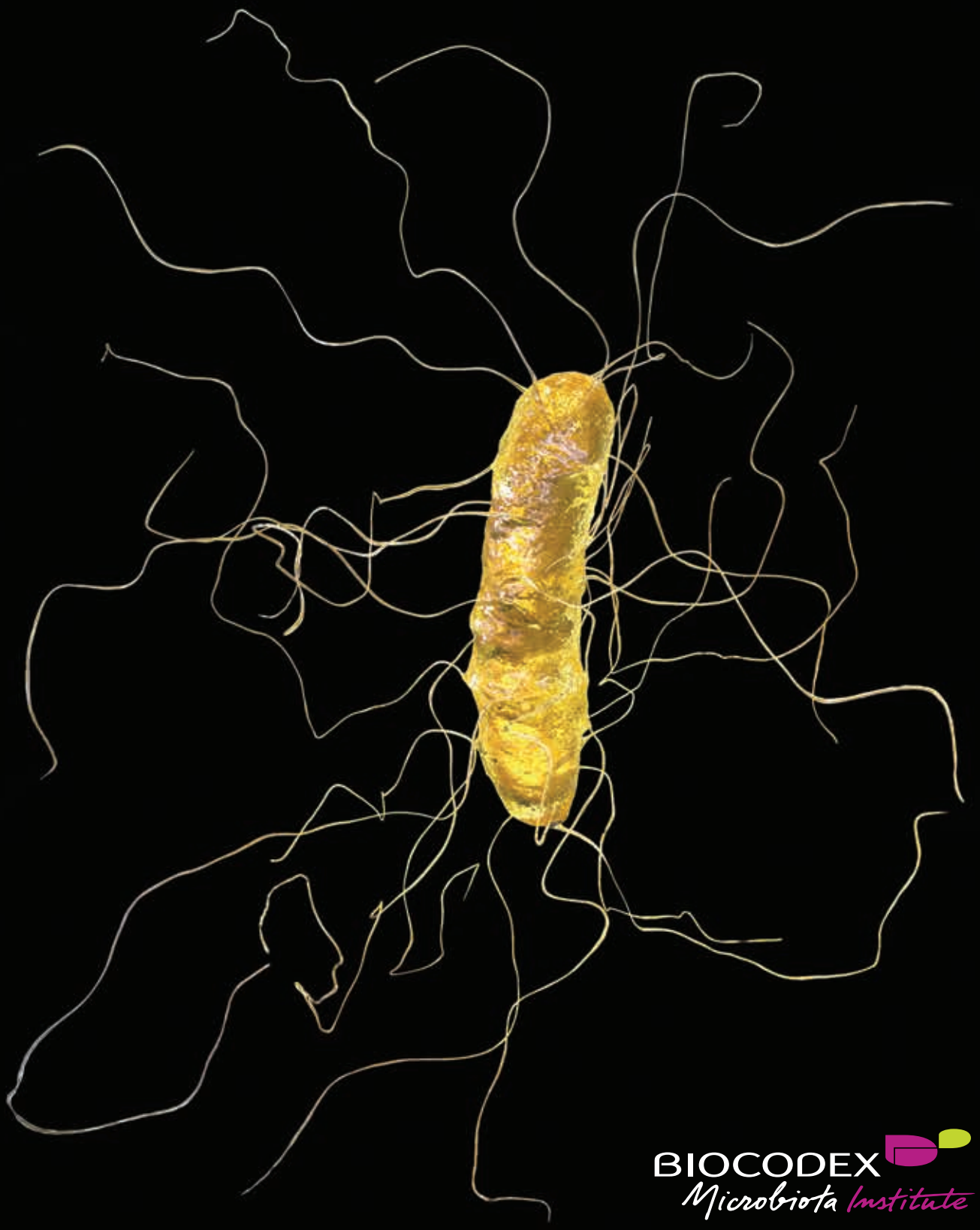
MICROBIOTA

5 BIOCODEX NEWSLETTER | OCTOBER 2018



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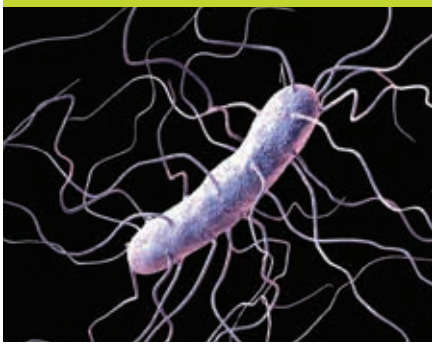

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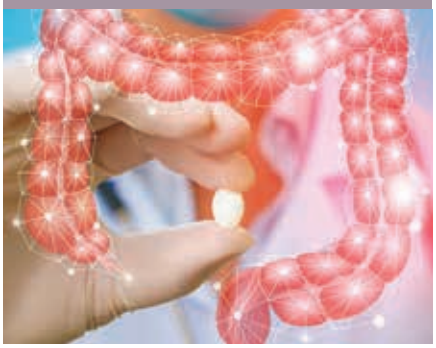

NEWS

CALL FOR PROPOSALS "PRO" OF THE NET

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EDITO

Dr Maxime Prost
France Medical Affairs Director

Camille Aubry, PhD
International Medical Affairs Manager

> **“PARKINSON’S AND ALZHEIMER’S DISEASES COULD ARISE FROM THE GUT”**

Dear readers, the gut microbiota is known for long to have the ability to act on our brain. Nevertheless, a bidirectional gut-brain communication system in which our gut microbiota participates has only been identified and studied as a “microbiota - gut - brain axis” since a dozen years. More recently, studies have suggested that dysbiosis could contribute to the pathophysiology of central nervous system diseases such as anxiety and depressive disorders, autism spectrum disorders, Alzheimer’s disease or Parkinson’s disease, while these last two diseases tend to be spontaneously related to brain damage.

In 2017, American researchers have named “mapranosis” (for Microbiota Associated PRoteopathy And Neuroinflammation) a concept relating the microbiota to Parkinson’s and Alzheimer’s diseases.

Their research has highlighted the involvement of gut bacteria producing amyloid proteins likely to increase the production of α-synuclein in the gut. This protein, in an inadequate conformation, could be transported *via* the gut-brain axis and promote the formation of aggregates in the brain, leading to neurodegenerative damage.

As part of this scientific stream, Prof. John F. Cryan (APC Microbiome Institute, Cork, Ireland) is particularly interested in the role of the microbiota in neurodevelopment, neuroinflammation and aging processes. In this newsletter, he explains how Parkinson’s and Alzheimer’s diseases could originate from the gut and describes dysbiosis associated with these neurodegenerative diseases, as well as the possible role of the vagus nerve.

For his part, Prof. Emmanuel Mas (Children’s Hospital, Toulouse, France) discusses the role of the microbiota - gut - brain axis in psychiatric diseases. He comments on the results of a recent Chinese study reporting gut dysbiosis in children with attention deficit hyperactivity disorder (ADHD). According to this work, the abundance of *Faecalibacterium* in the gut microbiota could be negatively correlated with the severity of ADHD.

As evidenced by these contributions, the research and application perspectives appear promising, although the mechanisms by which the microbiota influences - or is correlated with - these diseases remain to be further explored.

Enjoy your reading.

