately decreasing fasting blood glucose levels, conferring a protective effect against T2D. However, he acknowledged that more studies are warranted to confirm the working hypothesis. Collectively, dietary treatments such as the consumption of resistant starches to enrich the *Prevotella* enterotype to treat T2D are promising approaches and could pave the way for the treatment of such diseases.

Predicting Microbial and Physiological Responses to Diet

To understand the effects of diet on the gut microbiome, it is important to realise that nutrient intake is multi-dimensional [61]. These include macronutrient distribution, energy intake, the quality of each nutrient component, and its periodicity. The response of the gut microbiome to diet in each individual is unique and highly personalized. Consequently, understanding how the gut microbiome interacts with different diet dimensions across unique individuals proves to be difficult, and hence predicting microbial and physiological responses remain challenging.

The functional capacity of the gut microbiome is highly conserved, indicating a high level of functional redundancy. Andrew Holmes observed systematic patterns within the gut microbiome, possibly explaining how diet could drive microbial and physiological responses. He presented his work which investigated the nutritional determinants of the gut microbiome, and elucidated the mechanisms associated with the interaction between diet and gut microbiome [62]. Based on generalized additive models (GAM), intake of protein and carbohydrates were the major drivers of microbial response in terms of relative abundance. The concept of trophic response guilds was proposed given that only two main microbial responses were observed: either an increase or decrease in relative abundance. Trophic response guilds are aggregation of taxa that demonstrated similar response patterns to nutrient availability. Based on model simulations, he hypothesized that alterations to the relative importance of nutrients may drive the observed responses in microbial community, and that the endogenous host secretions could represent as a source of carbon or nitrogen for the bacteria. This hypothesis was confirmed by analysis of microbial community in mice fed with protein and carbohydrate of different energy density (4kcal/g versus 2kcal/g). He demonstrated an increase in uptake of endogenous nitrogen under different diets, resulting in a selective advantage during conditions of low nutrient intake. Microbes regulate their foraging strategies and are able to exploit endogenous sources of nitrogen during low nutrient intake conditions. This resource-based regulation allows for a mechanistic modelling of dietary effects on the gut ecosystem. Microbial and physiological responses are associated with resource use by microbes, and nutrient intake patterns. Different ratios of dietary protein, fats, and carbohydrates and resource use by the gut microbes differentially drive metabolic health and immuno-metabolic outcomes. Adiposity was driven by an increase in total caloric intake and was reversed with a low protein intake pattern. However, macronutrient distribution dictates the extent to which health was associated with adiposity. Health was improved under carbohydrate-dominated intake, whereas a protein or fat-dominated intake pattern leads to poor cardio-metabolic health outcome. Protein intake pattern strongly dictates the immuno-regulatory state of the host, suggesting that the relative amount of dietary protein is essential in realizing the benefits of carbohydrate intake. Taken together, this study highlights a crucial role for endogenous nitrogen sources in the way diet affects the host microbial and physiological outcomes. Holmes concluded that the recognition of dietary macronutrient profile and intake patterns could pave the way to better understand the complex interaction of diet, gut microbiome, and the host through simpler mechanistic modelling. This could possibly, give rise to a more contextualized approach with stratified cohorts to explore the specific impacts of individual macronutrient.

Gut Microbiome, Cognitive and Mental Health

The bi-directional communication between the gut and the brain is complex and highly dynamic. The concept of the gut-brain axis, although not new, has not been extensively studied. The mechanisms whereby the gut microbiome communicates with the brain are progressively being discovered, and there has been an increasing body of evidence suggesting a major role of the gut microbiome in many cognitive and psychiatric disorders. In the following sections, different cognitive and psychiatric disorders are being explored. The role of the gut microbiome in these diseases, and evidence of treatments are also discussed.

Understanding Huntington's Disease

Huntington's disease (HD) is a fatal neurodegenerative disorder with autosomal dominant inheritance. Affected individuals experience psychiatric symptoms of depression, cognitive deficits, and movement disorders. HD is caused by a CAG repeat expansion in exon 1 of HTT gene in the encoded huntingtin protein, resulting in dysfunction and eventual death of specific neuronal populations in the cerebral cortex and striatum [63]. It has been reported that R6/1 mice expressing the human exon 1 transgene encoding expanded polyglutamine in the huntingtin N-terminal fragment experienced progressive neurodegenerative phenotype, including

cognitive and motor deficits [64]. This finding suggests that exon 1 of HTT carrying CAG repeats is sufficient to induce symptoms characteristic of HD. A pioneering study revealed that the onset of HD in mice could be delayed with environmental enrichment (EE), suggesting environmental modulation of HD [65]. This study provides the foundation for subsequent studies exploring EE, cognitive and motor stimulation in HD mice [66,67], further justifying the role of environmental modulators in the pathogenesis of HD. Given that the gut-brain axis acts bi-directionally, alterations in the gut microbiome may implicate neurological and psychiatric disorders. Hence, the question can be asked whether the gut microbiome can be a modulator of pathogenesis and progression of HD? The following section examines the experimental evidence that may suggest a role of the gut microbiome in the pathogenesis and progression of HD.

