

GUT-BRAIN-AXIS AND THE MICROBIOME

G.M. Moloney¹, G. Clarke^{2,3}, J.F. Cryan^{1,3}

¹Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

²Department of Psychiatry and Neurobehavioural Science, University College Cork, Cork, Ireland

³APC Microbiome Ireland, University College Cork, Cork, Ireland

Corresponding Author: John F. Cryan, MD; email: j.cryan@ucc.ie

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The gut microbiome consists of a vast array of bacteria, viruses and fungi that are beneficial or symbiotic, and a smaller amount that are pathogenic. With the advance of sequencing technologies and the expansion in knowledge about function, the gut microbiome is implicated in every facet of life, including the development and maintenance of brain function and behaviour. The gut is also home to a reservoir of precursors and metabolites, some of microbial origin and others that are sourced directly from the food we eat^{1,2}. In the last year, several papers^{1,2} were published that identify the gut microbiome as a tractable target for positive intervention in many disorders of the brain across the lifespan.

Early-life is a crucial timepoint in the development of the microbiome and delay to the initial colonisation of the gut can have profound effects later in life. This was clearly demonstrated by Seki et al³, who showed that extremely premature infants, born on the verge of the third trimester, were more likely to have profound brain damage if they had an overgrowth of *Klebsiella* in their gut. Overgrowth of *Klebsiella* was associated with an expansion of $\gamma\delta$ T-cells influenced by the cytokine IL17A³. This paper underscores the sensitivity of the microbiota-gut-brain axis to atypical signals along its axis and should lead to further work targeting the overgrowth of *Klebsiella* and its connection with the brain⁴. At the other extreme, the aged are predicted to form 65% of the world's population by 2050, providing an enormous challenge for healthcare. Along with decline of immune function, the composition and function of the microbiome also regresses with normal aging, with a decline in cognition also. Cognitive decline may be reversible through manipulation of the gut microbiome. Utilising faecal microbiota transplantation (FMT) from young mice into old mice, Boehme et al⁵ were able to reverse the increase of CD8⁺ T-cells and CD103⁺ dendritic cells in aging along with a restoration of hippocampal metabolites that are reduced in aged animals⁵. FMT from young to old mice reversed behavioural deficits common to aged mice such as long-term memory. This has now been reproduced by two other research groups^{6,7}. Future studies will examine in greater detail the specific bacterial species responsible for these changes, and the mechanisms responsible. The importance of this data is echoed in a paper published in *Nature Metabolism*⁸ showing the importance of blood metabolites in healthy aging associated with an ever developing microbiome thought to be a hallmark of healthy aging offering hope that the microbial metabolites may offer a pathway to reversing the detrimental effects of aging.

Depression is a significant public health concern and the leading cause of disability globally. Rates of depression in men increase as levels of testosterone decrease due to advancing age⁹. A large body of preclinical and clinical data³⁻⁶ supports the hypothesis that the gut microbiome can influence the trajectory of depression. This suggests that, by modifying the microbiome, we may be



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able to treat depression by modifying the disease-causing agent(s) in our gut. Li et al¹⁰ were able to isolate *Mycobacterium neoaurum* from the faeces of patients with depression as a result of testosterone deficiency¹⁰. They showed that this strain could degrade testosterone *in-vitro* and in rodents, they found that gavaging this strain reduced peripheral and brain levels of testosterone along with inducing behaviours characteristic of depression. Further work on this strain needs to validate its role in other forms of depression and confirm that its effects are not restricted to phenotypes of depression with low testosterone.

The brain-gut-microbiome axis is heavily investigated with regard to autism spectrum disorder (ASD) with a subset of people with ASD displaying gastrointestinal symptoms and, a large collection of studies^{11,12} examining the composition of the gut microbiome and metabolome in adult and paediatric populations. Studies^{7,8} have focussed on the role of diet in the cause and treatment of ASD and a paper from Australia⁶ examined the role of diet in ASD focussing on some of the shortcomings of previous papers in the field, such as low N numbers, a failure to adjust analysis for confounders. Using a cohort of 247 children, 99 of which were diagnosed with ASD, 51 paired siblings and 97 unrelated children they found little evidence of an association between the microbiome and an autism diagnosis¹³. Behaviours associated with autism, such as sensory preferences and repetitive behaviours were associated with a less diverse diet. While this paper suggests that the microbiome is not directly linked with the developmental origins of ASD, future work should identify the role of the microbiome if any in the expression of specific symptoms of autism or in the ontogeny of the picky eating behaviour in the first place¹⁴.