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OVERVIEW

THE GUT MICROBIOME AND NEURODEGENERATION

The past decade has seen an explosion of research in the role of the gut microbiota in modulating brain health and disease. Although most research has focused on stress-related disorders, such as anxiety, depression and irritable bowel syndrome, a growing body of research, albeit largely preclinical, also implicates the microbiota as a disease moderator in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. In tandem, research has shown that the microbiome plays a critical role in key brain processes involved in neurodevelopment, neuroinflammation, and aging. Currently, research is heavily focused on a better understanding of the precise mechanisms of how the gut talks to the brain and how it may lead to increased susceptibly to brain disorders.

By Prof. John F. Cryan
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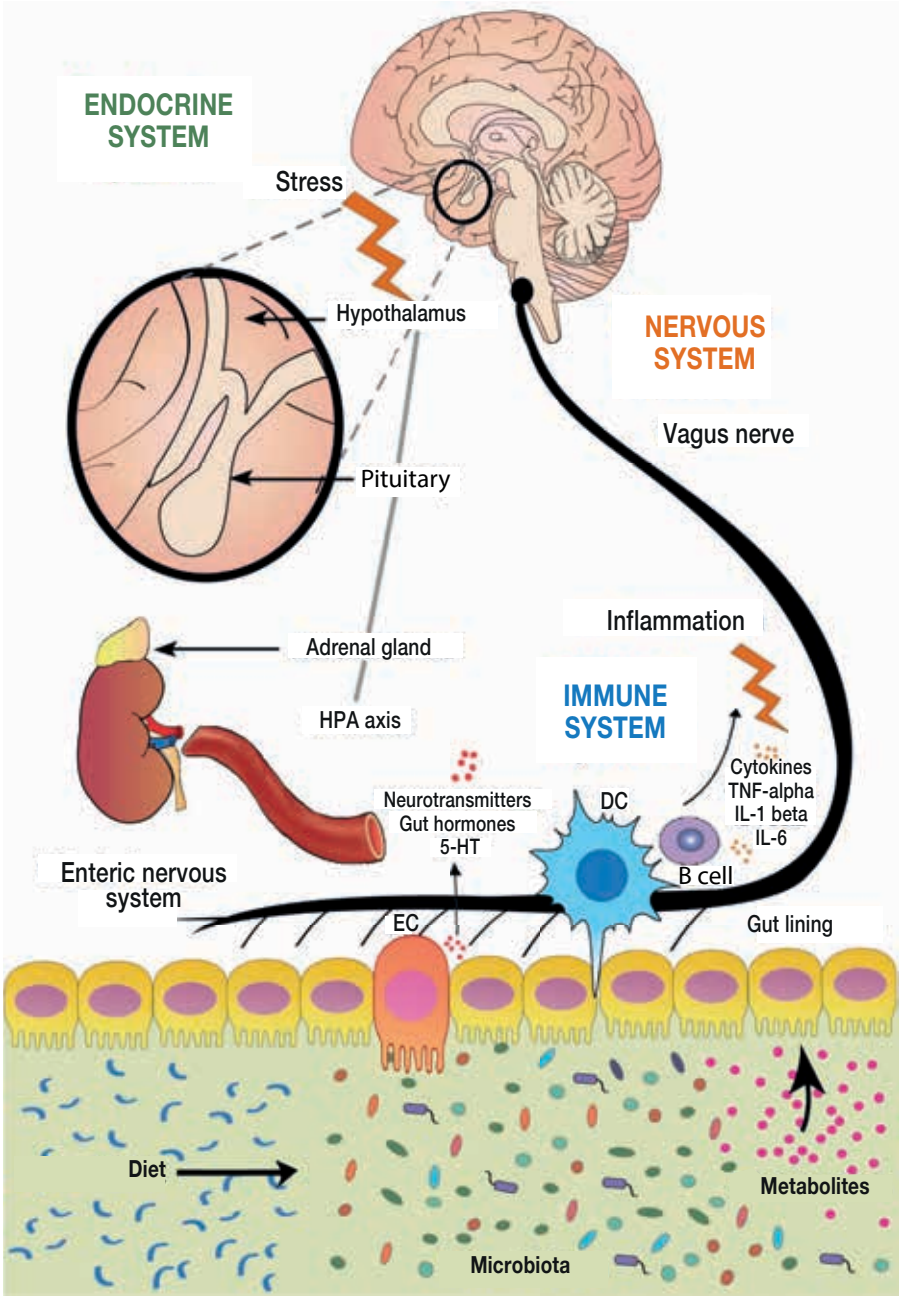
In medicine, the disciplines of neurology and microbiology have largely matured along distinct parallel trajectories, only interfacing in pathological situations when direct infections of the central nervous system occur. However, over the past decade, there has been a revolution in biomedicine with the realisation that the gut microbiota (the trillions of bacteria that reside within the gut) plays a key role in maintaining homeostasis and in programming the major body systems, including even the brain.

A growing body of research is focused on illuminating the bidirectional communication pathways between gut bacteria and the central nervous system, the microbiota–gut–brain axis, however, this is a field in its relative infancy [1]. Changes in the microbiome, its metabolites, and its interaction with the gut-brain axis are associated with a wide array of illnesses including brain disorders. Studying the microbiome requires close collaborative efforts of clinicians with basic scientists and bio-informaticians and works best when traditional discipline barriers between neurology, gastroenterology, and microbiology are broken down.

In preclinical research, a number of experimental models have proven essential to evaluate the microbiome within the context of brain and behaviour, including prebiotic and probiotic intervention, antibiotic administration, faecal transplantation, and the use of germ-free and gnotobiotic animals [1]. In clinical research, most data, especially in the area of neurology, have relied on cross-sectional studies of the microbiome in patients with disease versus healthy age-matched individuals.

▶ FIGURE 1

Pathways of communication between the microbiome and the brain.
Multiple direct and indirect pathways exist through which the gut microbiota can modulate the gut–brain axis. These include endocrine (cortisol), immune (cytokines), and neural (vagus and enteric nervous system) pathways. Neuroactive bacterial metabolites can also modulate the brain and behaviour. Figure produced by Dr. Kiran Sandhu.



HPA axis: hypothalamic–pituitary–adrenal axis; EC: enteroendocrine cell; DC: dendritic cell

THE MICROBIOTA-GUT-BRAIN AXIS-MECHANISMS OF COMMUNICATION

Much experimental effort is being placed at trying to dissect the pathways of communication between the gut and the brain. The gut bacteria influences central processes through a variety of mechanisms (**Figure 1**). Firstly, the microbiota's ability to synthesize neurotransmitters (i.e. γ-amino butyric acid (GABA), noradrenaline, and dopamine) is an important avenue of communication. Secondly, microbes play a key role in activation of the immune system which can play a fundamental role in ageing, neurological disorders, and neurodegeneration. Finally, microbes produce metabolites including short-chain fatty acids (SCFAs) which are essential for the gut, the immune system, and potentially brain health. Moreover, the gut microbiota and the brain are linked through the vagus nerve, and through the modulation of key dietary amino acids, such as tryptophan.

Given the close association between the gut microbiota and the brain, it is not at all surprising that gut bacteria play key roles in neurological and psychiatric diseases. The strongest evidence for a role of the microbiome in brain function derives from germ-free mice. Studies from a number of research groups in Canada, Sweden, and Ireland have shown that, in germ-free animals, the brain fails to develop normally in the absence of the gut microbiome [2]. Moreover, fundamental brain processes, such as myelination, adult neurogenesis, and microglia activation, have also been shown to be critically dependent on microbiota composition.