Role of Gut Microbiome in the Pathogenesis and Progression of Huntington's Disease

Anthony Hannan presented work elucidating the role of the gut microbiota as a modulator of pathogenesis and progression of HD. Recently, gut dysbiosis was reported for the first time in the R6/1 transgenic mouse model of HD. These mice had a significant increase in Bacteroidetes and a decrease in Firmicutes in the gut microbiome composition [68]. Metagenomic profiles of the gut microbiome of HD were characterized and computational analysis revealed that the gut microbiome of the HD mice was highly unstable in its taxonomic and functional composition as compared to that in wild type (WT) mice [69]. Further analysis of metagenomic and metabolomic profiles examined the correlation between particular gut microbiota and their metabolites in HD and WT mice. It was shown that ATP and pipecolic acid were highly correlated with several key species of the gut microbiota, namely *Bacteroides pyrogenes, Bacteroides oleiciplenus*, and *Prevotella ruminicola*. The gut microbiome could contribute to these metabolites that travel in the circulatory system and reach other organs including the brain. Matsumoto *et al.,* reported that the gut microbiome contributed to cerebral levels of pipecolic acid and ATP in ex-germ-free mice [70]. Hence, gut-derived metabolites are able to communicate with the brain via neural signaling through the vagus nerve, endocrine signaling through the HPA axis, and the immune system though production of cytokines, ultimately affecting the cognitive and behavioural aspects of the brain [71].

Given the bi-directional communication between the gut and the brain, the modulation of the gut microbiome by environmental factors has been suggested, potentially explaining the link between environmental modulators and progression of neurodegenerative disorders such as HD. Hannan hypothesised that EE, which are reported to delay the onset and progression of HD in R6/1 HD transgenic mice, may modulate the dysbiotic gut microbiome in HD mice. Specific alterations of the gut microbiome were observed between HD and WT mice, and between male and female mice when housed in different housing conditions (standard housing, EE, and exercise) [72]. HD mice housed with EE conditions had distinct gut microbiome signatures; Bacteriodales, Lachnospirales, Oscillospirales. These bacteria are linked to a healthy gut microbiome environment, conferring protective effects on the host. These results demonstrate the effects of the different housing conditions and how EE affects dysbiotic gut microbiome of HD mice. Hannan and his team have demonstrated that EE may modulate HD via the microbiome gut-brain axis.

Understanding Schizophrenia

Schizophrenia is a multi-factorial and complex psychiatric disorder characterized by the combined effects of high genetic heritability and a number of non-genetic environmental risk factors [73]. Hannan also studied how genetic, epigenetic, and environmental factors could modulate brain maturation and specific neural circuitry, leading to cognitive deficits, and psychotic symptoms. In particular, the phospholipase C-β1 (PLC-β1) signaling pathway appears to be implicated in schizophrenia. Prior studies investigating the effects of PLC- β1 knock-out in mice revealed disruption in the neocortex during brain development [74], and in the development of normal cortical circuitry [75]. These studies provide evidence that specific neocortical metabotropic glutamate receptors (mGluRs) and muscarinic acetylcholine receptors (mAChRs) are involved in the PLC-β1 signaling pathway, and have been implicated in the pathogenesis of schizophrenia. PLC-β1 and mGlu5 knock-out mice showed endophenotypes homologous to psychotic symptoms of schizophrenia, such as hyperactivity in locomotor function, and deficits in sensorimotor gating. Similar to HD, discrimination in housing conditions of PLC-β1 and mGlu5 knock-out mice revealed beneficial effects of EE. In addition, EE ameliorates cognitive deficits by rescuing deficits in sensorimotor gating [76], and improving learning in PLC- β1 and mGlu5 knock-out mice, respectively [77]. Given that current therapeutics for schizophrenia involves the use of anti-psychotic drugs that do not treat the cognitive deficit in schizophrenia, Hannan proposed that environmental modulation through EE may facilitate the development of novel therapeutic strategies.

Role of Gut Microbiome in the Pathogenesis of Schizophrenia

In a recent paper in which a dysbiotic gut microbiome was reported, Hannan described a model of relevance to schizophrenia, drawing interesting parallels to clinical evidence of gut dysbiosis [71]. Results from the study revealed a difference in beta diversity but not alpha diversity between mGlu5 KO mice and WT mice. In addition, these phenotypes were discriminated by unique microbial signatures; Bacteroidales order for mGlu5 KO phenotype, Erysipelotrichales, Bacteroidales and Clostridiales order for WT phenotype. Interestingly, the presence of gut dysbiosis in mGlu5 KO mice was associated with metabolic dysfunction characterized by reduced body weight, despite non-significant changes in food and water intake, when compared to WT mice. This study provided the first evidence of a genetic model of relevance to schizophrenia, showing both face and predictive validity that a dysbiotic gut microbiome was implicated in the cognitive development of the brain.