Continuing with the theme of translational research, two papers from the same lab illuminated a path from bench to bedside. Gene X environment interactions are thought to influence some ASD phenotypes and no approved treatments for any of the symptoms associated with ASD, such as restricted repetitive behaviours and reduced social interest exist. Hsiao et al⁹ in 2013 showed that metabolites produced and regulated by the gut microbiota were able to traverse the blood-brain-barrier (BBB). One specific microbial metabolite, 4-ethylphenyl sulfate (4EPS), was increased in a mouse model of atypical neurodevelopment and more recently¹⁵, increases in 4EPS were reported in the plasma of ASD individuals¹⁶.

Subsequently, using a mouse model of ASD (CNTNAP2) that again shows increased plasma levels of 4EPS, the authors examine the biochemical pathways responsible for 4EPS production and find that this metabolite is non-existent in germ-free animals providing strong evidence that the gut microbiota is responsible for the presence and function of this key metabolite. Furthermore, in the brain, they also show that 4EPS influences how oligodendrocytes interact with neurons, the overall oligodendrocyte number and reduces the quantity and thickness of myelination on the surface of oligodendrocytes in the corpus callosum. Interestingly, the authors also demonstrate that 4EPS mediates changes in emotional behaviours such as anxiety, using several behavioural paradigms. Using AB-2004, an orally administered GI-restricted adsorbent that mops up 4EPS and other phenolic metabolites in the gut, the authors demonstrate reduced anxiety-like behaviours in the marble burying test, open-field and elevated plus maze tests of anxiety in CNTNAP2 mice¹⁵ providing convincing evidence that AB-2004 may be a viable intervention in ASD.

In parallel, the authors performed a Phase I intervention in humans diagnosed with ASD with AB-2004 and demonstrated its safety, and tolerability when given orally. They showed increased levels of 4EPS, and following an 8-week treatment regime with AB-2004 the authors show a reduction in the levels of 4EPS in the plasma and urine of study participants with improvement in GI symptoms and ASD associated behaviours. This represents the first-in-class treatment for the core symptoms of ASD and provides further proof of the importance of the gut-brain-microbiome axis in ASD. It is likely that AB-2004 will only prove a feasible treatment in a subset of people with ASD given the diverse spectrum of symptoms in this population. Both papers in tandem provide an excellent example of how pre-clinical data can influence the development of viable interventions that target the microbiome¹⁷.

While dietary preferences may influence symptom development in many conditions, undernutrition, and lack of access to food are still prevalent. Chen et al¹⁰ published a randomized controlled feeding study on undernourished Bangladeshi children using a microbiota directed food (MDCF-2). This paper showed that MDCF-2 induced plasma proteins associated with neurodevelopment and bone growth alongside a restoration of the gut micro-

biome. Importantly, several measures of weight-for-age also improved following dietary intervention supporting the concept that microbiota targeted interventions can prevent malnutrition¹⁸. Though the sugar content differed between the diets tested, the caloric density was similar, and future studies will need to fully interrogate the precise mechanism behind these effects. Barratt et al¹⁹ found that the abundance of *Bifidobacterium longum* subspecies *infantis* in children with severe acute malnutrition was almost non-existent compared to the levels seen in healthy age-matched controls. Using the *B. infantis* strain EVC001 in a single-blind placebo-controlled trial, they noted increased weight gain, and reduced markers of gut inflammation in these infants¹⁹. These results provide an important validation of the idea that the microbiome is modifiable through dietary interventions, or supplementation with specific beneficial strains such as *B. infantis*. It will be interesting to see in future experimental characterisations of these interventions if they can influence verbal and spatial cognitive processes that can be affected by malnutrition. This paper proves a decisive link between the gut microbiome and diet, and how modifying the microbiome can have profound effects on society.

Understanding how nutrition influences brain structure and development in early life is crucial and represents an area that will provide key microbiome mediated therapies. The composition of the infant gut microbiota is formed postnatally and is influenced by maternal antibiotic usage, mode of delivery and feeding patterns. Challenges to the infant in this critical period have serious consequences for neurodevelopment. However, there is still much to learn about how our diet influences the composition of our microbiota. We now understand that there is a co-operative relationship between us and our gut microbes. We depend on these microbes to digest complex plant fibres, and similarly, they depend on our food intake for their survival. What was unclear though, was, can our diet result in genetic modifications that can be detected in the life cycle of these microbes and if so, can they influence health outcomes in the host. Mapping the human gut commensal *Bacteroides thetaiotaomicron* over a 3-month period they show that host dietary intake does in fact result in genetic adaptations in a member of the gut microbiome²⁰. Employing 3 different dietary regimes; a low-fat high fibre diet (Standard), a high-fat low-fibre diet (Western) and a diet that alternates between the 2, they demonstrate that *thetaiotaomicron* adapts rapidly to the host environment following gavage. Indeed, they found 72 adaptations in total, 2 of which confer a growth advantage to *thetaiotaomicron* allowing it to subsist in the mucosal lining. The consequence of these adaptations for humans are yet to be discovered but are unlikely to be beneficial. Furthermore, this paper shows us that new microbial adaptations are a strong marker of past host diet.