MICROBIOTA & AGEING

The relationship between the microbiome and the ageing brain is also receiving much attention, which is of particular interest in the field of neurology as many neurological and neurodegenerative disorders occur in old age. Once again, the concept of linking the microbiome to healthy ageing is not new, and this was championed over 100 years ago by Nobel prize-winning immunologist, Elie Metchnikoff, who observed that the villagers in a certain region of Bulgaria lived unusually long lives, a fact he attributed to the presence of lactic acid bacteria in their diet. We have recently revisited Metchnikoff's original study [3], and shown that the behavioural deficits in aged animals coincide with changes in the microbiome. Moreover, the ELDERMET study has shown that the composition of the gut bacteria in the elderly correlated with their overall health – frailty and the immune system [4]. The greater the microbiome diversity, the better the health outcomes. These investigators went one step further to investigate what is driving the diversity of the microbiome, which they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determined to be a diverse diet. As people eat processed bland food (often in nursing homes), the diversity of their microbiomes is reduced, whereas those with a diet rich in fruit and vegetables have better outcomes [4]. A decline in microbial diversity is associated with a concomitant increase in microglial activation which is correlated with brain mass differences in the mouse. This contributes to an age-associated inflammatory response known as "inflammaging", which in turn has been associated with neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD). Furthermore, the microbiome has been shown to regulate microglia activation; germ-free mouse brains were shown to express defective microglia, which was partially rescued upon restoration of the microbial community to control levels [5].

PARKINSON'S DISEASE (PD)

There is a growing realisation that the aetiology of PD may actually originate in the gut [6]. Indeed, α-synuclein, the protein aggregate hallmark of PD pathology in the brain, has also been identified in the mucosal and submucosal nerve fibres and ganglia of Parkinsonian patients, with some preclinical evidence even suggesting that α-synuclein in the gut can be transported to the brain via the vagus nerve. Moreover, functional gut symptoms, such as constipation, often occur as prodromal symptoms years before any motor symptoms emerge.

Since Scheperjans and colleagues first showed that there are specific alterations in microbiome composition in PD [7], many more studies have emerged [8]. However, to date, there is no consensus as to whether a specific microbial signature exists. When mice were colonized with the microbiota of PD patients via faecal microbiota transplantation, they developed motor deficits and neuroinflammation; two hallmark symptoms of PD [9]. Additionally, symptoms improved when the mice were treated with antibiotics. These studies implicated short-chain fatty acids as drivers of the neuroinflammatory processes in PD [9].

The vagus nerve is particularly well placed as the conduit for signals from the gut to the brain, involving either microbial or prion-like translocation of α-synuclein. Indeed, epidemiological studies based on Danish and Swedish patient registries have shown that truncal vagotomy is protective against PD. Although there has been much excitement in the field, caution is needed when examining the available data as it is largely derived from small cohorts and lacks a longitudinal perspective. Many more mechanistic studies are needed to understand how changes in the microbiota can moderate both the motor and non-motor symptoms of PD and its co-morbidities [10].

ALZHEIMER'S DISEASE (AD)

The concept that microbes may play a role in the pathophysiology of AD is not new and the notion that amyloid, the aggregation of which is one of the key hallmarks of AD, may act as an antimicrobial peptide in the brain is an intriguing concept [11]. However, it is ethically difficult from the

perspective of Koch's postulate to prove whether there is any infective cause of neuroinflammation and neurodegeneration. As in PD, the relationship between gut proteins and brain health is receiving increased attention with the realization that amyloid-like proteins can be produced by bacteria which has been shown to increase α-synuclein pathology in aged rats and worms [12]. Much more work is needed to validate such strategies in humans.

Recently, cross-sectional studies have identified that *Escherichia/Shigella* bacterial taxa, which are associated with mediating inflammation, were increased in faecal samples from AD patients relative to control subjects. Moreover, the microbiota changes correlated with pro-inflammatory cytokine levels in whole blood [13]. Such results suggest a causal link between dysregulation of the microbiota and systemic inflammation, which may initiate or exacerbate neurodegeneration in the brain in AD. However, these are still relatively small studies and much more research is needed in larger cohorts to assess the causal relationship between the gut microbiome and AD.

In parallel, a number of transgenic mouse models of AD have been shown to have an altered microbiome [14]. Seminal studies in germ-free mice showed that there is a marked absence of amyloid plaque build-up and neuroinflammation when microbes are not present [14]. Similarly, chronic treatment of APP/PS1 transgenic mice with an antibiotic cocktail reduced microglial and astrocyte accumulation surrounding amyloid plaques in the hippocampus and led to a decrease in insoluble Aß plaques [15]. Together, these studies unequivicolly place the microbiome as a regulator of key molecular components of AD.

FUTURE PERSPECTIVES

It is clear that the microbiome is critically important for the appropriate development and maintenance of brain function. Moreover, as outlined above, there is accumulating evidence from both animal and clinical studies implicating the microbiome in a variety of neurological and neurodegenerative diseases. Given the marked effects of the microbiota in regulating brain function, it is plausible that its composition affects the progression, susceptibility, and treatment of almost all neurological disorders. Nonetheless, there are marked gaps in our knowledge regarding the role of the microbiome in other neurodegenerative diseases, such as amyotrophic lateral sclerosis or Huntington's disease, and caution is needed to not over-interpret such studies. The field needs to move away from corelative studies towards mechanistic causal approaches. Moreover, more interventional studies are needed using probiotic strains and prebiotics, and even faecal microbiota transplants may potentially be important in the field. It is possible that similar approaches could target different disorders, for example, the modulation of T lymphocyte signalling to the brain may be useful in dampening down the neuroinflammatory status in patients following stroke, as well as in patients with AD and during ageing.

With regards to clinical neurology, many patients are on multiple medications and there is a growing understanding of the relationship between the microbiome and drug action. Thus, all studies should aim to differentiate between the impact of drugs and that of disease on the microbiome. Moreover, temporal studies with presymptomatic individuals will be important to determine the potential role of the microbiome as a biomarker of disease.

Diet is perhaps one of the greatest factors that influences the microbiome. As many neurological disorders affect appetite, swallowing, and diet in general, it is essential to have good nutritional data for all human studies in the future. In addition, this will further enable a better understanding of the relationship between diet, the microbiome, and the brain, which is critical both in early life and as we age.