Role of Gut Microbiome in Clinical Anxiety and Depression

Anxiety and depression are psychotic conditions that affect approximately 10% of the population globally every year. There has been an increasing number of studies exploring the bidirectional communication of the gut-brain axis. The gut microbiome being a key regulator in host health and disease prevention, may be implicated in such psychotic conditions of anxiety and depression through production of neurotransmitters, precursors, neuropeptide and metabolites. Over the years, several preclinical models have attempted to highlight the presence of a dysbiotic gut microbiome, and modulation of these behaviours in mice induced with anxiety and depression-like behaviours. The following section presented by Caitlin Cowan aims to highlight the use of preclinical models in understanding the gut microbiome in relation to psychotic disorders of anxiety and depression.

Germ-free (GF) models represent a foundational model commonly used to understand the role of the gut microbiota on the host. GF animals are completely devoid of microorganisms and bred in sterile conditions to prevent microbe colonization. Cowan described several studies that demonstrated the use of GF models to study the role of gut microbiota in psychotic disorders. A recent paper by Sudo *et al*., showed that GF mice exhibited a significant increase in plasma ACTH and corticosterone levels in response to restraint stress, resulting in an exaggerated hormonal stress response [78]. Diaz-Heijtz *et al*., showed that GF mice had decreased anxiety-like behavior [79] while Chu *et al*., reported that GF mice impaired fear extinction following Pavlovian fear conditioning [80]. From these studies, it can be suggested that the complete absence of a gut microbiome may affect behaviours relevant to anxiety and depression. However, Cowan pointed out that GF models are extreme due to the complete absence of microbes, and the findings may not translate well to human studies. She explained that other preclinical models that may have better translatability and equivalency to humans include the study of early-life stress. Maternal separation of pups from their mother over prolonged period is commonly used as an adverse event in early-life stress models. In a study conducted by O'Mahony *et al*., pups were maternally separated each day from their mothers for up to two weeks. These pups exhibited increased corticosterone, increased anxiety and depression-like behaviour, reminiscent of the observations made in humans exposed to early-life stress [81]. These changes were accompanied by alterations of the gut microbiome and were found to be responsive to microbiota-targeted interventions, indicating the role of gut microbiome in psychotic conditions such as anxiety and depression. Paramount to these models is the use of fecal microbiota transplant (FMT), in which a donor fecal sample is transferred into the gastrointestinal tract of the recipient. In a study conducted by Bercik *et al*., FMT was carried out between two strains of mice: NIH Swiss mice strain which exhibited an exploratory behaviour, and BALB/c mice strain which exhibited a cautious and anxious behaviour [82]. The ex-germ-free BALB/c mice colonized with microbiome from NIH Swiss mice exhibited an increased exploratory behaviour, whereas the colonization of germ-free NIH Swiss mice with microbiome from BALB/c mice resulted in increased anxious behaviour. Such adaptive transfer of phenotypic behaviour was also observed in cross-species FMT conducted by Kelly *et al.* [83]. The study reported an adaptive transfer of depressive phenotype in mice that received FMT from patients who were depressed. These mice also exhibited increased anxiety-like behaviours. These studies successfully employed pre-clinical animal models to understand the gut microbiome in psychotic disorders such as anxiety and depression.

Despite the wide use of pre-clinical models to draw associations between psychotic disorders and the gut microbiome, clinical findings in humans have showed that there is indeed microbiome differences associated with anxiety and depression [84]. Cowan and her team reported that individuals with depressive disorders had lower abundances of specific microbes such as Bacteroidetes, *Prevotellaceae, Faecalibacterium,* and a higher abundance of Actinobacteria, when compared to control individuals. On the other hand, individuals with generalized anxiety disorder (GAD) reported higher abundances of *Enterobacteriaceae*, and lower abundances of Firmicutes and *Ruminococcaceae*, when compared to control individuals. Although the differences in taxa were not significant, these taxa may be associated with anxiety and depression by the production of mediators that act through the gut-brain axis. It has been widely hypothesised that inflammation could contribute to the pathogenesis of depression and anxiety [85]. The changes in gut microbiome found in individuals with depression and anxiety could contribute to increased levels of pro-inflammatory mediators that may result in peripheral inflammation via signaling across the blood-brain barrier. The inflammatory state of the GI tract may be further exacerbated by the loss of SCFA-producing species such as *Faecalibacterium* which produces butyrate that has anti-inflammatory properties, and increased levels of microbial species with inflammatory potential such as *Enterobacteriaceae*. These data suggest that individuals with anxiety and depression have changes in the gut microbiome that support sustained levels of peripheral inflammation, which eventually lead to the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in higher levels of stress hormones such as cortisol. However, these findings are often confounded by several important factors such as diet, psychiatric medication, and clinical stratification criteria. Therefore, further research should consider these exogenous factors that could deepen our understanding of the gut microbiome in relation to anxiety and depression pathophysiology.