Previously, we discussed oscillations in our feeding, and how they affect the oscillations of the microbiome. There is a growing relationship between the microbiota-gut-brain axis and circadian rhythms too²¹. Hooper et al¹¹ have shown that the microbiota and the circadian cycle coordinate daily to influence the expression of several genes associated with the innate immune system as well as antimicrobial proteins (AMP), such as *Reg3g*. The circadian fluctuation of these AMP's is guided by the attachment of short filamentous bacteria (SFB) to the epithelial cell layer in the intestine which in turn was controlled by the central circadian clock²². These results provide an indication of how much the microbiome, and how it fluctuates, can interact with other physiological systems to control innate immunity and other systems yet to be discovered. It will be interesting to see if these mechanisms operate in a similar manner in humans. This may offer therapeutic strategies that target the microbiome and alleviate some of the many detrimental impacts to the health and wellbeing of long-term night and shift workers.

Increasingly the virome, specifically bacteriophage, as part of the gut microbiome are being recognised as important regulators of host physiology and bacterial phenotypes. In 2022, a fascinating paper examined the interplay between the gut virome, gut bacteriome and executive function in 4 human cohorts²³. Examining the host interaction with 2 separate domains of life in flies' mice and humans, they link the ratio of the bacteriophage Caudovirales/Microviridae with host cognition through experiments that link host and bacterial metabolism with the presence or absence of these phage. This paper will represent the start of a new chapter in the gut-brain-microbiome axis story, with a clearer understanding of the phage that live within the bacteria that live within us and their potential role as a microbiome targeted therapy.

In 2014, our lab established that the microbiota was required for social development in the mouse²⁴. This finding was replicated in zebrafish by the Eisen lab²⁵. They showed that a subset of neuronal projections from the vTely²³¹ nucleus are modified by the microbiome in early life and that this phenomenon is required for adult social behaviour. Microglia are intrinsically linked with the gut microbiota, with the microbiome being necessary for the appropriate quantity of microglial infiltration into this brain region, and while there, these microglia are important for the modification of vTely²³¹ neurites. These microglia have another role in this region, while in this nucleus, they promote the expression of the complement protein C1qa, a gene with a role in both innate and adaptive immune processes and in the context of this paper neuronal complexity. This paper represents an increasingly common theme in microbiome-brain-gut research, and that is the holistic approach using an expanding range of model systems²⁶ to disentangle the mechanisms that govern the communication between the microbes in our gut, and our brain.

What we learn from the many species now studied in this field, including rodents, zebrafish, *Drosophila melanogaster*, and *Caenorhabditis elegans* can be transferable to human. Microbiome-brain-gut research must move towards understanding the fundamental mechanisms that govern this communication, and, how it can be modified, reversed, or enhanced²⁵. Alongside this quest for definitive mechanisms and enhanced treatments for many neurological disorders, researchers must harness the advances in technology that are on the horizon. Another important tool in the elucidation of essential mechanisms is CRISPR, given that recently, it was used to edit species, and sites on bacterial genomes in complex microbial systems²⁷ and also a study published from the Turnbaugh group using CRISPR edited phage M13 to deliver DNA to *Escherichia coli*, a technique that will likely be an important part of the important molecular toolkit for editing the microbiome²⁸.

Research in the microbiome-brain-gut axis must focus on translational research and explore key mechanisms. While compositional studies have provided important leads in the quest to find microbiome-based therapeutics, current and future studies should focus research across the lifespan harnessing new technologies and analytical methods incorporating host genetics²⁹.

Conflict of Interest

G.C. has received honoraria from Janssen, Probi, and Apsen as an invited speaker; is in receipt of research funding from Pharmavite and Fonterra; and is a paid consultant for Yakult, Zentiva, and Heel pharmaceuticals. J.F.C. has been an invited speaker at conferences organized by Mead Johnson, Alkermes, Janssen, Ordesa, and Yakult, and has received research funding from Mead Johnson, Cremo Nutricia, Pharmavite, Dupont, and 4D Pharma. This support neither influenced nor constrained the contents of this review. GM declares no conflicts.

Acknowledgements

None.

Informed Consent

None.

Authors' Contribution

GM, GC and JFC compiled and reviewed the manuscript prior to submission.

Funding

This paper was funded by Science Foundation Ireland [SFI/12/RC/2273_P2]; Government of Ireland Postdoctoral Fellowship [GOPID/2021/298].

ORCID ID

Gerard Moloney (0000-0002-3672-1390). Gerard Clark (0000-0001-9771-3979). John Cryan (0000-0001-5887-2723).

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