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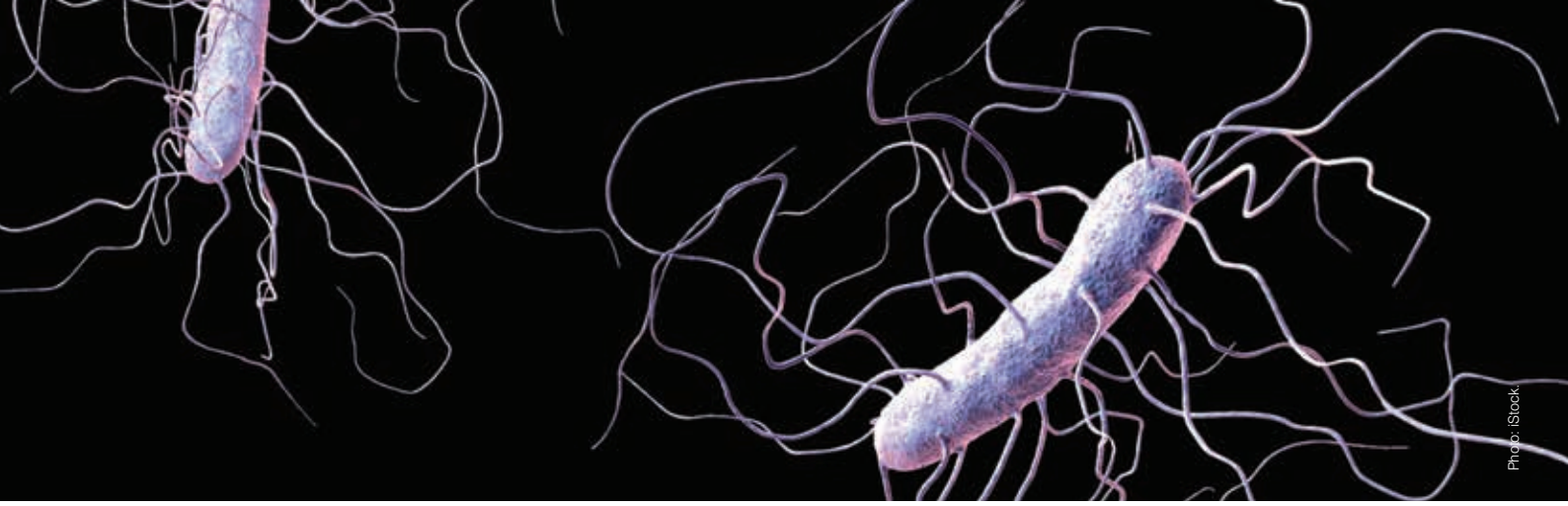

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COMMENTED ARTICLE
ADULTS' SECTION

THE FOOD ADDITIVE TREHALOSE INCREASES THE VIRULENCE OF EPIDEMIC CLOSTRIDIUM DIFFICILE

Comments of the original article by Collins et al. (Nature 2018) [1]

By Prof. Harry Sokol
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Clostridium difficile **has recently increased to become a dominant nosocomial pathogen in North America and Europe, although little is known about what has driven this emergence. Here the authors show that two epidemic ribotypes (RT027 and RT078) have acquired unique mechanisms to metabolize low concentrations of the disaccharide trehalose. RT027 strains contain a single point mutation in the trehalose repressor* that increases the sensitivity of this ribotype to trehalose by more than 500-fold.**

Furthermore, dietary trehalose increases the virulence of a RT027 strain in a mouse model of infection. RT078 strains acquired a cluster of four genes involved in trehalose metabolism, including a PTS permease that is both necessary and sufficient for growth on low concentrations of trehalose. The authors propose that the implementation of trehalose as a food additive into the human diet, shortly before the emergence of these two epidemic lineages, helped select for their emergence and contributed to hypervirulence.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

Whole-genome sequencing analysis of *C. difficile* RT027 strains has shown that two independent lines have emerged in North America between 2000 and 2003 [2]. A comparison with pre-epidemic RT027 strains has shown that the epidemic strains have acquired a mutation in the *gyrA* gene, that has led to an increased resistance to fluoroquinolone antibiotics. While the development of this resistance has certainly played a role in the spread of RT027 strains, it has also been observed in non-epidemic *C. difficile* ribotypes and identified in strains from the middle of the 1980s. Thus, other factors have probably contributed to the emergence of epidemic RT027 strains. The prevalence of a second *C. difficile* ribotype, RT078, has increased tenfold in hospitals and clinics between 1995 and 2007, and has been associated with increased severity [3]. However, the mechanisms involved in the increased virulence remain unknown. Since RT027 and RT078 lines are phylogenetically distant from each other, the changes that have simultaneously led to an increase in prevalence and severity of infection might be due to independent mechanisms.

WHAT ARE THE MAIN RESULTS OF THIS STUDY?

RT027 strains have been shown to have a competitive advantage over other strains both *in vitro* and in mouse models of *C. difficile* infection. To investigate the mechanisms involved, the authors examined the use of different carbon sources by the various strains and highlighted an increased capacity of RT027 strains to metabolise the disaccharide, trehalose. By comparing the

* A repressor is a protein that negatively regulates one or more genes by binding to a specific sequence on the DNA. This binding prevents transcription of the messenger RNA by the RNA polymerase, and therefore suppresses the expression of downstream genes.

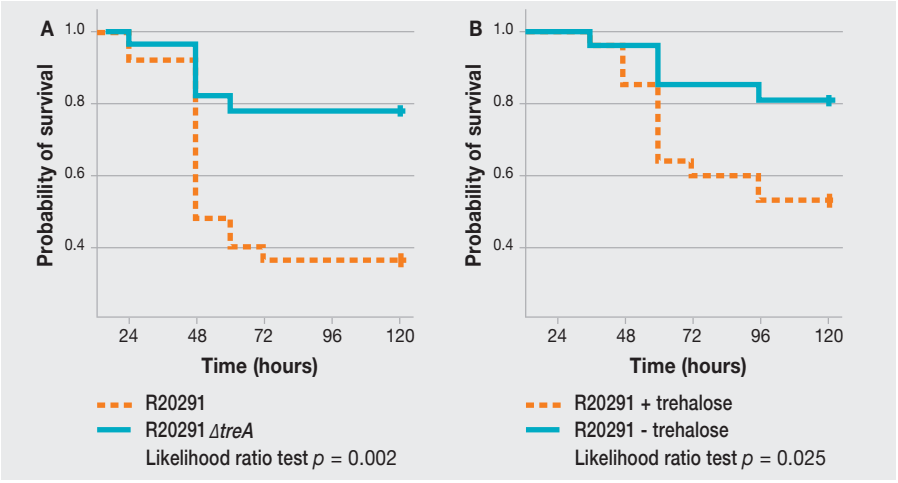
KEY POINTS

- *C. difficile* infection outbreaks with hypervirulent epidemic strains (RT027 and RT078) emerged in the early 2000s.
- Trehalose is a highly resistant disaccharide that has been used in the food industry since 2000.
- RT027 and RT078 strains have acquired a competitive advantage that allows them to metabolise trehalose, even at low concentrations, which increases their virulence.

▼ FIGURE 1

The metabolism of trehalose increases virulence

A: *Mice infected with RT027 R20291Δ treA strain, unable to metabolise trehalose, have a significantly reduced risk of mortality compared to mice infected with R20291.* **B**: Mice infected with R20291 have a higher risk of mortality when trehalose is added to food.



genomes of many *C. difficile* strains, the authors identified a putative responsible enzyme, phosphotrehalase enzyme (TreA), which metabolises trehalose-6-phosphate into glucose and glucose-6-phosphate. The authors then observed that this gene was activated in RT027 strains at a concentration of trehalose 500-fold lower than that for the other *C. difficile* strains. More detailed analyses have identified a polymorphism in the TreA transcriptional repressor (TreR) in all RT027 strains and in other closely related strains responsible for epidemics in Europe and Australia. To determine whether the capacity to metabolise trehalose has an impact on virulence, the authors administered it orally to mice transplanted with human microbiota and infected with either a RT027 strain (R20291) or the same strain deleted for the *TreA* gene (R20291Δ TreA), and therefore unable to metabolise trehalose. Mortality was much lower with the R20291Δ TreA strain (**Figure 1**). In a second experiment, the authors infected mice transplanted with human microbiota with the RT027 strain (R20291) in the presence or absence of trehalose in the drinking water (given at a dose equivalent to that received during a standard human meal). Mortality was much higher in the presence of trehalose. The two combined experiments confirm the assumption that trehalose in food contributes to the severity of RT027 strains.