Evidence of Treatments

In recent years, the use of probiotics as a treatment for psychotic disorders such as anxiety and depression has been gaining traction [86]. Probiotics may affect the health and mood of the host, mainly by regulating the gut-brain axis. It has been proposed that probiotics which elicit a health benefit for individuals suffering from psychotic disorders, be called psychobiotics [87]. Cowan described research into the use of psychobiotics to treat anxiety and depression. A study showed that the effects of psychobiotics

significantly improved the mood in individuals with depression, evidenced by better depression scores in the psychobiotic treated group when compared to placebo treated group [86]. A recent meta-analysis review exploring the effects of psychobiotics on anxiety and depression from 34 controlled clinical trials revealed that psychobiotics elicited a small but significant benefit for individuals with anxiety and depression [88]. These findings support the modest effectiveness of psychobiotics for depression, however, she emphasized that these findings should be regarded as preliminary and further studies are warranted to understand the potential efficacy of psychobiotics for anxiety and depression.

Apart from probiotics, diet plays an important role in modulating the health and mood of the host through the gut-brain axis. It has been suggested that a poor-quality diet may lead to a dysbiotic gut microbiome in subjects suffering psychotic disorders [89]. There is increasing evidence that poor diet is a risk factor for psychotic disorders. The popularization of a 'westernized' diet, characterized by high intakes of fat, sugar, and low fiber exposure, coupled with a sedentary lifestyle may result in a dysbiotic gut microbiome [90]. Loss of certain bacterial members such as *Bifidobacterium*, *Prevotella*, and *Bacteroides* may promote an inflammatory state of the GI tract, contributing to increased levels of anxiety, depression, and cognitive impairments. A meta-analysis showed that a healthy diet consisting of high intakes of fruit, vegetables, whole grains, and fish was associated with reduced risk of depression in adulthood [91]. In addition, adherence to a Mediterranean diet was also associated with 30% reduced risk for depression. These findings suggest that dietary intervention could confer protective effects against psychotic disorders, potentially treating anxiety and depression. A 'Supporting the Modification of lifestyle In Lowered Emotional States' (SMILES) study exploring the efficacy of dietary treatment for depression demonstrated that adherence to a modified Mediterranean diet for 12 weeks significantly improved depressive symptoms, when compared to the control group without dietary intervention but with social support [92]. These individuals also reported significant improvements in their depressive and anxiety symptoms, and on the Clinical Global Impressions Improvement scale. This study supports the hypothesis that improving one's diet may treat depressive symptoms, calling for dietary intervention as an efficacious and accessible strategy to treat psychotic disorders. Although these treatments of probiotics and diet for psychotic disorders are promising, the findings are preliminary and more clinical studies are warranted.

Transgenerational Impacts on Cognitive Behaviour

Anthony Hannan discussed the growing body of evidence that the transgenerational impact of phenotypic traits is increasingly associated with psychiatric disorders such as anxiety and depression. These disorders could be partly inherited due to changes in regulation of epigenetic marks, including DNA methylation. A recent review conducted by Yeshurun *et al.,* explored the evidence of paternal transgenerational epigenetic inheritance [93]. It has been suggested that parental environmental exposures may lead to changes in DNA methylation profiles, and these profiles are transferred to the offspring during spermatogenesis, resulting in a modified phenotype observed in the offspring. Furthermore, several loci that are associated with psychotic disorders have been identified to be sites of DNA methylation, potentially leading to transgenerational epigenetic inheritance of psychotic disorders. Parental stress and social isolation activated the HPA axis responsible for inducing changes in DNA methylation profile of the sperm, leading to an altered DNA methylation and elevated gene expression of stress hormones in the offspring. Furthermore, the offspring exhibited a dysregulated HPA axis. In addition to a dysregulated HPA axis, cognitive abilities of the offspring were affected. Adult male mice that were exposed to unpredictable maternal separation combined with unpredictable maternal stress (MSUS) had offspring that demonstrated impaired memory performance and synaptic plasticity [94].

This data suggests the transgenerational effects of parental environmental exposures to the offspring cognitive behaviour, possibly explaining the epigenetic mechanisms contributing to psychiatric disorders such as anxiety and depression. Following up on these findings, the transgenerational effects of paternal diet were observed in a study conducted by Bodden *et al*. [95]. Male mice were fed a "westernized" diet characterized by a high intake of fats and sugars, and the behaviour and physiology of the offspring were assessed. In comparison with the offspring from male mice that were fed with normal chow, the offspring from adult male mice fed with "westernized" diet showed a significant increase in abundance of actinobacteria in the gut microbiome. Such changes in the gut microbiome may ultimately impact the cognitive behaviour of offspring phenotype. The increase in relative abundance of actinobacteria has been associated with psychoactive effects, resulting in depressive-like behaviours, when compared to healthy control individuals [96, 97]. These shifts in paternal diet and environmental exposures have been shown to influence the offspring cognitive abilities and behaviours, and such mechanisms could explain the inheritance and prevalence of psychotic disorders in offspring.

