The microbiota-gut-brain axis – pathways to better brain health

Perspectives on what we know, what we need to investigate and how to put knowledge into practice

Chakrabarti, Anirikh[1]; Geurts, Lucie[2*]; Hoyles, Lesley[3]; Iozzo, Patricia [4]; Kraneveld, Aletta D.[5]; La Fata, Giorgio[6]; Miani, Michela[2]; Patterson, Elaine[7]; Pot, Bruno[8]; Shortt, Colette[9]; Vauzour, David[10].

1. Cargill R&D Centre Europe, Vilvoorde, Belgium. ORCID: 0000-0001-7804-209X.
2. International Life Sciences Institute, European Branch, Brussels, Belgium. ORCID: 0000-0003-2975-9590, 0000-0001-6392-2342.
3. Department of Biosciences, Nottingham Trent University, Nottingham, UK. ORCID: 0000-0002-6418-342X.
4. National Research Council (CNR), Institute of Clinical Physiology, Pisa, Italy. ORCID: 0000-0001-6443-7074.
5. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands. ORCID: 0000-0001-9819-383X.
6. DSM Nutritional Products Ltd., Kaiseraugst, Switzerland.
7. IFF Health & Biosciences, Kantvik, Finland. ORCID: 0000-0002-2679-2072.
8. Yakult Europe BV, Almere, The Netherlands. ORCID: 0000-0001-5995-6929.
9. Shortt, C. NICHE, Ulster University, Coleraine, Co Londonderry, NI. ORCID: 0000-0002-4845-7339.
10. Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK. ORCID: 0000-0001-5952-8756.

*Corresponding author: publications@ilsieurope.be.

Acknowledgements

The authors would like to acknowledge and thank Cath Mersh for her services in writing the manuscript based on the authors' input and comments. The contents of this paper were debated during a workshop organised on 5 December 2019 in Brussels by ILSI Europe. The authors wish to thank all workshop participants for their valuable input. The research question addressed in this publication and contributing experts were identified by the Nutrition & Brain Health, Prebiotics, Probiotics, Early-Life & Long-Term health, Health Benefits Assessment of Foods, and Nutrition & Immunity Task Forces. Members of these task forces are listed on the ILSI Europe website. The expert group then refined the research question and carried out the work of discussing and collecting/analysing data and information for this scientific paper, independent of other task force's activities. The research reported is the result of a scientific evaluation in line with ILSI Europe's framework to provide a precompetitive setting for public-private partnerships. ILSI Europe facilitated scientific meetings and coordinated the overall project management and administrative tasks relating to the completion of this work. For further information about ILSI Europe, please email info@ilsieurope.be or call +3227710014. The opinions expressed herein, and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies, nor any regulatory authority.

Abstract

The gut and brain link via various metabolic and signalling pathways, each with the potential to influence mental, brain and cognitive health. Over the past decade, the involvement of the gut microbiota in gut-brain communication has become the focus of increased scientific interest, establishing the microbiota-gut-brain axis as a field of research.

There is a growing number of association studies exploring the gut microbiota's possible role in memory, learning, anxiety, stress, neurodevelopmental and neurodegenerative disorders. Consequently, attention is now turning to how the microbiota can become the target of nutritional and therapeutic strategies for improved brain health and wellbeing.

However, while such strategies that target the gut microbiota to influence brain health and function are currently under development with varying levels of success, still very little is yet known about the triggers and mechanisms underlying the gut microbiota's apparent influence on cognitive or brain function and most evidence comes from pre-clinical studies rather than well controlled clinical trials/investigations. Filling the knowledge gaps requires establishing a standardised methodology for human studies, including strong guidance for specific focus areas of the microbiota-gut-brain axis, the need for more extensive biological sample analyses, and identification of relevant biomarkers. Other urgent requirements are new advanced models for *in vitro* and *in vivo* studies of relevant mechanisms, and a greater focus on omics technologies with supporting bioinformatics resources (training, tools) to efficiently translate study findings, as well as the identification of relevant targets in study populations.

The key to building a validated evidence base rely on increasing knowledge sharing and multi-disciplinary collaborations, along with continued public-private funding support. This will allow microbiota-gut-brain axis research to move to its next phase so we can identify realistic opportunities to modulate the microbiota for better brain health.

Keywords: microbiome, cognitive performance, nutrition, inflammation, ageing, mental health

1. Introduction: A field of growing scientific interest

The microbiota-gut-brain axis and the potential to support cognition and brain health

Does the gut hold the key to brain development and health? Through decades of research, scientists have established the strong connection between the gut and brain, modulated by neurons, neurotransmitters, hormones, and immune mediators (for details, we kindly direct readers towards extensive reviews [1-3]. More recently, focus has been extended to the role of the gut microbiota (referring to the trillions of microorganisms and viruses residing in the gut) [2, 4-6], creating considerable excitement with findings that suggest specific intestinal microorganisms (the greatest amount of information comes from studies of bacteria) may be associated with memory [7], learning [7], stress [8], and mood [6, 9, 10] – and even neurodevelopmental [11, 12] and neurodegenerative disorders [2].

Today, the so-called microbiota-gut-brain axis is an area of multidisciplinary research that has captured international attention. Scientists specialised in neurology, endocrinology, immunology, microbiology, and bioinformatics have all found a niche worthy of exploration. Interest is such that international journals publish as many as 30 new studies a day related to this field.

While there is now considerable evidence that the microbiota-gut-brain axis plays an important role in mental and cognitive health, human clinical studies have as yet provided few clear answers to one burning question. How?

How does the gut microbiota influence brain development [13] and function [14]? Are brain disorders potentially shaped by the gut microbiota [15]? What role does diet play and what is its scope in influencing the microbiota-gut-brain axis [16, 17]? How do dietary supplements exert their apparent effect(s) on stress, mood, and cognition [18, 19]? What physiological mechanisms are at play [20]? And do alterations in microbiota-gut-brain interactions through life reflect the cause or symptom of an underlying brain condition [21]? Answering these questions is critical to harnessing the intestinal microbiota as a tool for ameliorating or preventing brain disorders, determining potential links with metabolic and cardiovascular diseases and for developing nutritional and therapeutic strategies that support and strengthen the brain health of the individual.

This perspective paper offers a short introduction to the microbiota-gut-brain axis, the knowledge and research so far and the considerable remaining gaps in the understanding of causes and mechanisms. Finally, the paper proposes how future meaningful progress can be made, which should benefit researchers active in fundamental and clinical gut-brain research from a multi or transdisciplinary perspective (including doctors and possibly patients/care takers), professionals in the mental health care, as well as research funders, food industry and investors. Once the mechanisms of gut microbiota modulation of brain health are unravelled, the potential for improving human quality of life and wellbeing is vast.

2. The two-way street between gut and brain

An introduction to microbiota-gut-brain communication, research, and potential therapeutic strategies

A 'gut feeling' or the sensation of 'butterflies' in the stomach are common illustrations of how a response in the brain is felt in the gut. Beyond that, microbiota-gut-brain interactions are much more complex to describe – as is abundantly clear from the intense research efforts to document them and propose links with brain development, physiology, function, and health.

As a highly complex community, the gut microbiota has a myriad of functions including education of the immune system, protection against pathogens, energy homeostasis and metabolite production. It is acknowledged that diet is a key determinant of composition of gut microbial populations and that it impacts on gut transit time and gut environmental conditions, and critically determines the supply of substrates for microbial growth [22, 23]. The gut microbiota has the potential to be both a mediator of the effect of diet and an effect modifier of the metabolic response to diet. In the case of the microbiota acting as a mediator, the dietary intervention acts directly on the microbiota, modifying the microbiota's composition and function. In contrast, as an effect modifier, the effect of diet on metabolism depends on the microbiota but the effect is not due to diet-induced changes in the microbiota. Thus, the gut microbiota is modifiable by diet and specific dietary components, and it plays a key role in shaping the composition and activity of the microbiota from birth, which impacts lifelong health [24-27].

In relation to brain development and brain health, up until now, many of the studies examining the microbiota-gut-brain axis have been performed in animal models; for example, germ-free, antibiotic-treated, genetically modified, or humanised mice, and behavioural models (for further details, we kindly direct readers towards extensive reviews [1]. Far fewer clinical studies have investigated whether the interactions observed in rodents are also observed in humans [6]. Due to a heavy reliance on association studies, there is still little evidence of the triggers and mechanisms linking the microbiota to gut-brain communication.

The extensive reviews by Cryan *et al.* (2019) [1] and Margolis *et al.* (2021) [6] are recommended reading for a detailed overview for the development of the microbiota-gut-brain axis, the pathways of communication involved, the modulating factors and the potential health implications [1, 6]. As the primary objective of this paper is to highlight the means for taking research to the next level of discovery, current microbiota-gut-brain axis knowledge is only briefly summarised here.