The genetic analysis of RT078 strains demonstrated an insertion of 4 genes, encoding a second copy of phosphotrehalase (TreA2) and its repressor (TreR2), as well as 2 other related genes. A mutation- and overexpression-based approach confirmed that this insertion was responsible for the capacity of RT078 strains to grow in the presence of trehalose.

WHAT ARE THE PRACTICAL CONSEQUENCES?

Trehalose is an extremely stable sugar that is resistant to both high temperatures and hydrolysis. Considered ideal for use in the food industry, it has been mainly used since 2000, when a new low-cost production process was discovered [3]. Its use has been authorized in food by the Food and Drug Administration (FDA) in 2000 and by European institutions in 2001. The wide adoption of trehalose coincides with the emergence of infection outbreaks with RT027 and RT078 strains. Overall, the results suggest a causal role for trehalose in food in the emergence of these hypervirulent epidemic strains of *C. difficile*.

CONCLUSION

The wide adoption of trehalose in the food industry coincides with the emergence of infection outbreaks with *C. difficile* RT027 and RT078 strains. These strains have acquired the capacity to metabolise trehalose at low concentrations, conferring them a selective advantage over other strains in an ecosystem in which trehalose has been introduced. This capacity to metabolise trehalose increases their virulence. Overall, the results suggest a causal role for trehalose in food in the emergence of these hypervirulent epidemic strains of *C. difficile*.

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COMMENTED ARTICLE
CHILDREN' SECTION

By Prof. Emmanuel Mas
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GUT MICROBIOTA PROFILES IN UNTREATED CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER

Comments of the original article by Jiang et al. (Behavioural Brain Research 2018) [1]

Although increasing evidence suggests a role for the gut microbiota in neurodevelopment, the actual structure and composition of microbiota in children with attention-deficit/hyperactivity disorder (ADHD) remain unclear.

Thus, the present study aimed to define the characteristics of gut microbiota in treatment-naive children with ADHD and to assess their relationship with the severity of ADHD symptoms. High-throughput pyrosequencing was used to investigate the microbiota composition in fecal matter from 51 children with ADHD and 32 healthy controls (HC).

An operational taxonomical unit (OTU)-level analysis revealed a significant decrease in the fractional representation of *Faecalibacterium* in children with ADHD compared to HC. In individuals with ADHD, the abundance of Faecalibacterium was negatively associated with parental reports of ADHD symptoms. However, there was no significant difference in alpha diversity between the ADHD and control groups.

This present findings support the involvement of microbiota alteration in psychiatric diseases and *Faecalibacterium* may represent a potential novel marker of gut microbiota in ADHD. Future studies are needed to validate these findings and to elucidate the temporal and causal relationships between these variables.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

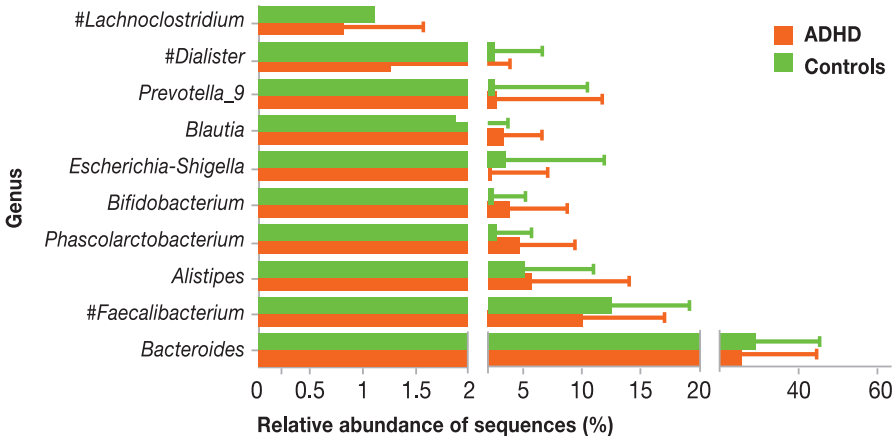
In recent years, the gut and gut microbiota have gained important status in human biology, perceived by some authors as a "second or third brain". The gut-brain axis is being studied more precisely, and impairments in its function are being investigated with regards to various neurological and psychiatric disorders. Gut microbiota impairment has been highlighted in autism as well as in other psychiatric disorders. Regarding ADHD, no study has precisely analyzed the gut microbiota, but some authors suggest that a dysbiotic gut microbiota may play a role (symptoms improve under probiotics and worsen under antibiotics, and delivery by Caesarean section is a risk factor for the disease).

WHAT ARE THE MAIN RESULTS OF THIS STUDY?

The authors included 51 children with ADHD, aged 6-10 years, and 32 matched controls from a Chinese hospital, from May 2015 to December 2016. The diagnosis of ADHD was based on the Kiddie-SADS-PL questionnaire that is included in the diagnostic manual of mental disorders: Diagnostic and Statistical Manual of Mental Steenvoorde-IV classification sys-

▼ FIGURE 1

Distribution of bacterial genera (≠ p <0.05).



KEY POINTS

- The gut-brain axis plays an important role in various neurological and psychiatric disorders.
- For ADHD, a disturbance of the gut microbiota (including a decrease in *Faecalibacterium*) may play a role, as suggested by this pilot study.
- Further studies are needed to confirm this result and to assess whether correcting this dysbiosis will improve ADHD symptoms.

tem (DSM-IV). Parents completed a questionnaire to assess the severity of ADHD symptoms (Conners Parent Rating Scales). Children with specific diets, probiotic or antibiotic treatment within the previous 2 months, digestive disorders, depressive or anxious symptoms, obesity, an atopic background, and/or medically treated for their ADHD were excluded.

No difference was identified between the two groups for age, sex, BMI, delivery, or feeding formula (breast-feeding). The analysis of the gut microbiota, carried out through pyrosequencing of 16S RNA and OTU analysis, showed no difference in bacterial diversity (alpha and beta). The four major phyla in all samples were *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinetobacteria*, with no difference between ADHD children and controls. However regarding genera, the levels of *Faecalibacterium, Lachnoclostridium* and *Dialister* were reduced in ADHD children (**Figure 1**). The abundance of *Faecalibacterium* negatively correlated with the severity of ADHD and the hyperactivity index (**Figure 2**).

WHAT ARE THE PRACTICAL CONSEQUENCES?

If disturbance of the gut microbiota is involved in ADHD (as in other disorders), care should be taken to provide appropriate prescription of antibiotics in children, *a fortiori* in infants, to prevent further development of these disorders.