Future of the Gut Microbiome and Health

In the following sections, Emad M El-Omar discussed the future of the gut microbiome. In particular, explore the relevancy of the gut microbiome in early life, the confounders of the gut microbiome, the influence of medications on the gut microbiome, and the modulation of the gut microbiome to impact health. The future of medicine and what it means for the field of gut microbiome are also discussed.

Relevancy of Gut Microbiome in Early Life

El-Omar discussed the relevance of the gut microbiome in early life as it has been influenced by the traditional view that the prenatal environment was considered to be sterile [98]. Over the last few years, this view has been challenged by next-generation sequencing studies suggesting the presence of microbial signatures in the prenatal environment. In a recent study, analysis of human fetal tissues in the second trimester of gestation has identified live

bacteria that are capable of priming immune cells, thus promoting development of the fetal immune system [99]. Although this data further challenges the dogma of a sterile womb, several scientists have remained skeptical and attributed the presence of live bacterial strains at prenatal sites to be contamination and inconsistencies in studying early microbiome assembly at those sites.

The future of human health and its relevance to the gut microbiome depends on the ability of humans to produce healthy progenies of the next generation, and pregnancy is that process that provides an end to that means. El-Omar questioned if there could be evidence that the microbiome might be dysbiotic during pregnancy, and that outcomes be predicted and thereby prevented to ensure healthy progenies? He shared that there has been an increasing number of publications exploring the gut microbiome of pregnant mothers. In the study conducted by Torres *et al.,* the gut microbiome of mothers with IBD was analyzed [100]. The study demonstrated that pregnant mothers with IBD have a dysbiotic gut microbiome, with an increase in Gammaproteobacteria and decrease in Bacteroidetes. The dysbiotic gut environment persisted throughout the pregnancy, and infants born to the mothers with IBD showed a similar dysbiotic microbiome profile.

The composition of an infant gut microbiome showed a reduction in bacterial diversity that persisted during the first three months of development. Moreover, an altered gut microbiome transplanted from a pregnant mother with IBD into germ-free mice resulted in an impaired immune system in these ex-germ-free mice, suggesting that microbes and the associated metabolites could be essential in immune system priming and development. These findings indicate that maternal IBD is the main predictor of the gut microbiome and the infant gut microbiome, and this proved to be crucial as early intervention of dysbiosis in pregnant mothers could foster proper development of healthy infant gut microbiome and immune system in infants, thus mitigating the risk of IBD in these infants. Another study of mothers with pre-eclampsia (PE), a metabolic disease which can develop during pregnancy, demonstrated dysbiosis in the gut microbiome [101]. There was an increased abundance of several opportunistic pathogens including *Fusobacterium* and *Veillonella*, while the abundance of beneficial bacteria such as *Faecalibacterium* was significantly decreased. Furthermore, the FMT from patients with PE administered to germ-free mice induced PE phenotype and a dysregulated immune system, suggesting that a dysbiotic gut microbiome contributes to PE pathogenesis. Similarly, the gut microbiota of mothers during early pregnancy has been associated with risk of gestational diabetes mellitus (GDM) [102]. Several opportunistic pathogens and beneficial bacteria were found to be correlated with the risk of development of GDM. These data suggest the possibility of uncovering the microbial signature that predicts adverse outcomes such as PE and this may provide a new approach for pre-emptive measures for treating the gut microbiome to prevent these diseases.

Confounders of the Gut Microbiome

Omar pointed out that studies of the gut microbiome and its role in host health and diseases focused on identifying the gut microbial contributors to such diseases are often confounded by interindividual variability in the gut microbiota. These variations may obscure the differences between individuals with disease and healthy individuals in cross-sectional study designs. A recent study conducted by Vujkovic-Cvijin *et al.,* aimed to resolve these confounders that hinder the understanding of the gut microbiome across several studies [103]. Using machine learning strategies, lifestyle and physiological factors were identified to be sources of heterogeneity in the gut microbiome that confound most analysis. Interestingly, the frequency of alcohol consumption and bowel movement quality were shown to have a major impact on outcomes, indicating significant differences between the gut microbiome of diseased individuals and healthy individuals. This study suggests that such factors should be considered and accounted for when analyzing the gut microbiota across cross-sectional studies in order to better resolve the true microbial contributors to human diseases. In a separate study conducted by Kurilshikov *et al.,* it has been observed that cross-replication across studies investigating the effects of host genetics on the gut microbiome composition using genome-wide association studies (GWAS) were limited [104]. The low frequency could be attributed to differences in methodologies employed, and that these studies were underpowered and under-represented. A large molecular biology consortium, MiBioGen, investigated the effects of host genetics on the gut microbiome composition. Large variability in the gut microbiome composition was identified across 18,000 individuals and GWAS of host genetic variation identified 31 unique loci that affects gut microbiome composition that was deemed significant at genome-wide level. In particular, a lactase gene locus was found to significant across the be study, demonstrating an association between age and abundance of *Bifidobacterium*. Moreover, a phenome-wide association study identified associations between genetic variants affecting gut microbiome composition and several host characteristics such as psychiatric, immunological and metabolic traits. Mendelian randomization employed in this study also identified that *Bifidobacterium* may have protective effects in ulcerative colitis, indicating casual links between gut microbiome composition and complex disease traits. This finding further supports the potential of microbiome-targeting treatments.