Pathways for communication

At a fundamental level, the gut-brain axis is a bi-directional communication pathway composed of the central, enteric, and autonomic nervous systems and the hypothalamic-pituitary-adrenal (HPA) axis. The microbiota-gut-brain axis includes the gut microbes – comprising bacteria, viruses, fungi, and archaea – and their metabolites and by-products as factors in this bi-directional communication.

The vagus nerve, the immune and neuroendocrine systems, the neurotransmitters and metabolites along with the gut microbiota are currently the key pathways of interest in microbiota-gut-brain axis research [28].

The vagus nerve – the physical connection between brain and gut

The tenth cranial nerve that extends from the brain to the abdomen is responsible for regulating internal organ functions such as digestion, heart rate and respiratory rate. Comprising efferent and afferent neurons, the vagus nerve carries motor signals between the brain and organs, including the intestinal cells, which are also subject to the influence of the gut microbiota. The brain is, in this way, able to 'sense' the environment in the gut [29, 30].

The immune system – firm roots in the gastrointestinal tract

Evidence of the immune system's crucial role in gut-brain signalling is growing [31]. Today, it is widely recognised that most neurological conditions, including autism spectrum disorders (ASD), epilepsy, Alzheimer's disease, Parkinson's disease and cerebrovascular diseases, have low-grade systemic inflammatory components [32]. This low-grade inflammation is indicative of a malfunctioning immune response and dysbiotic microbiota.

Studies of germ-free mice and mice treated with broad-spectrum antibiotics have documented the gut microbiota's involvement in intestinal immunity related to bacterial infections and inflammation [33]. Here, the microbiota was seen to regulate both innate and adaptive immunity – locally in the gastrointestinal (GI) tract and throughout the body. Scientists have similarly used such animal models to investigate the immunological effects of specific microbes in the gut microbiota.

From a brain health perspective, microbiota-immune interactions are of interest due to the systemic low-grade inflammation often seen in neurodegenerative, neuropsychiatric, and metabolic disorders. For example, there have been extensive studies of the causal role of the microbiota in inflammatory bowel disease (IBD), which is associated with an increased susceptibility to Parkinson's disease [33, 34].

The neuroendocrine system – gut hormones and the regulation of wellbeing

Recent studies suggest that gut hormones are involved in the physiological processes that lead to disorders such as anxiety and depression – with indications that mood disorders and obesity often co-exist [35]. Scientists focus increasingly on the ability of the microbiota to modulate gut hormones and, through that, their potential to regulate mood.

Increasing evidence supports the concept of bidirectional communication between the neuroendocrine system and gut microbiota. Disturbances in both systems have been associated with disorders such as depression and irritable bowel syndrome [36]. Findings further indicate that the gut microbiota can activate the HPA axis [36] – one of the body's major neuroendocrine systems that controls responses to stress and is involved in regulating, for example, mood and emotions [37] and the immune system [38].

A growing body of research suggests that a number of neurotransmitters function as hormones and *vice versa*. Dopamine and serotonin, for example, are known to have hormonal properties [39]. Although these hormone-like neurotransmitters are not solely produced in the gut, the gut microbiota is thought to play a role in their modulation.

Field Code Changed

Field Code Changed

Formatted: Font color: Red

Field Code Changed

Field Code Changed

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Neurotransmitters and metabolites

Evidence from animal studies suggests the host's physiology is affected in various ways by the ability of gut microorganisms to produce and metabolise a range of neurotransmitters, although this remains to be documented in human subjects [13]. In the context of the microbiota-gut-brain axis, noteworthy neurotransmitters include dopamine, serotonin, noradrenaline, and gamma-aminobutyric acid (GABA). The neuroactive amino acids tyramine and tryptophan, short-chain fatty acids (SCFA), and bile acids are other molecules of interest.

GABA

GABA is believed to have a role in behaviour, cognition and the body's response to stress, anxiety and fear [40], while low GABA levels are associated with psychiatric illnesses, including schizophrenia, autism and depression [41]. Although the regulatory importance of the microbiota is not yet fully mapped, studies of germ-free animals suggest that the microbiota influences circulating GABA levels [42]. GABA is also produced by some *Lactobacilli* [43] and specific strains of *Bifidobacterium* [13, 44].

Serotonin and tryptophan

Much research has linked the microbiota with serotonin regulation in the gut [45, 46]. Serotonin is involved in mood, cognition, sleep, and appetite control [46]. Today, selective serotonin reuptake inhibitors (SSRI) are commonly prescribed treatments for depression as they increase the level of available serotonin in the brain [47]. Studies also focus on the amino acid tryptophan as the sole precursor of serotonin. It has been proposed that gut microbiota may influence tryptophan uptake and, in that way, serotonin synthesis [47].

In addition, 90% of tryptophan in the intestinal tract is metabolised along the kynurenine pathway. Of particular interest are the neuroactive metabolites quinolinic and kynurenic acids that affect the enteric nervous system (ENS) and central nervous system (CNS) (for review see [48, 49]).

Dopamine

Dopamine is a major neurotransmitter associated with the brain's reward system and is a precursor for epinephrine, also known as adrenaline, and norepinephrine, which contributes to arousal and alertness as well as behaviour and cognition [13]. Disorders associated with dopamine deficiency include addiction, schizophrenia, and Parkinson's disease. Research suggests that certain bacteria produce [13] or metabolise [50] dopamine.

SCFAs

The SCFAs propionate, butyrate and acetate are metabolites mainly produced and regulated by the bacterial fermentation of complex plant-based polysaccharides in the gut [51]. In recent years, research has explored the potential role of SCFAs in gut-brain communication with and across the blood-brain barrier (BBB) [52] and in supporting BBB integrity – a progressively leaky BBB being seen in Alzheimer's disease [15].

Studies have led to a wide range of findings that connect butyrate, for example, with memory, cognition, mood, and metabolism [53]. Acetate has been associated with appetite regulation [54], and propionate may be involved in protecting against type 2 diabetes and obesity and reducing stress behaviours [55].

Gut microbiota – the omnipresent factor, modulated by diet

Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Formatted: Font color: Red
Formatted: Font color: Red
Field Code Changed
Field Code Changed
Formatted: Font color: Red
Formatted: Font color: Red
Field Code Changed
Field Code Changed
Field Code Changed

Research has repeatedly revealed new aspects of the microbiota's contribution to gut-brain crosstalk, beginning with maternal nutrition [56] and the colonisation of the infant gut at birth [15]. It is also known that age, gender, genetics, environmental factors, geography, disease, exercise, fasting [57] and diet influence the microbiota's composition – diet and nutritional status being among the most influential factors [28, 58]. Recent reviews give a comprehensive overview of the role of diet in shaping the gut microbiota [59-61]. The gut microbiota itself can influence dietary preferences via the mesocorticolimbic system, responsible for the hedonic response to food intake [62].

Greater knowledge of the gut microbiota represents exciting possibilities to track changes in microbiota composition, activity, and behaviour in relation to the development and progression of brain disorders. Another promising avenue of exploration is the modulation of the gut microbiota by specific dietary components such as probiotics, prebiotics, postbiotics, synbiotics, and parabiotics. Such work could lead to novel therapeutic strategies, fuelled by so-called microbiotic medicinal products (MMPs) [63].

The potential for nutritional and therapeutic strategies

Research has established many links, associations, and hypotheses about the lifelong influence of the gut microbiota on brain health. Underlining this critical role, one review ranks the gut microbiota as the fourth key factor in early-life programming of brain health and disease, alongside prenatal and postnatal environment, and host genetics [64]. The scientific challenge is to identify opportunities to alter and fine-tune the microbiota and, through that, enhance human health and wellbeing.

To this end, animal and human clinical trials have explored dietary supplementation with pro-, pre-, syn- and postbiotics, omega-3 polyunsaturated fatty acids [64] and phytochemicals, such as polyphenols, which may act as prebiotics [65]. High-fibre diets - promoting SCFA production by the gut microbiota - are a promising intervention to overcome maternal-obesity-induced impairment of cognitive and social functions [66]. Faecal microbiota transplants are another potential therapeutic opportunity, having already been shown to influence hedonic food intake in mice [62]. Here, important regulatory differences apply whether developing strategies for clinical therapies or foods.

Regulation of stress, mood, and anxiety

Research has associated the gut microbiota with a range of stress- and mood-related conditions [8]. In relation to stress, several clinical studies have linked probiotic and prebiotic supplementation with a positive outcome [67-69]. The majority of mood and anxiety studies, on the other hand, have relied on preclinical animal models [8]. Healthy mice that received a probiotic formulation with *Lactobacillus rhamnosus,* for example, were seen to perform best in tests designed to provoke anxiety, depression, and stress [70].