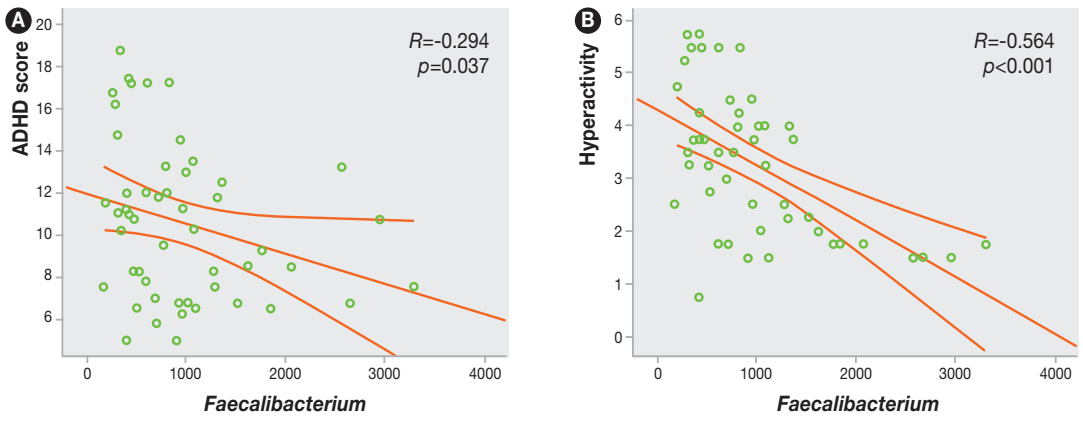
In ADHD, it might be useful to target *Faecalibacterium* by increasing its level in the gut. Dietetically, this is promoted by a Mediterranean diet and probably reduced by a Western diet. In addition to this dietary approach targeted on *Faecalibacterium*, it is also necessary to reduce gut inflammation, which is supported by a decrease in *Faecalibacterium*.

CONCLUSION

This pilot study has shown that the gut microbiota is disturbed in ADHD. This dysbiosis was more specifically related to *Faecalibacterium*, a genus that negatively correlated with the severity of ADHD symptoms.

▼ FIGURE 2

Correlation between *Faecalibacterium* and symptoms of ADHD (A) and hyperactivity (B).



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ESPGHAN 51ST ANNUAL MEETING

of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition

CONGRESS REVIEW

ESPGHAN

By Dr. Solange Heller Rouassant
Pediatrician with specialty in Pediatric Gastroenterology and Nutrition México City, México
Mexican Councilor of NASPGHAN

51ST ANNUAL MEETING ESPGHAN

9-12 MAY 2018

GENEVA,SWITZERLAND

GASTRIC MICROBIOTA AND *HELICOBACTER PYLORI*

Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and *Fusobacteria* are the most abundant phyla in *H. pylori* positive and *H.pylori*-negative patients and this gastric microbiota may play a role in the *H. pylori*-associated carcinogenicity [1]. Alarcón [2] characterized gastric microbiota in children with and without *H. pylori*; when detected, *H. pylori* dominated the microbial community, but when absent, there was a higher bacterial richness and diversity.

GUT MICROBIOTA IN EARLY LIFE

Development of gut microbiota in early life is influenced by delivery mode, breast milk or formula feeding, antibiotic use, timing of introduction of solid foods and cessation of milk feeding. The gut microbiota of a newborn is transiently dominated by *Enterobacteriaceae* and *Staphylococcus* and very soon by *Bifidobacterium* and lactic acid bacteria. *Bifidobacterium*-dominated microbiota continues until complementary feeding starts [3].

• Dukanovic [4] showed that C-section delivery and exclusive breast-feed infants have a relatively low abundance of *Bacteroides* in the infant stool; *Bacteroides* species were detected in 73% vaginal and 16% C-section samples.
• Collado [4] demonstrated shared features between microbiota in maternal-infant pair and breast milk, suggesting a microbial transfer during lactation. Specific strains of *Bacteroides, Bifidobacterium, Staphylococcus* and *Enterococcus* genera were isolated from maternal infant gut and specific strains of *Staphylococcus, Lactobacillus, Enterobacter* and *Acinetobacter* from paired breast milk at 2 months of age.

SPROBIOTICS AND SYNBIOTICS SUPPLEMENTATION IN EARLY LIFE

• It has been reported that association between gut microbiota composition in early life and development of diseases exists [5]. Studies on early infant gut microbiome have shown that *antibiotic resistant gene carriage* is acquired early and may have long term sequelae.

• Exclusively breastfed infants were supplemented with *Bifidobacterium longum subsp. infantis* EVC001 (Casaburi [4]) as a targeted probiotic capable of remode-

ling gut microbiome with potential reduction of antibiotic gene reservoirs. It was concluded that colonization of high levels of this strain is a safe and non-invasive method to decrease a reservoir of genes that confer antibiotic resistance.

• High levels of *Bifidobacterium longum* subsp. *infantis* in breast-fed infants, regardless of delivery mode, remained stable through the first year of life if breastfeeding was continued [4].

INFANT FORMULA SUPPLEMENTATION WITH PREBIOTICS AND PROBIOTICS

Human milk oligosaccharides (HMOs) are unconjugated, solid and abundant compounds breast milk. The spectrum of HMOs in mother's milk especially related to the mother's secretor status modulates the bifidobacterial composition of the infant's gut.

Gut of formula-fed infants has a lower relative abundance of *Bifidobacteria* and a higher microbial diversity. The use of prebiotics in infant formula increases the bifidobacterial fraction in the infant's gut. Currently, available prebiotics (galacto- (GOS) and fructooligosaccharides (FOS)) are metabolized by *Bifidobacteria*, but not by the human host [5].

• Puccio [6] supplemented an infant formula with 2 "fucosyllactose and lacto-N-neotetraose", commonly found in human milk, with good results. An infant formula with GOS, FOS and *Bifidobacterium breve* M-16V compensates the delayed *Bifidobacterium* colonization in C-section-delivered infants, modulates gut microbiota and emulates conditions observed in vaginally born infants [6].

• Comparison of two different infant formula supplemented with prebiotics only or prebiotics and probiotics showed similar gut microbiota profiles than breast-fed infants (Tims & Phavichir [4]).

COW'S MILK ALLERGY (CMA) PREVENTION AND MANAGEMENT

• Probiotics have been recommended for prevention of CMA even though more evidences are needed. *Lactobacillus rhamnosus* HN001 or *Bifidobacterium lactis* HN019 were administered daily from 35 weeks gestation to 6 months postpartum in mothers, and from birth until the age of two in children. Children that ingested *Lactobacillus rhamnosus* had a significant reduction of prevalence of eczema in childhood (Wickens [4]).

• Extensive hydrolyzed infant formulas have been supplemented with *L. rhamnosus* GG for the management of IgE mediated CMA and development of immune tolerance. Clinical studies in healthy infants and infants suffering CMA showed that synbiotic-supplemented aminoacid-formulas (AAF) are hypoallergenic, well tolerated, and warranty normal growth.

• Results of a multicenter, double blind, randomized controlled trial in infants with non IgE mediated CMA was presented (Candy [7]). Infants received a hypoallergenic, AAF formula containing a prebiotic blend of chicory-derived neutral oligofructose and long-chain *Bifidobacterium breve* M-16 V. At week 8, significant differences on gut microbiota composition were present between groups, with higher percentages of *Bifidobacteria* in the symbiotic-AAF supplemented group. Modulation of gut microbiota using specific synbiotics may improve symptoms in infants with CMA.

INFANTILE COLIC

Evidence suggests that altered gut microbiota affects gut motor function and induce gas production in infants, resulting in abdominal pain/colic. Manipulation of gut microbiota may play a role in the management and prevention of infantile colic.