Influence of Medications on the Gut Microbiome

Medications are pharmaceutical agents that elicit beneficial and undesirable effects on human health. It has been suggested that the gut microbiome will be altered by the use of medications. In a systematic drug screen against a panel of 40 human gut microbes, 24% of non-antibiotics had a direct killing effect on some of the components of the microbiota, suggesting that non-antibiotic medications can be harmful to the commensal bacteria [105]. Interestingly, anti-psychotics treatments were found to have the greatest killing effect. These findings highlight the need to elucidate drug-microbiota interactions, and to aid future drug development, including mitigating off-target effects, improving drug efficacy, and modulating gut microbiome, before translational application of

these drugs. A complex bi-directional interaction exists between the gut microbiome and drugs [106]. Pharmaceutical agents may influence the gut microbiota composition and colonization, leading to increased infections, but at the same time, the gut microbiota possesses enzymes capable of transforming drugs, affecting its efficacy, bioavailability and toxicity. Furthermore, it is important to note that non-antibiotic medications could also alter the microbiota, leading to diseases and illnesses. Maier and co-workers explored the use of antibiotics and their collateral damage on the gut microbiome [107]. These workers showed that with time antibiotics can develop broad-spectrum activities that alter the gut microbiota, and may cause unintended gastrointestinal side effects. Hence, it is of great interest to elucidate the collateral damage of medications on the gut microbiome. The study conducted by Maier *et al.,* characterised 144 antibiotics against a panel of 38 human gut microbes, providing a useful resource of the impact of antibiotics on commensal bacteria. The study also suggests strategies to circumvent the adverse effects of such antibiotics on the gut microbiota. These studies highlight new avenues for precision medicine to allow specific selection of medication for the host.

An important class of pharmaceutical drugs that is of great interest are the gastric acid inhibitors. Proton pump inhibitors (PPIs) are drugs used to reduce gastric acids. Although it is conceivable that gastric acid aids digestion, absorption of Fe, Ca ions, vitamin B12, and protects against enteric infections, the fundamental role of gastric acid remains the first line of defense against ingested microbes. However, 10 to 20% of humans have lost the protective effects conferred by gastric acid due to the use of PPIs. Hence, the unphysiological state of the "acid-free" stomach may further induce hallmarks of a dysbiotic gut microbiome, and ultimately lead to disease. The use of PPIs and its association with diseases is further corroborated by a recent study conducted by Abrahami *et al.* [108]. These authors found that first-time users of PPIs are at a 45% increase risk of gastric cancer, compared to first-time users of histamine-e receptor antagonist (H2RA), with a number needed to harm of 2121 for 5 years and 1191 for 10 years. Globally, this accounts for a considerable amount of people in the world. Similarly, the prolonged use of PPIs has also been associated with an increased risk of colorectal cancer, with a number to harm of 5343 for 5 years and 792 for 10 years [109]. In another study, regular use of PPIs was associated with a 24% increased risk of diabetes, and the risk increased with prolonged usage [110]. The association between PPIs use and adverse outcomes such as type 2 diabetes, gastric cancer, and colorectal cancer could be mediated by several biological factors, including an altered microbiome. Previous studies have shown that PPIs use significantly reduced diversity and promoted oralisation of the gut microbiome [111]. Furthermore, the PPI-associated increase in abundance of *Lactobacillus*, and the decrease in *Bifidobacterium* have been associated with type 2 diabetes. Other PPI-associated changes in the abundance of bacterial taxa have been reported, and were associated with an increased susceptibility to a myriad of diseases including *C. difficile* infection (CDI). Overall, the findings from these studies highlight the pressing need for physicians to reassess the necessity of PPIs, so that such medications will be prescribed appropriately. Essentially, the prescription medications that humans use can impact on the gut microbiome, and this in turn has profound effects on efficacy and potential side effects.