Clinical trials have often produced conflicting results. While some have observed a significant reduction in stress and anxiety following probiotic intervention with *Lactobacillus* (*sensu lato)* and *Bifidobacterium* strains [58], others have not [70]. Reviews of clinical trials found probiotics had a limited effect on psychological outcomes – although this could be partly explained by an incomplete evidence base along with a large heterogeneity in the population, cognitive tests, and interventions. [70]. Another study reported a positive probiotic effect on mood and anxiety in patients with IBD [71].

Implications for autism spectrum disorder

Formatted: Font color: Red

Commented [DV(S1]: Wolfgang Marx, Andrew Scholey, Joseph Firth, Nathan M. D'Cunha, Melissa Lane, Meghan Hockey, Melanie M. Ashton, John F. Cryan, Adrienne O'Neil, Nenad Naumovski, Michael Berk, Olivia M. Dean, Felice Jacka,

Prebiotics, probiotics, fermented foods and cognitive outcomes: A meta-analysis of randomized controlled trials,

Neuroscience & Biobehavioral Reviews,

Volume 118, 2020, Pages 472-484,

https://doi.org/10.1016/j.neubiorev.2020.07.036

The microbiota has been demonstrated to have a clear role in autism spectrum disorder (ASD). One study has observed how the transplantation of microbes from a human diagnosed with ASD induced -like behaviour in mice [72]. Conversely, several clinical studies of ASD have found that microbiota modulation through antibiotic, prebiotic and probiotic and faecal transplantation treatments can improve social behaviour [73-76]. Researchers have further reported a reduction in anxiety behaviour, hyperactivity and defiance behaviours [73].

Other findings show that children diagnosed with ASD are four times more likely to have GI symptoms, including inflammation and abdominal pain [73] and that faecal transplantation may have long-term beneficial effects on intestinal and behavioural symptoms [76].

Learning and memory

A number of studies have explored the relationship between the gut microbiota and the development of learning and memory systems in childhood [77]. This has led to a growing appreciation that sensitive periods of development occur across the microbiota-gut-brain axis.

From animal studies, there is increasing evidence that changes in the gut microbiota alter performance in relation to visual-spatial learning and memory tasks [78]. Although there are still few human data, one study has associated microbial diversity with cognitive functioning in infancy [77].

A new approach to cognitive development research is required, including the microbiota-gut-brain axis as a peripheral force among the complex biological systems that act on behaviour. By improving understanding, this may lay the foundation for innovative therapies for learning and memory disorders [77].

Cognitive performance and age-related disorders

Many scientists now believe in the close relationship between microbial diversity and healthy ageing. Studies in mice have shown that faecal microbiota transplantation can correct age-related defects in immune function [33] – and that a similar transplant from aged to young mice has a detrimental impact on key functions of the CNS [79, 80]. These and other findings highlight the importance of the microbiota-gut-brain axis during ageing and raise the possibility that a 'young' microbiota may maintain or improve cognitive functions in life's later years [81, 82].

Neurological research suggests the microbiota also play a role in neurodegenerative diseases [83]. This supports the idea that an ageing gut microbiota could be linked to immune and neuronal dysfunction in Parkinson's and Alzheimer's disease. Indeed, studies of faecal microbiota transplants in transgenic mouse models point to a causal relationship between intestinal microbiota, protein aggregation and cognitive problems [84-86]. More studies are necessary to confirm this.

Knowledge with potential

Whether changes in the microbiota are key to detecting and understanding the physiological processes that lead to brain disorders is still unknown. But the possibilities are undeniable. Research has uncovered positive indications that therapeutic interventions may have a beneficial impact, for example in neurodevelopmental disorders, such as ASD, and age-related neurodegenerative disorders [15]. And there is every reason to be optimistic about the potential to reduce stress and anxiety. The task now is to overcome the barriers to further discovery.

3. Shortfalls and challenges – the bottlenecks to progress

The need for more knowledge and comprehensive study designs

Research in the microbiota-gut-brain axis has reached a crossroad. The gut microbiota's omnipresence and overlapping influence on physiological systems has made it progressively challenging to discuss individual aspects of the microbiota-gut-brain axis in isolation – underlining the need for a multidisciplinary, multi-system research approach to uncover the mechanisms and opportunities for improving human quality of life and wellbeing, as is being done for metabolic diseases [87, 88]. Multidomain interventions combining diet, with other health-promoting lifestyle approaches, have been demonstrated to be effective strategies as they target endogenous and environmental factors (such as genetics, age, diet, and lifestyle) that modulate the gut microbiota activity and composition, underlining enormous variability between individuals [89, 90].

Consequently, while many of the tools and methodologies in use until now have significantly advanced our knowledge and understanding of the role of the microbiota-gut-brain axis in brain health and disease, the large majority of studies to date have been limited to animal models and have mostly been observational in a clinical setting. There are still many unanswered questions within the field which require more clarity in order to drive further meaningful progress towards microbiota-targeted strategies for improving brain health. Some of the gaps in current knowledge are fundamental and must be bridged by skilful scientific investigation.

Understanding changes and mechanisms

The characteristics and function of a 'healthy' gut microbiota are still unknown. Although studies have frequently documented a reduction in functional diversity and compositional alterations in relation to a variety of disorders [61], there is as yet little understanding of how the microbiota changes over time and may reflect the impending onset of disease. Recent data from more than 9,000 adults of different ages show that, as individuals age, the gut microbiome becomes increasingly unique, increasingly different from others, starting in mid-to-late adulthood. A better understanding of this phenomenon may open the way to an improved understanding of what is a 'healthy ageing microbiota pattern' [91]. Similarly, there is lack of knowledge about disease biomarkers and whether they may be reversed through treatment or dietary interventions. Several systematic reviews and meta-analyses, albeit with different search criteria, have investigated the effects of probiotics, prebiotics, and even fermented foods on symptoms of depression, anxiety, and mood, as well as on cognition. Interestingly, while the majority of studies did conclude there were some positive effects of dietary interventions or supplements on depression and anxiety symptoms [18, 19, 70, 92], others concluded that the data to support the role of dietary interventions on mood and cognitive function were insignificant [93, 94]. In addition, some studies reported that targeting the gut microbiome to alleviate symptoms of anxiety and depression were more pronounced in clinical patient populations compared with healthy adults [95]. Finally, most studies did suggest that additional double-blind, randomised, placebo controlled clinical trials in clinical populations are warranted to further assess efficacy.

Numerous association and correlation studies have identified links between the gut microbiota and the CNS [96-99]. Further targeted studies are required to identify and confirm the mechanisms of action in humans. Complex gaps in existing knowledge include:

- The immunological effects of specific microbes in the human gut microbiota and their role in neurodevelopmental, neurodegenerative, and neuropsychiatric disorders.
- A precise mapping of microbiota-regulated neurotransmitters in human subjects, the hormonal properties of these neurotransmitters and the mechanisms by which they activate the HPA axis.
- How microbial by-products, such as SCFAs, branched-chain fatty acids, methylamines, and peptides, influence brain function in tandem with immunological and neurological signalling molecules.
- The contribution of specific microbes to brain development during early life.

Few and varied clinical studies

Intervention studies in humans and preclinical studies in humanised mice and rats are a fundamental requirement. In the early days of this research field, most research was limited to *in vitro* or preclinical studies, and there was a high prevalence of review articles and meta-analyses of the microbiota compositions [96, 100-103]. Since, clinical intervention studies have been performed more frequently although often characterised by a low number of human subjects and short timeframes [94, 104].

As typical in nutritional intervention studies, the non-standardised approaches often used means that the authors of review articles frequently struggle to find suitable clinical studies for meaningful comparisons. Wide variations in test subjects, cognitive and mental test designs, intervention formulations and the filtering of data stand in the way of general conclusions – with many studies being low on statistical power [94].

Overall, clinical studies are held back by a lack of disease- and microbiota-specific biomarkers, absence of clinically relevant behavioural phenotypes and poor tools for cohort stratification. Still, over the last year a number of meta-analyses have appeared which show a moderately positive evaluation on the use of psychobiotic [104] interventions for anxiety [105], schizophrenia [106] or cognitive functions [107, 108], pointing to the diversity and complexity of - and the numerous confounding factors that may affect - the gut microbiota [21, 109].

Furthermore, when trying to establish cause and consequence relation, it might also be important to better understand the effects of traditional drugs, including psychotropics, on the microbiota and the potential health consequences [110].

A general tendency to conduct preclinical and clinical studies within the silos of individual disciplines also compounds these limitations and, at the same time, rules out the opportunities created by multidisciplinary collaboration. The time has clearly come for a new approach.

4. Beyond hypotheses to validated nutritional and therapeutic strategies

Practical proposals for moving microbiota-gut-brain axis research forward

As the microbiota-gut-brain axis continues to attract scientific attention, a whole-system, multi-disciplinary approach is necessary to progress from hypotheses to validated therapeutic strategies of benefit to brain health. Scientists have successfully documented countless associations between the gut microbiota and brain disorders. However, correlation does not equal causation. The next step is to understand the mechanisms behind those associations and how they are influenced by dietary

habits, lifestyle, and genetic risk factors. This will require new methods, skills, and collaborations. An overview of the gaps and needs is represented in *Figure 1*.