• A Cochrane systematic review [4] of prophylactic probiotics in infantile colic included studies of *Lactobacillus reuteri* DSM, multi-strain probiotics, *Lactobacillus Rhamnosus, Lactobacillus paracasei* and *Bifidobacterium animalis*. This meta-analysis showed no differences in the use of several probiotics on primary outcome. However, a wider analysis suggested efficacy of probiotics for infantile colic (Ong [4]).

Photo: 123RF

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CONGRESS REVIEW

By Dr. Jari Punkkinen
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3RD MEETING OF THE FEDERATION OF NEURO-GASTROENTEROLOGY

SEPTEMBER 2018

AMSTERDAM, THE NETHERLANDS

Selected excerpts about Irritable Bowel Syndrome and Gut Microbiota

GUT MICROBIOTA DYSBIOSIS IN IBS

Microbiota dysbiosis and its relation to irritable bowel syndrome (IBS) was discussed during various sessions of the conference. In particular, Prof. Magnus Simrén and Prof. Uday Ghoshal highlighted some features related to microbiota composition of IBS patients. Based on several studies, IBS patients have been shown to have a low microbial richness compared to healthy individuals, and *Methanobacteriales* may be undetected and methane production low in such patients [1]. Furthermore, a subset of IBS patients present dysbiosis with increased *Firmicutes* and *Bacteroides* enterotypes compared to healthy individuals with enriched Clostridiales and Prevotella enterotypes. However, an important question is not only which bacteria are associated with IBS but what they do in the gut and how they are involved in mechanisms of visceral hypersensitivity, neuro-motor dysfunction, increased permeability, and low-grade inflammation. Small intestine bacterial overgrowth (SIBO) may be the cause of IBS in some patients and a current challenge is to improve screening for these patients as upper gut aspirate culture is difficult to perform and not always available. The glucose hydrogen breath test may be used to identify such patients, and this would appear to be more accurate than the lactulose hydrogen breath test [2].

GUT MICROBIOTA MODULATION IN IBS

Can the gut microbiome be changed for therapeutic purposes and could it relieve IBS symptoms? Options to modulate gut microbiota include antibiotics, probiotics, synbiotics, altering gut motility, dietary manipulation, fecal transplantation, and use of bacteriophages. These therapies were discussed in the presentations of Prof.

Uday Ghoshal and Prof. Giovanni Barbara. A role for antibiotics is most apparent in IBS-patients with SIBO. Both norfloxacin and rifaximin are significantly more effective in reducing IBS symptoms in SIBO-positive than SIBO-negative patients.

In IBS patients without constipation, according to Target 1 and 2 studies, rifaximin relieves global IBS-symptoms, and bloating. Target 3 and further studies have shown that rifaximin can be used repeatedly in relapsing IBS-D without loss of effect or appearance of bacterial resistance [3, 4]. Moreover, rifaximin transiently reduces bacterial counts in the feces but it also seems to have an eubiotic effect increasing *Lactobacillaceae* abundance.

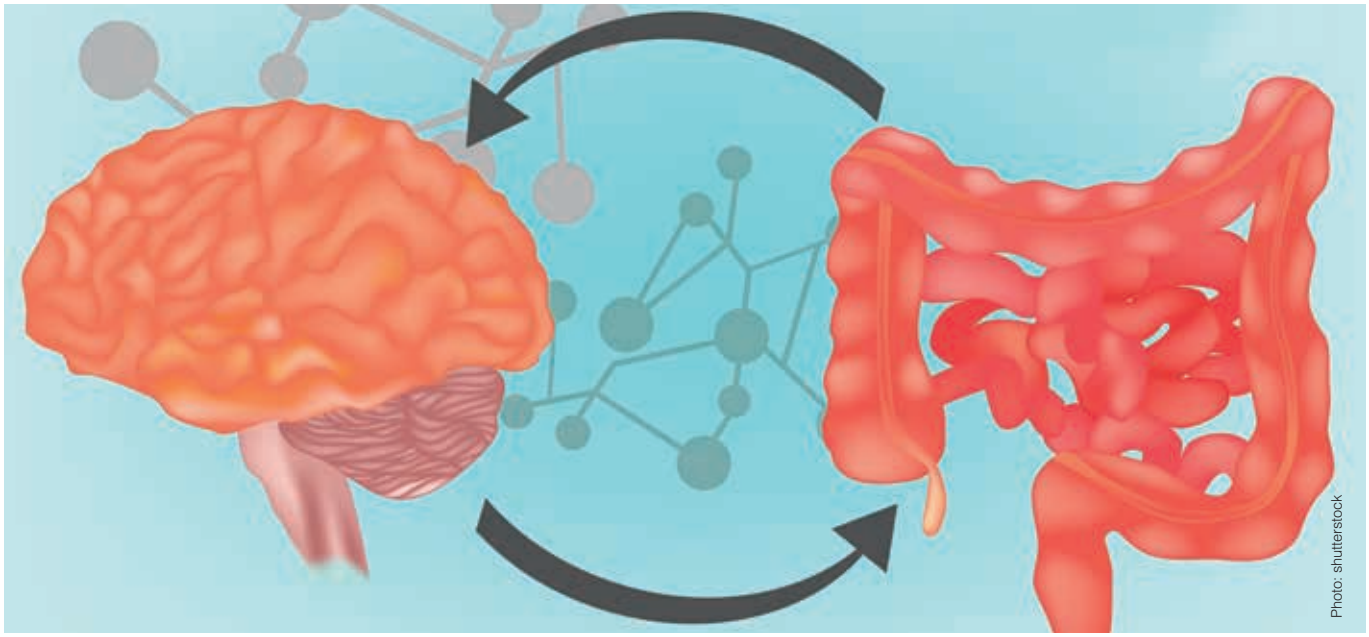
The low FODMAP diet seems to reduce symptoms in some IBS patients but it also results in lower Bifidobacterium counts in the feces. In IBS patients responding to a low FODMAP diet the dysbiosis index increases, thus responsiveness to the diet may be predicted by faecal bacterial profiles. The efficacy of fecal microbial transplantation in IBS remains controversial as this was demonstrated in only one of the two large randomized controlled studies [5, 6].

As stated by Prof. Giovanni Barbara, the American College of Gastroenterology, based on a meta-analysis of 53 randomized controlled trials, concluded that probiotics reduce global IBS symptoms as well as bloating and flatulence [7]. To be consolidated, this recommendation should be founded on new, high-quality data.

However, not all probiotics are similar. *Bifidobacterium infantis 35624* was shown by prof. Eamonn Quigley to relieve abdominal pain, bloating, and bowel function and improve quality of life in patients with all IBS-subtypes, and this appears to have anti-inflammatory and immunomodulating properties reducing CRP and TNFα in conditions like psoriasis, chronic fatigue syndrome, and ulcerative colitis. Furthermore, preliminary results suggest that in combination with *Bifidobacterium longum 1714* it might also relieve depression in patients with IBS.