Modulation of the Gut Microbiome to Impact Human Health

Many strategies could be employed to modulate the gut microbiome to impact health and prevent disease, such as diet, exercise, prebiotics, probiotics, synbiotics, post-biotics, antibiotics, and FMT. The following section discusses clinical studies exploring diet, FMT, and probiotics as strategies to modulate the gut microbiome. The NU-AGE dietary intervention study conducted by Gosh TS *et al.,* studied the effects of a Mediterranean diet (MedDiet) administered to elderly for 1 year across five European countries [112]. The data indicates that adherence to a Mediterranean diet altered the dynamics of the gut microbiome, increasing abundance of specific bacterial taxa known to be positively associated with improved cognition, reduced risk of frailty and inflammation. Predictive metabolite profiling further substantiates the positive impacts of MedDiet on host health as adherence to MedDiet enriched SCFAs-producing bacterial taxa, and diminished bile acid dysregulation associated bacterial taxa. In a separate prospective study investigating the effects of high-fiber or fermented food diet on the human gut microbiome, it was shown that these dietary interventions resulted in altered gut microbiome profiles [113]. The high-fiber diet increased the relative abundance of microbial proteins detected in stool, suggesting that a high-fiber diet increases microbial density and numbers within the gut microbiome. In contrast, the fermented food diet increased alpha diversity of the gut microbiome, attributed to gut ecosystem remodelling rather than consumed microbes present in the fermented food. This data suggests that fermented food has an indirect effect on the gut microbiome. Collectively, these clinical studies highlight the microbiome-host relationship and the responsiveness of the microbiome to dietary intervention, further supporting the use of dietary intervention to improve human health.

The use of FMT has been demonstrated to be an effective therapeutic treatment for diseases associated with an altered gut microbiome such as CDI, IBD, and metabolic diseases. FMT involves administering a donor stool preparation into the GI tract of the patients, most commonly through the ingestion of pills filled with the faecal preparation. Other routes of administration include endoscopy, nasogastric or nasointestinal tubes, and colonoscopy. These administration routes have been shown to be effective but with varying degrees of comfort for the patients, and the choice of administration depends on the clinical situation. Several studies have elucidated the mechanisms of action of FMT in recurrent CDI, and it has been that patients with recurrent CDI have a dysbiotic gut microbiome. Furthermore, antibiotic use represents a major risk factor for CDI as antibiotic treatment alters the gut environment, increasing primary bile acids, sugar alcohols, and amino acids, creating a gut environment that favours *C. difficile* proliferation.

Bacterial phyla Bacteroidetes and Firmicutes are reported to be deficient in these patients, as compared to healthy individuals [114]. These phyla are important in maintaining gut homeostasis, and are hypothesised to inhibit *C. difficile* proliferation. The administration of FMT has been associated with shifts in microbial community structure, restoring key phyla Bacteroidetes and Firmicutes and decreasing Proteobacteria. These changes are accompanied with an increase in secondary bile acid production, significantly affecting the proliferation of *C. difficile*. In a clinical study exploring the therapeutic potential of FMT in patients with active ulcerative colitis (UC), there was strong statistical evidence that FMT induce remission in UC when compared to patients who received a placebo (nine patients who received FMT and two who received placebo were in remission after 7 weeks) [115]. Interestingly, a clinical study conducted by Rossen *et al.,* reported that FMT did not significantly induce remission in patients with UC as compared to patients who received placebo [116]. However, two recent trials employing a short duration of FMT therapy have demonstrated significant benefits in patients with active UC [117,118]. Although remission of UC was observed in these two studies, these benefits dissipated over time, ultimately reducing the efficacy of the FMT in UC. The reduced efficacy of FMT in UC could be attributed to limited colonization of the donor bacterial populations in the gut environment of the patients and the particular donor material utilised. Therefore, further clinical studies are warranted to assess the long-term benefits and remission of FMT therapy. The conflicting clinical findings of FMT in patients with UC could be explained by differences between donors, and this led to the super-donor concept. A recent study conducted by Magdy El-Salhy *et al.,* redefined the term super-donor as an individual with a normobiotic gut microbiome, in which the deviation of microbial signatures does not cause dysbiosis, and showed a positive microbial signature [119]. Faecal material obtained from a super-donor and used as FMT in patients with IBS over 3 months resulted in an improved efficacy of the treatment, as significant improvements in fatigue and quality of life were reported when compared to patients who received placebo. Furthermore, the gut microbiome profile of these patients changed significantly, and the changes were positively associated with the clinical improvements.

FMT-based intervention could also be applied to brain health and neuroimmunity in aging. Studies have elucidated the mechanistic underpinnings of the gut microbiota during aging and FMT-based intervention has been proposed to be a therapeutic strategy to target the gut microbiome to achieve healthy aging. In a recent study investigating the use of FMT for treating aging-associated cognitive, neural, and immune impairments, FMT was transferred from young mice into aged mice [120]. Alterations in the gut microbiome, immunity, metabolomics, and behaviour were analysed before and after FMT. There were significant differences in gut microbiome diversity between young and aged mice before FMT, but differences were not significant following FMT, indicating the restoration of the gut microbiome. Furthermore, FMT reduced propionate synthesis III and acetate degradation. Modulation of the peripheral and hippocampal immunity was observed in aged mice that received FMT. Early activated CD8+ T cells, CD103+ dendritic cells, and aging-associated CD11b expression on Ly6+ neutrophils were reduced in these aged mice, while reversing the aging-associated increase in peripheral IL-10. Aged mice that received FMT also reported significant improvements in hippocampal metabolomes, as evidenced by the restoration of retinol, GABA, and N-glycolylneuraminate metabolites. These metabolites are known to protect against aging-associated cognitive decline and impairment, and neuroinflammation in the aged mouse brain. FMT also rescued aging-associated, hippocampal-dependent behavioural abnormalities in these aged mice. These mice showed improvements in the Morris water maze probe trials, in areas of mean distance from platform, average velocity, and path length. These improvements indicate the attenuation of aging-associated cognitive decline and behavioural abnormalities, suggesting that FMT drove restorations in hippocampus-related functions.