Fig. 1 *Key gaps and needs in microbiota-gut-brain axis research on the journey towards nutritional and therapeutic strategies for improved quality of life.*

Insert figure 1 here

More targeted, gold standard clinical studies with reproducible results

Experiences so far highlight the need to rethink and redesign the approach to clinical studies in a way that facilitates the integration of standardised methods and models from all fields of study related to the microbiota-gut-brain axis. The emphasis on 'standardised' is important. In this context, human clinical studies should be robust, employing a design that includes randomisation, controlled with a suitable placebo, and conducted at least double-blind. Clinical trials should always be conducted in accordance with the Declaration of Helsinki [111] and the guidelines for Good Clinical Practice (GCP) [112] to ensure ethical and scientific quality requirements are followed throughout the study design, conduct, recording of information, and reporting of data. Compliance with this standard ensures not only that the rights, safety, and wellbeing of trial subjects are protected, but also that the data is credible. Finally, only by conducting repeated studies that provide comparable and reproducible results will it be possible to build a critical mass of scientific evidence to drive real progress.

A new research framework should include strong guidance on specific areas of the microbiota-gut-brain axis to investigate, which biological samples to collect and the biomarkers or surrogate biomarkers to measure – with regard to sampling and analysis, the NIH Human Microbiome Project website already provides some guidance [113]. Standard operating procedures should also be established for the collection, transport, storage, and analysis of biological samples and for the sequencing and filtering of data, reducing the variables that can influence study outcomes. Equally important are the identification and stratification of relevant cohorts to support cross-study comparisons and consolidate research findings (e.g. The Quadram Institute website released for best practice in microbiome research [114]).

Robust human studies must be conducted in real-life settings using calibrated dietary habit assessments and validated test methods to investigate potential windows for nutritional strategies [115]. At present, studies of dietary habits rely on subjects to provide data by filling out food frequency questionnaires, 24h recalls, food checklists, diet histories, and food diaries which require large and complicated data analyses and experienced dieticians or nutritionists to accurately extrapolate the data [116]. To improve the quality of these data, there is a need to replace self-reporting with new and emerging objective tools. The emergence of food intake biomarkers holds great promise for nutrition research in this regard [117, 118]. Another possibility is to recruit subjects who share the same household or live in a care home, for example, where they tend to eat the same foods. One recent study by Valles-Colomer *et al.* assessed gut microbiota compositional covariation with quality-of-life indicators and depression in the Belgian Flemish Gut Flora Project population cohort [119]. While *Faecalibacterium* and *Coprococcus* were consistently associated with higher quality of life indicators, both of these genera were depleted in depression and inflammatory bowel disease. Interestingly, *Coprococcus* and *Dialister* decreased with depression. These results were validated in other large microbiome cohorts. To investigate the link between microbial neuroactive capacity with quality of life and depression, the authors constructed the first catalogue of gut microbiota

neuroactive potential using a module-based analytical framework. Specific covariations were discovered between pathways of neurotransmission, mental quality of life and specific genera such as *Coprococcus* [119]. New investigative tools such as the gut–brain module analysis of faecal metagenomes described by Valles-Colomer *et al.* could provide greater insight into the associations between pathways regulating brain health and function, the gut microbiota, and symptoms of mood disorders commonly found across different population cohorts. Clinical studies of the role of microbiota in disease must account for the natural variations in microbiota composition from one individual to the next. Age, sex, body mass index, medications, and lifestyle are among the host variables that confound microbiota analyses and limit the capacity to draw valid conclusions. For example, research has shown that patients with depression have an altered gut microbial profile compared with healthy adults [119-122]. However, each study describes unique microbial changes in these patients due to huge inter-individual microbial differences in the general human population. This variability between studies makes it extremely difficult to interpret whether the microbial changes described are a hallmark of depression or whether they are unique to one individual study. Indeed, this is an important limitation to consider before drawing conclusions on the role of the gut microbiome in mental disorders such as depression. Furthermore, investigations into the gut microbial profile of patients with depression do not indicate whether these changes are causal to disease state or consequential of disease. In studies of personalised interventions based on intestinal microbiota composition and activity, an unhealthy diet, for example, may negate the potential beneficial effects of a dietary supplement. Nutrition, physical activity, psychological and physical stress, sleep restrictions, socioeconomic status, antibiotics use, exposure to pets, noise, and temperature have been all reported to associate with changes in human microbiota [123-125]. It is, therefore, essential that human microbiota studies capture such host variables to secure reproducible evidence about the relationship between specific gut microorganisms and biomarkers of disease [126]. The appropriate timing of an intervention is an additional factor to account for, considering that the impact of lifestyle and environments may vary along the lifespan. Intervening during sensitive time-windows, e.g., when microbiota and brain are still developing and their plasticity is high, may increase the likelihood of a persistent effect. Studies in the first 1,000 days of life indicate that exposure to antibiotics [127, 128], pets, siblings [129], specific maternal intakes (sweeteners [130]) and environmental toxicants [131] affecting the infant's microbiota are likely targets. On the other hand, since diet and lifestyle are such strong drivers of microbiota composition and activity [132], this opens the possibility to help patients to take their own responsibility to improve their brain health. Indeed, there is accumulating evidence in nutritional psychiatry regarding the importance of diet for realising mental health [133], however, the causational role of the gut microbiome needs to be established. This challenge cannot be tackled by observational studies and interventional studies examining the effects of dietary and/or lifestyle changes as well as interventions with nutraceuticals. It needs to be designed in a different way, because the classical double blinded approach does not work. A combination of alternative interventional study approaches, such as cross-over studies (for example [134], or citizen science (for example [135]) combined with mechanistic studies using new models and tools might be the way forward.

Robust new models and elegant tools

Future progress further relies on the development of new models and elegant tools for studying bi-directional communication pathways. While animal models have proven invaluable in establishing the

current knowledge base, it is inescapable that the gut microbiota of rodents is substantially different from that of humans. To overcome this limitation, there is a need for robust and reliable humanised rodent models [136].

From the perspective of *in vitro* models, three-dimensional brain and gut organoids and advanced co-culture systems including the ENS, vagus nerve and the BBB provide alternative methods for investigating realistic conditions for unravelling the mysteries of microbiota-gut-brain mechanisms [137, 138]. Used in combination with models for digestion, such organoids and co-cultures could form *in vitro* workflow models for studying the gut-brain axis in context. A number of so-called organ-on-a-chip *in vitro* models have already been developed for this purpose, though they still have limitations [139].

Great opportunities also lie in the development of methods that track, for example, how neurotransmitters travel from the gut through the BBB in response to neuroinflammatory processes. Some of this methodology is becoming available, with human brain imaging representing a possibility to track the influence of microbiota on neurotransmission [13]. Metabolomic, metaproteomic and metagenomic analyses and gut biopsies are other possible methodologies.

Many research studies today involve statisticians from their inception to assure the quality of the study's design. Computational and data scientists are similarly vital to maximise the value of research through comprehensive data analysis. Specialised computer programs are already able to provide next-level precision when generalising and stratifying results in relation to specific population groups, such as those at risk of brain disorders [140].

Machine learning technology will become increasingly essential to improving the efficiency and accuracy of study findings. Indeed, bioinformatics holds the key to integrating large, multi-dimensional datasets and, from that, gaining a better understanding of their clinical significance. At the current pace of technological development, it is now possible to imagine the potential of such tools to identify high-risk patients at an early stage, determine which microbial/immunological imbalances may cause such risks and suggest possible interventions to mitigate them [141].

An emphasis on collaboration

More sharing and collaborative work is essential to extract maximum knowledge from available data and build a truly validated evidence base. This requires the establishment of new biobanks to facilitate the sharing of material from human and animal studies. Deep phenotyping databases, standardised data formats [142] and new methodologies for preserving microbiome samples [143] are essential for such biobanks to play a meaningful role. By the same token, *in vitro* models must become more easily available for use across labs.

The competition for funding is one explanation for the low level of scientific collaboration to date. However, a number of programmes and initiatives are, today, moving research in this direction. Within Europe, they include the Community Research and Development Information Service (CORDIS) [144], which gathers and disseminates results from projects funded by the EU's framework programmes for research and innovation.

One such project is the five-year multi-centre GEMMA project funded by the EU's Horizon 2020 programme [145]. Launched in January 2019, GEMMA explores interactions between the gut

microbiome, metabolome, epigenome, and immune function to discover useful biomarkers for early diagnosis of autism, along with potential targets for preventive therapies [146]. Other examples are the ONCOBIOME [147] and MICROB-PREDICT [148] projects, funded by Horizon 2020 to investigate the microbiome's role in cancer development and chronic liver disease, respectively.