THE MICROBIOME AND GUT-BRAIN AXIS

Based on preclinical studies, products of gut bacteria have been shown to change brain responses to stimuli, however the challenge is to translate these studies to clinical relevance. Huiying Wang showed in her recent study that *Bifidobacterium longum 1714* strain modulates brain activity during social stress (associated with a cyberball game) in healthy volunteers based on evaluation using magnetoencephalography and QOL questionnaires. Besides an effect on neural oscillations, the strain also enhances a feeling of vitality and reduces mental fatigue compared to placebo over a four-week follow-up period. Prof. Paul Enck described the relationship between stress or anxiety and IBS as two-way as the symptoms can be both the cause and result of IBS. Based on a study of patients with IBS, *Bifidobacterium longum* was shown to correlate with a decrease in depression and anxiety scores, but at onset, these scores were insufficiently high to establish a diagnosis of depression or anxiety [8]. Thus, it is more appropriate to state that this probiotic affects mood rather than depression or anxiety. Similar to *Bifidobacterium longum*, rifaximin has also been shown to modulate brain activity and increase relaxation and reduce anxiety during social stress based on a double blindedrandomized trial of healthy volunteers evaluated by magnetoencephalography [9].



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LITERATURE SELECTION

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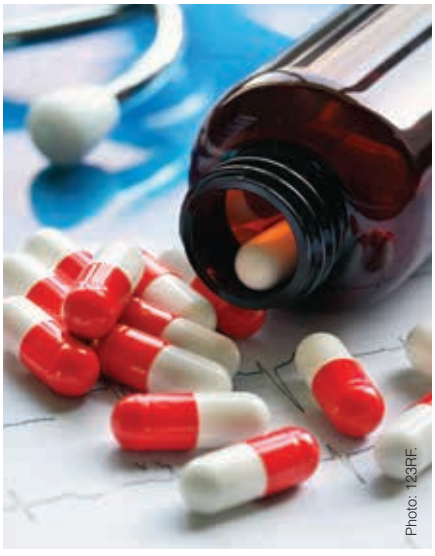
MICROBIOTA AND NON-ANTIBIOTIC DRUGS INTERACTIONS: FRIENDS OR FOES?

Most of the recent studies about the effects of drugs on the intestinal microbiota composition have focused on antibiotic use in different age groups.

Previous studies showed that metformin, proton pump inhibitors, NSAIDs, and atypical antipsychotics have an effect on the intestinal microbiota composition. However, these studies presented general results for drug classes instead of specific drugs. Lisa Maier and colleagues published their new study results in *Nature* in 2018, and they aimed to generate a comprehensive resource of more than 1,000 drugs actions on the microbiome (against 40 representative gut bacterial strains), which could facilitate more in-depth clinical and mechanistic studies, ultimately improving therapy and drug design. Maier *et al.* [1] showed that 24% out of these 1,000 drugs inhibited the growth of at least one strain *in vitro*. The effects of human-targeted drugs on gut bacteria are reflected on their antibiotic-like side effects in humans, like previously published human studies. This study showed that susceptibility to antibiotics and human-targeted drugs correlates across bacterial species, suggesting common resistance mechanisms, and highlighted the potential risk of non-antibiotics promoting antibiotic resistance. Widespread worldwide use of pharmaceutical drugs might be related with the dysbiosis, especially in modern Western societies.

This recent trial also showed that the intestinal microbiota composition can also modulate drug efficacy and toxicity and might be a new platform for further drug development; however, further *in vivo* clinical trials are necessary to better understand the mechanism of action. A comprehensive understanding of how therapeutics interact with gut microbes will open up the path for further mechanistic dissection of such interactions, and ultimately improve not only our understanding of the gut microbiome, but also drug safety and efficacy [2].



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MICROBIOTA AND CYSTIC FIBROSIS

Cystic fibrosis (CF) is a progressive, genetic disease that causes persistent lower respiratory infections and is related to different systemic symptoms and signs. More than 70,000 people live with CF worldwide [1].

The types and severity of symptoms can differ widely from person to person and are mainly related to the age of the patient as well as age at diagnosis. Children and adolescents with CF have a wide range of symptoms and signs including gastrointestinal manifestations. Recent studies have shown that dysbiosis is a feature of CF, leading to reports focusing on the relationship between the composition of the airway microbiota and clinical features and pulmonary function in patients with CF [2].

Dysbiosis-associated CF may be related to the natural course of the disease (including gastrointestinal involvement or respiratory microbiota alterations). However, patients require multiple courses of antibiotic treatment and these antibiotics may change the composition of the microbiota.

Studies have shown that patients with CF typically have decreased amounts of *Bifidobacterium spp., Bacteroides-Prevotella* group, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Eubacterium rectale*, whereas *Enterobacteriaceae* and *Clostridia* are increased. De Freitas and colleagues published a recent study in PLOS One, aimed at evaluating the effect of CF and antibiotic therapy on the intestinal microbiota composition in 19 children and adolescents with CF relative to 17 age and sex-matched controls [3]. The level of fecal calprotectin (an intestinal inflammation marker) was higher in the CF group (irrespective of antibiotic treatment) compared to the healthy controls. The authors showed that *Bacteroides, Firmicutes, Eubacterium rectale* and *Faecalibacterium prausnitzii* were significantly decreased, whereas *Clostridium difficile, Escherichia coli* and *Pseudomonas aeruginosa* were significantly increased in the CF group, relative to the healthy controls. The main differences in microbiota composition between patients with CF and controls, irrespective of antibiotic treatment, were noted for *Eubacterium rectale, Bifidobacterium, Escherichia coli, Firmicutes, Pseudomonas aeruginosa* and *Clostridium difficile*. The results of this study therefore demonstrate that the intestinal microbiota composition in CF patients is different from that of healthy controls and that the frequent use of antibiotics has no additional effects on these alterations.

Photo: 123RF.

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NEWS

CALL FOR PROPOSALS

BIOCODEX MICROBIOTA FOUNDATION

The Foundation's Website allows you to discover - or rediscover - the calls for research proposals currently open:

- The 2019 international call for proposals focuses on the topic «Gut microbiota and drug metabolism». The deadline for submission of applications is November 30, 2018.
- Two national calls for proposals (reserved for scientists working in the relevant countries) are also still ongoing:

Country	Topic	Application form to be returned before
FRANCE	Gut microbiota and human health	December 31, 2018
FINLAND	Gut microbiota in human health and diseases	January 13, 2019

- **For further information, please visit the website www.biocodexmicrobiotafoundation.com**.

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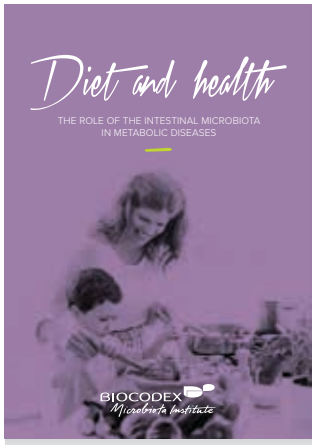

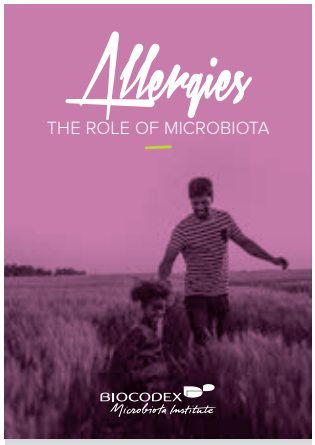

The Institute's Website makes available to the general public two new thematic files:

- **Food and Health**: the role of the gut microbiota in metabolic diseases
- **Allergies**: the role of microbiota

It has also been enriched with a new section entitled «To learn more». It allows improving the knowledge on all the addressed topics. Without forgetting, of course, news which will be added each week to an already rich corpus.

- **For further information, please visit the website www.biocodexmicrobiotafoundation.com**.

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