The Microbiome and the Future of Medicine

Advancement in technology over the years enabled scientists to study the gut microbiome with greater resolution, employing advanced molecular profiling techniques such as 16s rRNA sequencing, and shotgun sequencing [121]. These tools have contributed to the expanding knowledge of the gut microbiome in both healthy and diseased states, supporting the role of the gut microbiome in human health and disease prevention. However, El-Omar emphasized that questions need to be asked as to what constitutes a "healthy" microbiota, and should the universal definition be based on composition alone? The gut microbiome is unique in each individual, and the different phyla, family, genus, or strain may respond differently to the same strategy across individuals [122]. Therefore, it has been postulated that the gut microbiome structure and function could be utilised when developing personalized medicine. In order to identify the right treatment for a patient, subtyping of patients based on host genetics factors, and many clinical characteristics are often conducted. This results in large amount of data sets, and hence the clinical relevance could be underestimated due the lack of analysis tools to interpret them. The rise of artificial intelligence (AI) and machine learning (ML) tools allow complex data sets to be fed through neural networks, to be interpreted, and predicted in various ways as dictated by specific algorithms [123]. Powerful ML tools have predicted molecular mechanisms in *Pseudomonas* and risk of sepsis based on pathological markers [121]. Moreover, these ML algorithms have been used to develop an *in-silico* trial pipeline for assessing the efficacy of *C. difficile* treatments. It is apparent that the use of AI and ML will contribute to a better understand of clinical relevance and significance of the microbiome data. Ultimately, these tools have the potential to assist in clinical outcomes, as well as to allow the development of tools for personalized medicine.

Conclusion

The ILSI SEA region conference series highlighted several key aspects of the gut microbiome, providing scientific evidences from recent scientific studies. It was shown that the infant gut microbiome

is highly malleable and the structure of the gut microbiome is greatly dependent on the diet consumed, as evidenced by the differences observed in gut microbiome composition in children from rural Africa and Europe. Furthermore, the role of infant diet and in particular, human breast milk, has been recognized to support the development of the infant immune system, drawing inspiration for formula milk recipes to incorporate bioactives found in human breast milk. The gut microbiome and its role in human health were also discussed. Dysbiosis of the gut microbiome has been implicated in various disease states, and the consumption of a Westernized diet was associated with the pathogenesis of these diseases through the gut microbiome. Dietary approaches such as a low FODMAP diet have been suggested as a therapeutic strategy to treat the dysbiosis. Although a low FODMAP diet reduced symptoms of IBS, it also led to a reduction in total abundance of gut microbes. This finding sparked several meaningful insights, and changed the way we perceive dietary intervention strategies as they could exacerbate the dysbiosis state of the gut microbiome. Moreover, this finding illustrates the need to identify unique microbial signatures so that responses to diet could be predicted on an individual level, paving the way for personalized treatments.

The effects of diet on the gut-immune axis were also discussed. Dietary fiber was shown to increase plasma acetate levels, and promotes B10 cell differentiation which is important for immune tolerance and homeostasis. Likewise, the gut microbiome in relation to metabolic diseases, and how diet can be used to treat and prevent such diseases were explored. The consumption of resistant starches was shown to enrich the *Prevotella* enterotype, known to reduce susceptibility towards metabolic diseases such as T2D. The use of probiotics has also been widely recognized as potential treatment for metabolic diseases, conferring protection through various mechanisms. Additionally, the gut microbiome is implicated in cognitive and mental health disorders such as HD, schizophrenia, and anxiety and depression. The use of dietary interventions has shown promising efficacy in reducing symptoms, paving the way for an efficacious and affordable strategy to treat psychiatric disorders. Interestingly, the prevalence of psychiatric disorders in offspring could be associated with the transgenerational impacts of paternal diet, possibly explaining the epigenetic mechanisms contributing to psychiatric disorders such as anxiety and depression. Lastly, the future of the gut microbiome in human health and medicine was discussed. The relevancy and importance of the gut microbiome in early life underscored the need for analytical tools capable of identifying microbial signatures that could predict adverse events during pregnancy. Advancement in technology over recent years has led to the innovative use of AI and ML to analyse gut microbiome data with precision and efficiency, and this appears to be increasingly relevant in assisting clinical outcomes, as well as to generate tools for personalized medicine.

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