Organisations such as the International Life Science Institute Europe (ILSI Europe) [149] and the International Scientific Association for Probiotics and Prebiotics (ISAPP) [150] bring together academic and industrial scientists involved in basic and applied research across multiple disciplines. Their purpose is to promote progress in the field by supporting scientific integrity and transparency, harmonising scientific efforts, and providing guidance for collaborative and multidisciplinary research.

ISAPP is setting an excellent example. Each one of its objectives is relevant to the progress of microbiota-gut-brain axis research at large and the ultimate development of dietary strategies where the gut microbiota is the primary target.

5. The dream destination – improved quality of life

The potential of the microbiota-gut-brain axis through future nutritional and therapeutic interventions

The microbiota-gut-brain axis represents an intricate network of systems which scientists are only beginning to understand. Given this complexity, the nutritional and therapeutic strategies with the best chances of success are likely to be those aimed at improving human quality of life. Some are even already on the market, including foods and supplements that promise to improve mood, sleep, or cognitive performance. The evidence behind some of these claims is, however, still in question. Very recently though, the European Food Safety Agency (EFSA) approved *Akkermansia muciniphila* as a novel food [151].

By expanding knowledge, scientists have recognised the potential to achieve much more. Although the prevention of brain disorders may remain out of reach for the foreseeable future, the mapping of healthy microbiota and communication pathways could enable their early prediction. The first signs of neurodegenerative conditions such as Alzheimer's and Parkinson's disease, for example, are known to develop many years before diagnosis. Imagine if it were possible to slow neurodegenerative processes by altering the microbiome.

A similar scenario is imaginable for children with ASD. What if dietary influences on the gut microbiota could both relieve GI irritation and calm anxiety and hyperactivity? And what if it were possible to complement drug and psychiatric therapy for schizophrenia with targeted foods such as probiotics?

These are, perhaps, realisable dreams. Over the past few years, they have inspired a growing number of scientists to found start-up companies that are now investigating small molecule therapeutics for treating neurological and other disorders through microbiome modulation. Private investors often support their clinical research.

Scientists have documented many links between the microbiota, gut, and brain. The time has come to dig even deeper through integrated, multidisciplinary research – aimed at understanding microbiota-gut-brain mechanisms and identifying true opportunities to adapt and adjust the

560 microbiota for better brain health through life. Continuous investment from the public and private
561 sector is vital to keep up the momentum.

6. Declarations

Funding

This work was conducted by an expert group (EG) of the European branch of the International Life Sciences Institute, ILSI Europe. According to ILSI Europe policies, the EG is composed of at least 50% external non-industry members. The complete composition of the EG can be found on the ILSI Europe website (https://ilsi.eu/task-forces/nutrition/nutrition-and-mental-performance/). Experts are not paid for the time spent on this work. However, non-industry members were offered support for travel and accommodation costs from the above-mentioned task forces when attending workshops/ meetings to discuss the manuscript. Journalist and communication consultant Cath Mersh received funding for her writing services from the above-mentioned Task Forces.

Competing interests

The following authors: LG, LH, PI, ADK, MM, CS, DV declare that they have no competing interests. AC, GLF, EP, BP are employees of the food industry, as declared under affiliation.

Author contributions

AC, GLF, LH, PI, ADK, EP, BP, CS, DV: conception, design, and revision of the work.
LG, MM: overall management, conception, design, and revision of the work.

Availability of data and material (data transparency)

Not applicable.

Code availability (software application or custom code)

Not applicable.

7. References

1. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV *et al*: **The Microbiota-Gut-Brain Axis**. *Physiological reviews* 2019, **99**(4):1877-2013.
2. Morais LH, Schreiber HL, Mazmanian SK: **The gut microbiota–brain axis in behaviour and brain disorders**. *Nature Reviews Microbiology* 2021, **19**(4):241-255.
3. Liang S, Wu X, Jin F: **Gut-Brain Psychology: Rethinking Psychology From the Microbiota-Gut-Brain Axis**. *Frontiers in integrative neuroscience* 2018, **12**:33.
4. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG: **The gut microbiome in neurological disorders**. *The Lancet Neurology* 2020, **19**(2):179-194.
5. Maiuolo J, Gliozzi M, Musolino V, Carresi C, Scarano F, Nucera S, Scicchitano M, Oppedisano F, Bosco F, Ruga S *et al*: **The Contribution of Gut Microbiota-Brain Axis in the Development of Brain Disorders**. *Frontiers in neuroscience* 2021, **15**:616883.
6. Margolis KG, Cryan JF, Mayer EA: **The Microbiota-Gut-Brain Axis: From Motility to Mood**. *Gastroenterology* 2021, **160**(5):1486-1501.
7. Novotný M, Klimova B, Valis M: **Microbiome and Cognitive Impairment: Can Any Diets Influence Learning Processes in a Positive Way?** *Frontiers in aging neuroscience* 2019, **11**:170.
8. Foster JA, Rinaman L, Cryan JF: **Stress & the gut-brain axis: Regulation by the microbiome**. *Neurobiology of stress* 2017, **7**:124-136.
9. Appleton J: **The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health**. *Integrative medicine (Encinitas, Calif)* 2018, **17**(4):28-32.
10. Liu L, Zhu G: **Gut-Brain Axis and Mood Disorder**. *Frontiers in psychiatry* 2018, **9**:223.
11. Tognini P: **Gut Microbiota: A Potential Regulator of Neurodevelopment**. *Frontiers in cellular neuroscience* 2017, **11**:25.
12. Lacorte E, Gervasi G, Bacigalupo I, Vanacore N, Raucci U, Parisi P: **A Systematic Review of the Microbiome in Children With Neurodevelopmental Disorders**. *Frontiers in neurology* 2019, **10**:727.
13. Strandwitz P: **Neurotransmitter modulation by the gut microbiota**. *Brain research* 2018, **1693**(Pt B):128-133.
14. Mohajeri MH, La Fata G, Steinert RE, Weber P: **Relationship between the gut microbiome and brain function**. *Nutrition reviews* 2018, **76**(7):481-496.
15. Dinan TG, Cryan JF: **Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration**. *The Journal of physiology* 2017, **595**(2):489-503.
16. Oriach CS, Robertson RC, Stanton C, Cryan JF, Dinan TG: **Food for thought: The role of nutrition in the microbiota-gut–brain axis**. *Clinical Nutrition Experimental* 2016, **6**:25-38.
17. Ceppa F, Mancini A, Tuohy K: **Current evidence linking diet to gut microbiota and brain development and function**. *International journal of food sciences and nutrition* 2019, **70**(1):1-19.
18. Wallace CJK, Milev R: **The effects of probiotics on depressive symptoms in humans: a systematic review**. *Annals of general psychiatry* 2017, **16**:14.
19. Liu RT, Walsh RFL, Sheehan AE: **Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials**. *Neuroscience and biobehavioral reviews* 2019, **102**:13-23.

20. Palacios-García I, Parada FJ: **Measuring the Brain-Gut Axis in Psychological Sciences: A Necessary Challenge**. *Frontiers in integrative neuroscience* 2019, **13**:73.
21. Osadchiy V, Martin CR, Mayer EA: **The Gut-Brain Axis and the Microbiome: Mechanisms and Clinical Implications**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2019, **17**(2):322-332.
22. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K: **Gut microbiota functions: metabolism of nutrients and other food components**. *European journal of nutrition* 2018, **57**(1):1-24.
23. Shortt C, Hasselwander O, Meynier A, Nauta A, Fernández EN, Putz P, Rowland I, Swann J, Türk J, Vermeiren J *et al*: **Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients**. *European journal of nutrition* 2018, **57**(1):25-49.
24. Graf D, Di Cagno R, Fåk F, Flint HJ, Nyman M, Saarela M, Watzl B: **Contribution of diet to the composition of the human gut microbiota**. *Microbial ecology in health and disease* 2015, **26**:26164.
25. Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR, Kim AD, Shmagel AK, Syed AN, Walter J *et al*: **Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans**. *Cell host & microbe* 2019, **25**(6):789-802.e785.
26. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA *et al*: **Diet rapidly and reproducibly alters the human gut microbiome**. *Nature* 2014, **505**(7484):559-563.
27. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H *et al*: **Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life**. *Cell host & microbe* 2015, **17**(5):690-703.
28. Sandhu KV, Sherwin E, Schellekens H, Stanton C, Dinan TG, Cryan JF: **Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry**. *Translational research : the journal of laboratory and clinical medicine* 2017, **179**:223-244.
29. Agostoni E, Chinnock JE, De Daly MB, Murray JG: **Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat**. *The Journal of physiology* 1957, **135**(1):182-205.
30. Prechtl JC, Powley TL: **B-Afferents: A fundamental division of the nervous system mediating homeostasis?** *Behavioral and Brain Sciences* 1990, **13**(2):289-300.
31. Salvo-Romero ES, P.; Gareau, M.G.: **Microbiota-immune interactions: from gut to brain**. *LymphoSign Journal* 2020, **7**(1):1-23.
32. Coyle PK: **Dissecting the immune component of neurologic disorders: a grand challenge for the 21st century**. *Frontiers in neurology* 2011, **2**:37.
33. Fung TC: **The microbiota-immune axis as a central mediator of gut-brain communication**. *Neurobiology of disease* 2020, **136**:104714.
34. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T: **Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014**. *Gut* 2019, **68**(1):18-24.
35. Sun LJ, Li JN, Nie YZ: **Gut hormones in microbiota-gut-brain cross-talk**. *Chinese medical journal* 2020, **133**(7):826-833.
36. Farzi A, Fröhlich EE, Holzer P: **Gut Microbiota and the Neuroendocrine System**. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 2018, **15**(1):5-22.

37. Bao AM, Swaab DF: **The human hypothalamus in mood disorders: The HPA axis in the center**. *IBRO reports* 2019, **6**:45-53.
38. Dunn AJ: **The HPA Axis and the Immune System: A Perspective**. In: *NeuroImmune Biology*. vol. 7: Elsevier; 2007: 3-15.
39. A. C, M. C: **Hormones and neurotransmitters: the differences and curious similarities.** . In: *Medium.* 2018.
40. Jewett BE, Sharma S: **Physiology, GABA**. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
41. Allen MJ, Sabir S, Sharma S: **GABA Receptor**. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
42. Mao JH, Kim YM, Zhou YX, Hu D, Zhong C, Chang H, Brislawn CJ, Fansler S, Langley S, Wang Y *et al*: **Genetic and metabolic links between the murine microbiome and memory**. *Microbiome* 2020, **8**(1):53.
43. Zheng J, Wittouck S, Salvetti E, Franz C, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J *et al*: **A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae**. *International journal of systematic and evolutionary microbiology* 2020, **70**(4):2782-2858.
44. Duranti S, Ruiz L, Lugli GA, Tames H, Milani C, Mancabelli L, Mancino W, Longhi G, Carnevali L, Sgoifo A *et al*: **Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA**. *Scientific reports* 2020, **10**(1):14112.
45. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY: **Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis**. *Cell* 2015, **161**(2):264-276.
46. Malinova TS, Dijkstra CD, de Vries HE: **Serotonin: A mediator of the gut-brain axis in multiple sclerosis**. *Multiple sclerosis (Houndmills, Basingstoke, England)* 2018, **24**(9):1144-1150.
47. Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP: **Influence of Tryptophan and Serotonin on Mood and Cognition with a Possible Role of the Gut-Brain Axis**. *Nutrients* 2016, **8**(1).
48. Kennedy PJ, Cryan JF, Dinan TG, Clarke G: **Kynurenine pathway metabolism and the microbiota-gut-brain axis**. *Neuropharmacology* 2017, **112**(Pt B):399-412.
49. Gheorghe CE, Martin JA, Manriquez FV, Dinan TG, Cryan JF, Clarke G: **Focus on the essentials: tryptophan metabolism and the microbiome-gut-brain axis**. *Current opinion in pharmacology* 2019, **48**:137-145.
50. Maini Rekdal V, Nol Bernadino P, Luescher MU, Kiamehr S, Le C, Bisanz JE, Turnbaugh PJ, Bess EN, Balskus EP: **A widely distributed metalloenzyme class enables gut microbial metabolism of host- and diet-derived catechols**. *eLife* 2020, **9**.
51. Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, Nauta A, Scott K, Stahl B, van Harsselaar J *et al*: **Short chain fatty acids in human gut and metabolic health**. *Beneficial microbes* 2020, **11**(5):411-455.
52. Hoyles L, Snelling T, Umlai UK, Nicholson JK, Carding SR, Glen RC, McArthur S: **Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier**. *Microbiome* 2018, **6**(1):55.

53. Silva YP, Bernardi A, Frozza RL: **The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication**. *Frontiers in endocrinology* 2020, **11**:25.
54. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S *et al*: **The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism**. *Nature communications* 2014, **5**:3611.
55. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G: **Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits**. *Cell* 2014, **156**(1-2):84-96.
56. Wang D, Zhang H, Zeng M, Tang X, Zhu X, Guo Y, Qi L, Xie Y, Zhang M, Chen D: **Maternal high sugar and fat diet benefits offspring brain function via targeting on the gut-brain axis**. *Aging* 2021, **13**(7):10240-10274.
57. Soares NL, Dorand VAM, Cavalcante HC, Batista KS, de Souza DM, Lima MDS, Salvadori M, Magnani M, Alves AF, Aquino JS: **Does intermittent fasting associated with aerobic training influence parameters related to the gut-brain axis of Wistar rats?** *Journal of affective disorders* 2021, **293**:176-185.
58. Mörkl S, Wagner-Skacel J, Lahousen T, Lackner S, Holasek SJ, Bengesser SA, Painold A, Holl AK, Reininghaus E: **The Role of Nutrition and the Gut-Brain Axis in Psychiatry: A Review of the Literature**. *Neuropsychobiology* 2018:1-9.
59. Grosso G: **Nutritional Psychiatry: How Diet Affects Brain through Gut Microbiota**. *Nutrients* 2021, **13**(4).
60. Kincaid HJ, Nagpal R, Yadav H: **Diet-Microbiota-Brain Axis in Alzheimer's Disease**. *Annals of nutrition & metabolism* 2021:1-7.
61. Clarke G, Sandhu KV, Griffin BT, Dinan TG, Cryan JF, Hyland NP: **Gut Reactions: Breaking Down Xenobiotic-Microbiome Interactions**. *Pharmacological reviews* 2019, **71**(2):198-224.
62. de Wouters d'Oplinter A, Rastelli M, Van Hul M, Delzenne NM, Cani PD, Everard A: **Gut microbes participate in food preference alterations during obesity**. *Gut microbes* 2021, **13**(1):1959242.
63. Cordaillat-Simmons M, Rouanet A, Pot B: **Live biotherapeutic products: the importance of a defined regulatory framework**. *Experimental & Molecular Medicine* 2020, **52**(9):1397-1406.
64. Codagnone MG, Spichak S, O'Mahony SM, O'Leary OF, Clarke G, Stanton C, Dinan TG, Cryan JF: **Programming Bugs: Microbiota and the Developmental Origins of Brain Health and Disease**. *Biological psychiatry* 2019, **85**(2):150-163.
65. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD *et al*: **Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics**. *Nature reviews Gastroenterology & hepatology* 2017, **14**(8):491-502.
66. Liu X, Li X, Xia B, Jin X, Zou Q, Zeng Z, Zhao W, Yan S, Li L, Yuan S *et al*: **High-fiber diet mitigates maternal obesity-induced cognitive and social dysfunction in the offspring via gut-brain axis**. *Cell metabolism* 2021, **33**(5):923-938.e926.
67. Kato-Kataoka A, Nishida K, Takada M, Kawai M, Kikuchi-Hayakawa H, Suda K, Ishikawa H, Gondo Y, Shimizu K, Matsuki T *et al*: **Fermented Milk Containing Lactobacillus casei Strain Shirota Preserves the Diversity of the Gut Microbiota and Relieves Abdominal Dysfunction in Healthy Medical Students Exposed to Academic Stress**. *Applied and environmental microbiology* 2016, **82**(12):3649-3658.
68. Takada M, Nishida K, Gondo Y, Kikuchi-Hayakawa H, Ishikawa H, Suda K, Kawai M, Hoshi R, Kuwano Y, Miyazaki K *et al*: **Beneficial effects of Lactobacillus casei**

strain Shirota on academic stress-induced sleep disturbance in healthy adults: a double-blind, randomised, placebo-controlled trial. *Beneficial microbes* 2017, **8**(2):153-162.
69. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW: **Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers**. *Psychopharmacology* 2015, **232**(10):1793-1801.
70. Romijn AR, Rucklidge JJ: **Systematic review of evidence to support the theory of psychobiotics**. *Nutrition reviews* 2015, **73**(10):675-693.
71. Agustí A, García-Pardo MP, López-Almela I, Campillo I, Maes M, Romaní-Pérez M, Sanz Y: **Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function**. *Frontiers in neuroscience* 2018, **12**:155.
72. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, Zink EM, Casey CP, Taylor BC, Lane CJ *et al*: **Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice**. *Cell* 2019, **177**(6):1600-1618.e1617.
73. Roussin L, Prince N, Perez-Pardo P, Kraneveld AD, Rabot S, Naudon L: **Role of the Gut Microbiota in the Pathophysiology of Autism Spectrum Disorder: Clinical and Preclinical Evidence**. *Microorganisms* 2020, **8**(9).
74. Liu J, Wan GB, Huang MS, Agyapong G, Zou TL, Zhang XY, Liu YW, Song YQ, Tsai YC, Kong XJ: **Probiotic Therapy for Treating Behavioral and Gastrointestinal Symptoms in Autism Spectrum Disorder: A Systematic Review of Clinical Trials**. *Current medical science* 2019, **39**(2):173-184.
75. Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, Leigh Gibson E, Nielsen DS, Costabile A: **A prebiotic intervention study in children with autism spectrum disorders (ASDs)**. *Microbiome* 2018, **6**(1):133.
76. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, Caporaso JG, Krajmalnik-Brown R: **Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota**. *Scientific reports* 2019, **9**(1):5821.
77. Callaghan B: **Nested sensitive periods: how plasticity across the microbiota-gut-brain axis interacts to affect the development of learning and memory**. *Current opinion in behavioral sciences* 2020, **36**:55-62.
78. Vuong HE, Yano JM, Fung TC, Hsiao EY: **The Microbiome and Host Behavior**. *Annual review of neuroscience* 2017, **40**:21-49.
79. D'Amato A, Di Cesare Mannelli L, Lucarini E, Man AL, Le Gall G, Branca JJV, Ghelardini C, Amedei A, Bertelli E, Regoli M *et al*: **Faecal microbiota transplant from aged donor mice affects spatial learning and memory via modulating hippocampal synaptic plasticity- and neurotransmission-related proteins in young recipients**. *Microbiome* 2020, **8**(1):140.
80. Kundu P, Lee HU, Garcia-Perez I, Tay EXY, Kim H, Faylon LE, Martin KA, Purbojati R, Drautz-Moses DI, Ghosh S *et al*: **Neurogenesis and prolongevity signaling in young germ-free mice transplanted with the gut microbiota of old mice**. *Science translational medicine* 2019, **11**(518).
81. Lach G, Fülling C, Bastiaanssen TFS, Fouhy F, Donovan ANO, Ventura-Silva AP, Stanton C, Dinan TG, Cryan JF: **Enduring neurobehavioral effects induced by microbiota depletion during the adolescent period**. *Translational psychiatry* 2020, **10**(1):382.
82. Boehme M, Guzzetta KE, Bastiaanssen TFS, van de Wouw M, Moloney GM, Gual-Grau A, Spichak S, Olavarría-Ramírez L, Fitzgerald P, Morillas E *et al*: **Microbiota**

from young mice counteracts selective age-associated behavioral deficits. *Nature Aging* 2021, **1**(8):666-676.
83. Bullich C, Keshavarzian A, Garssen J, Kraneveld A, Perez-Pardo P: **Gut Vibes in Parkinson's Disease: The Microbiota-Gut-Brain Axis**. *Movement disorders clinical practice* 2019, **6**(8):639-651.
84. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V *et al*: **Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease**. *Cell* 2016, **167**(6):1469-1480.e1412.
85. Sun J, Xu J, Ling Y, Wang F, Gong T, Yang C, Ye S, Ye K, Wei D, Song Z *et al*: **Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice**. *Translational psychiatry* 2019, **9**(1):189.
86. Kim MS, Kim Y, Choi H, Kim W, Park S, Lee D, Kim DK, Kim HJ, Choi H, Hyun DW *et al*: **Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model**. *Gut* 2020, **69**(2):283-294.
87. Koh A, Molinaro A, Ståhlman M, Khan MT, Schmidt C, Mannerås-Holm L, Wu H, Carreras A, Jeong H, Olofsson LE *et al*: **Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1**. *Cell* 2018, **175**(4):947-961.e917.
88. Hoyles L, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, Heymes C, Luque JL, Anthony E, Barton RH *et al*: **Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women**. *Nature medicine* 2018, **24**(7):1070-1080.
89. Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, Bäckman L, Hänninen T, Jula A, Laatikainen T *et al*: **A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial**. *Lancet (London, England)* 2015, **385**(9984):2255-2263.
90. Dardiotis E, Kosmidis MH, Yannakoulia M, Hadjigeorgiou GM, Scarmeas N: **The Hellenic Longitudinal Investigation of Aging and Diet (HELIAD): rationale, study design, and cohort description**. *Neuroepidemiology* 2014, **43**(1):9-14.
91. Wilmanski T, Diener C, Rappaport N, Patwardhan S, Wiedrick J, Lapidus J, Earls JC, Zimmer A, Glusman G, Robinson M *et al*: **Gut microbiome pattern reflects healthy ageing and predicts survival in humans**. *Nature Metabolism* 2021, **3**(2):274-286.
92. Noonan S, Zaveri M, Macaninch E, Martyn K: **Food & mood: a review of supplementary prebiotic and probiotic interventions in the treatment of anxiety and depression in adults**. *BMJ nutrition, prevention & health* 2020, **3**(2):351-362.
93. Ng QX, Peters C, Ho CYX, Lim DY, Yeo WS: **A meta-analysis of the use of probiotics to alleviate depressive symptoms**. *Journal of affective disorders* 2018, **228**:13-19.
94. Marx W, Scholey A, Firth J, D'Cunha NM, Lane M, Hockey M, Ashton MM, Cryan JF, O'Neil A, Naumovski N *et al*: **Prebiotics, probiotics, fermented foods and cognitive outcomes: A meta-analysis of randomized controlled trials**. *Neuroscience and biobehavioral reviews* 2020, **118**:472-484.
95. Chao L, Liu C, Sutthawongwadee S, Li Y, Lv W, Chen W, Yu L, Zhou J, Guo A, Li Z *et al*: **Effects of Probiotics on Depressive or Anxiety Variables in Healthy Participants Under Stress Conditions or With a Depressive or Anxiety Diagnosis: A Meta-Analysis of Randomized Controlled Trials**. *Frontiers in neurology* 2020, **11**:421.

96. Iglesias-Vázquez L, Van Ginkel Riba G, Arija V, Canals J: **Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis**. *Nutrients* 2020, **12**(3).
97. Nishiwaki H, Ito M, Ishida T, Hamaguchi T, Maeda T, Kashihara K, Tsuboi Y, Ueyama J, Shimamura T, Mori H *et al*: **Meta-Analysis of Gut Dysbiosis in Parkinson's Disease**. *Movement disorders : official journal of the Movement Disorder Society* 2020, **35**(9):1626-1635.
98. Safadi JM, Quinton AMG, Lennox BR, Burnet PWJ, Minichino A: **Gut dysbiosis in severe mental illness and chronic fatigue: a novel trans-diagnostic construct? A systematic review and meta-analysis**. *Molecular psychiatry* 2021.
99. Nguyen TT, Kosciolek T, Eyler LT, Knight R, Jeste DV: **Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder**. *Journal of psychiatric research* 2018, **99**:50-61.
100. Shen T, Yue Y, He T, Huang C, Qu B, Lv W, Lai H-Y: **The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis**. *Frontiers in aging neuroscience* 2021, **13**(40).
101. Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A: **Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation**. *npj Parkinson's Disease* 2021, **7**(1):27.
102. Xu M, Xu X, Li J, Li F: **Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis**. *Frontiers in psychiatry* 2019, **10**:473.
103. Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, Drago F, Caraci F: **Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials**. *PloS one* 2014, **9**(5):e96905.
104. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ: **Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals**. *Trends in neurosciences* 2016, **39**(11):763-781.
105. Cohen Kadosh K, Basso M, Knytl P, Johnstone N, Lau JYF, Gibson GR: **Psychobiotic interventions for anxiety in young people: a systematic review and meta-analysis, with youth consultation**. *Translational psychiatry* 2021, **11**(1):352.
106. Munawar N, Ahsan K, Muhammad K, Ahmad A, Anwar MA, Shah I, Al Ameri AK, Al Mughairbi F: **Hidden Role of Gut Microbiome Dysbiosis in Schizophrenia: Antipsychotics or Psychobiotics as Therapeutics?** *International journal of molecular sciences* 2021, **22**(14).
107. Zhu G, Zhao J, Zhang H, Chen W, Wang G: **Probiotics for Mild Cognitive Impairment and Alzheimer's Disease: A Systematic Review and Meta-Analysis**. *Foods (Basel, Switzerland)* 2021, **10**(7).
108. Białecka-Dębek A, Granda D, Szmidt MK, Zielińska D: **Gut Microbiota, Probiotic Interventions, and Cognitive Function in the Elderly: A Review of Current Knowledge**. *Nutrients* 2021, **13**(8).
109. Cussotto S, Strain CR, Fouhy F, Strain RG, Peterson VL, Clarke G, Stanton C, Dinan TG, Cryan JF: **Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function**. *Psychopharmacology* 2019, **236**(5):1671-1685.
110. Bastiaanssen TFS, Cryan JF: **The Microbiota-Gut-Brain Axis in Mental Health and Medication Response: Parsing Directionality and Causality**. *International Journal of Neuropsychopharmacology* 2021, **24**(3):216-220.

111. Association WM: **World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects**. *JAMA* 2013, **310**(20):2191-2194.
112. Group IEW: **ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6 (R1) 1996.** . *ICH, Geneva* 1996.
113. **NIH Human Microbiome Project. Institute for Genome Sciences, University of Maryland School of Medicine.** [https://www.hmpdacc.org/hmp/resources/]
114. **Quadram Institute Best Practice in Microbiome Research** [https://quadram.ac.uk/best-practice-in-microbiome-research/]
115. Healey GR, Murphy R, Brough L, Butts CA, Coad J: **Interindividual variability in gut microbiota and host response to dietary interventions**. *Nutrition reviews* 2017, **75**(12):1059-1080.
116. Ottaviani JI, Britten A, Lucarelli D, Luben R, Mulligan AA, Lentjes MA, Fong R, Gray N, Grace PB, Mawson DH *et al*: **Biomarker-estimated flavan-3-ol intake is associated with lower blood pressure in cross-sectional analysis in EPIC Norfolk**. *Scientific reports* 2020, **10**(1):17964.
117. Dragsted LO, Gao Q, Scalbert A, Vergères G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres Lacueva C *et al*: **Validation of biomarkers of food intake-critical assessment of candidate biomarkers**. *Genes & nutrition* 2018, **13**:14.
118. Kuhnle GG: **Nutritional biomarkers for objective dietary assessment**. *Journal of the science of food and agriculture* 2012, **92**(6):1145-1149.
119. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C *et al*: **The neuroactive potential of the human gut microbiota in quality of life and depression**. *Nature microbiology* 2019, **4**(4):623-632.
120. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, Wang W, Tang W, Tan Z, Shi J *et al*: **Altered fecal microbiota composition in patients with major depressive disorder**. *Brain, behavior, and immunity* 2015, **48**:186-194.
121. Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J, Kennedy PJ, Beers S, Scott K, Moloney G *et al*: **Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat**. *Journal of psychiatric research* 2016, **82**:109-118.
122. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, Zeng L, Chen J, Fan S, Du X *et al*: **Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism**. *Molecular psychiatry* 2016, **21**(6):786-796.
123. Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, Soares JW: **Effects of Psychological, Environmental and Physical Stressors on the Gut Microbiota**. *Frontiers in microbiology* 2018, **9**:2013.
124. Tun HM, Konya T, Takaro TK, Brook JR, Chari R, Field CJ, Guttman DS, Becker AB, Mandhane PJ, Turvey SE *et al*: **Exposure to household furry pets influences the gut microbiota of infant at 3-4 months following various birth scenarios**. *Microbiome* 2017, **5**(1):40.
125. He Y, Wu W, Wu S, Zheng HM, Li P, Sheng HF, Chen MX, Chen ZH, Ji GY, Zheng ZD *et al*: **Linking gut microbiota, metabolic syndrome and economic status based on a population-level analysis**. *Microbiome* 2018, **6**(1):172.
126. Vujkovic-Cvijin I, Sklar J, Jiang L, Natarajan L, Knight R, Belkaid Y: **Host variables confound gut microbiota studies of human disease**. *Nature* 2020, **587**(7834):448-454.

127. Gibson MK, Crofts TS, Dantas G: **Antibiotics and the developing infant gut microbiota and resistome**. *Current opinion in microbiology* 2015, **27**:51-56.
128. Nogacka A, Salazar N, Suárez M, Milani C, Arboleya S, Solís G, Fernández N, Alaez L, Hernández-Barranco AM, de Los Reyes-Gavilán CG *et al*: **Impact of intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates**. *Microbiome* 2017, **5**(1):93.
129. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Sears MR, Becker AB, Scott JA, Kozyrskyj AL: **Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity**. *Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology* 2013, **9**(1):15.
130. Laforest-Lapointe I, Becker AB, Mandhane PJ, Turvey SE, Moraes TJ, Sears MR, Subbarao P, Sycuro LK, Azad MB, Arrieta MC: **Maternal consumption of artificially sweetened beverages during pregnancy is associated with infant gut microbiota and metabolic modifications and increased infant body mass index**. *Gut microbes* 2021, **13**(1):1-15.
131. Iszatt N, Janssen S, Lenters V, Dahl C, Stigum H, Knight R, Mandal S, Peddada S, González A, Midtvedt T *et al*: **Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month**. *Microbiome* 2019, **7**(1):34.
132. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, Turroni S, Biagi E, Peano C, Severgnini M *et al*: **Gut microbiome of the Hadza hunter-gatherers**. *Nature communications* 2014, **5**:3654.
133. Loughman A, Staudacher HM, Rocks T, Ruusunen A, Marx W, A OAN, Jacka FN: **Diet and Mental Health**. *Modern trends in psychiatry* 2021, **32**:100-112.
134. Phillips MCL, Deprez LM, Mortimer GMN, Murtagh DKJ, McCoy S, Mylchreest R, Gilbertson LJ, Clark KM, Simpson PV, McManus EJ *et al*: **Randomized crossover trial of a modified ketogenic diet in Alzheimer's disease**. *Alzheimer's research & therapy* 2021, **13**(1):51.
135. Klimenko NS, Tyakht AV, Popenko AS, Vasiliev AS, Altukhov IA, Ischenko DS, Shashkova TI, Efimova DA, Nikogosov DA, Osipenko DA *et al*: **Microbiome Responses to an Uncontrolled Short-Term Diet Intervention in the Frame of the Citizen Science Project**. *Nutrients* 2018, **10**(5).
136. Park JC, Im S-H: **Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics**. *Experimental & Molecular Medicine* 2020, **52**(9):1383-1396.
137. Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG *et al*: **Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system**. *Nature medicine* 2017, **23**(1):49-59.
138. Le Berre-Scoul C, Chevalier J, Oleynikova E, Cossais F, Talon S, Neunlist M, Boudin H: **A novel enteric neuron-glia coculture system reveals the role of glia in neuronal development**. *The Journal of physiology* 2017, **595**(2):583-598.
139. Raimondi I, Izzo L, Tunesi M, Comar M, Albani D, Giordano C: **Organ-On-A-Chip in vitro Models of the Brain and the Blood-Brain Barrier and Their Value to Study the Microbiota-Gut-Brain Axis in Neurodegeneration**. *Frontiers in bioengineering and biotechnology* 2019, **7**:435.
140. Putignani L, Gasbarrini A, Dallapiccola B: **Potential of multiomics technology in precision medicine**. *Current opinion in gastroenterology* 2019, **35**(6):491-498.

141. Marcos-Zambrano LJ, Karaduzovic-Hadziabdic K, Loncar Turukalo T, Przymus P, Trajkovik V, Aasmets O, Berland M, Gruca A, Hasic J, Hron K *et al*: **Applications of Machine Learning in Human Microbiome Studies: A Review on Feature Selection, Biomarker Identification, Disease Prediction and Treatment**. *Frontiers in microbiology* 2021, **12**:634511.
142. Ryan MJ, Schloter M, Berg G, Kinkel LL, Eversole K, Macklin JA, Rybakova D, Sessitsch A: **Towards a unified data infrastructure to support European and global microbiome research: a call to action**. *Environmental microbiology* 2021, **23**(1):372-375.
143. Ryan MJ, Schloter M, Berg G, Kostic T, Kinkel LL, Eversole K, Macklin JA, Schelkle B, Kazou M, Sarand I *et al*: **Development of Microbiome Biobanks - Challenges and Opportunities**. *Trends in microbiology* 2021, **29**(2):89-92.
144. **CORDIS EU research results. European Commission. EU Publications Office.** [https://cordis.europa.eu]
145. **GEMMA Multi-Omics Research in Autism. Funded by EU Horizon 2020 research & innovation programme. Grant agreement no. 8205033. 2021.** [https://www.gemma-project.eu]
146. Troisi J, Autio R, Beopoulos T, Bravaccio C, Carraturo F, Corrivetti G, Cunningham S, Devane S, Fallin D, Fetissov S *et al*: **Genome, Environment, Microbiome and Metabolome in Autism (GEMMA) Study Design: Biomarkers Identification for Precision Treatment and Primary Prevention of Autism Spectrum Disorders by an Integrated Multi-Omics Systems Biology Approach**. *Brain sciences* 2020, **10**(10).
147. **ONCOBIOME Microbiota against cancer international research program. Funded by EU Horizon 2020 research & innovation programme. Grant agreement no. 825410. 2021.** [https://www.oncobiome.eu]
148. **MICROB-PREDICT. Funded by EU Horizon 2020 research & innovation programme. Grant agreement no. 825694.** [https://microb-predict.eu.]
149. **European Life Sciences Institute (ILSI Europe)** [https://ilsi.eu/]
150. **International Scientific Association for Probiotics and Prebiotics.** [https://isappscience.org]
151. EFSA Panel on Nutrition NF, Allergens F, Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ *et al*: **Safety of pasteurised Akkermansia muciniphila as a novel food pursuant to Regulation (EU) 2015/2283**. *EFSA Journal* 2021, **19**(9):e06780.