MICROBIOTA

SUMMARY

EDITO

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" THE USE OF ANTI-BIOTICS WEAKENING THE MICROBIOTA IS OFTEN SUGGESTED AS A RISK FACTOR BUT IT MAY NOW NO LONGER BE THE ONLY ONE. "

Dear readers, Isolated for the first time in 1935 by Hall and O'Toole in the faeces of a healthy infant, *Clostridium difficile*, recently renamed "*Clostridioides difficile*", is a commensal gut bacterium found in 5% of adults and from 15 to 70% of children. Its species name, *difficile*, reflects the difficulties that its discoverers encountered in isolating and growing it in culture. Its reclassification to *Clostridioides* a few years ago was also complex. The genus *Clostridium* was proposed in 1880 by Prazmowski based on the type species *Clostridium butyricum* and has become the "storehouse" of a large number of anaerobic, spore-forming Gram positive rod-shaped bacteria. Thanks to developments in sequencing technologies, the phylogeny of the genus *Clostridium* has been redefined, showing it to have a very wide phylogenetic diversity and the need to create new genera. The name *C. difficile*, or *C. diff.*, is used worldwide and a radical change of name would have had wide ranging consequences. Changes for hospitals (names of tests and results, pharmacy codes, epidemiological materials, training handbooks), for national and international health organisations (websites, official literature and documents) and also for the pharmaceutical and biotechnology industries would have proved very costly overall. It is for this reason that the letter "C" was kept as the first letter of the new family name Clostridioides, literally: *Clostridium*-like organism.

The responsibility de *C. difficile* in pseudomembranous colitis was demonstrated in 1978. Towards the end of the 20th century, the incidence of *C. difficile* infections rose considerably, to become the first cause of nosocomial infections, affecting all hospital departments, thus becoming a threat in need of urgent attention according to the CDC. The use of antibiotics weakening the microbiota is often suggested as a risk factor but it may now no longer be the only one: a 2018 study (discussed in our October 2018 *Microbiota* newsletter) demonstrated the possible role of trehalose, a widely used food additive, in the virulence of some epidemic lines of *C. difficile*. It is suggested that some of these strains are able to use small amounts of this sugar as a single carbon source for their metabolism, thus aiding their emergence and contributing to their virulence.

In this issue, Professor Ianiro evokes the importance of the gut microbiota in the physiopathology and also in the treatment of *C. difficile* infection: some risk factors (broad spectrum antibiotics, proton pump inhibitors) are associated with dysbiosis and therefore modulation of the microbiota (probiotics, faecal transplantation) is currently one of the options which can be envisaged to prevent or treat this infection.

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OVERVIEW

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⁞ GUT MICROBIOTA AND *CLOSTRIDIUM DIFFICILE*

C. difficile infection (CDI) has become in recent years a clinical and socioeconomical burden worldwide, due to its increase in morbidity, severity, mortality, and likelihood to recur. There is a considerable involvement of gut microbiota in CDI, for many reasons. First, most risk factors associated with the development of CDI, including the overuse of broad-spectrum antibiotics or proton pump inhibitors, are associated with an imbalance of gut microbiota. Moreover, specific microbiota modulators are involved in the prevention (specific probiotics) or treatment (fecal microbiota transplantation) of CDI. In this paper, we will review epidemiology, risk factors, and approved therapies of CDI, with a microbiota-centric view.

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Clostridioides difficile (*C. difficile*, previously called *Clostridium difficile*) is a gram-positive, spore-forming, obligate anaerobe. Spores allow *C. difficile* to persist in environments, and to be spread from infected subjects. Under specific circumstances (*e.g.,* antibiotic-driven dysbiosis), spores are driven to germination in the large bowel, and present in a vegetative form that leads to clinical infection (*Clostridium difficile* infection [CDI]). In the infection phase, *C. difficile* produces two toxins, enterotoxin A and cytotoxin B that both cause damage to colonocytes and trigger the inflammatory response, leading to a variety of clinical pictures, from mild colitis to pseudomembranous colitis and toxic megacolon [1].

In recent years, CDI has become a considerable healthcare and economical burden in most countries. Studies from the United States report an incidence of nearly 453,000 cases and of nearly 29,000 CDI-related deaths in 2011, while the incidence in Europe is 124,000 cases/year, with nearly 3,700 deaths/year. Increased morbidity, hospitalization length and mortality, contribute to the considerable economic burden of CDI, which accounted for nearly $ 5 billions in the US in 2011, and for nearly € 3.7 billions in Europe in 2013 [2, 3]. These figures show that the CDI incidence has risen worldwide, for several reasons. First, the increased use of antibiotics, which are a known as risk factors for CDI development. Furthermore, the spreading of specific ribotypes (mainly the virulent ribotype 027, but also the 017 in Asia, the 018 in Italy, the 17,621 in Eastern European countries, 24,422 in Oceania) has let CDI clusters develop. Additionally, there was also an increased number of diagnoses, due to the development of highly sensitive diagnostic tests (*e.g.,* PCR), and the risen awareness of CDI among healthcare professionals.

Overall, the main cause of the overall increase in CDI incidence appears to be the increased rate of recurrences. From 2001 to 2012, the annual incidence of recurrent CDI has increased by nearly 189%, while the increase in overall CDI incidence in the same time period was nearly 43% [2]. As recurrent infection is less likely than first episode to be cured by antibiotics, it is associated with longer hospitalization, increased morbidity and mortality too.

Despite this increase in diagnoses, the misdiagnosis/underdiagnosis of CDI is still relevant, as observed in the EUCLID study.

This finding suggests that a considerable number of patients with CDI is still not diagnosed, increasing the risk of disease diffusion.

> CDI is widely known to be the main cause of healthcare-associated infectious diarrhoea, but recent evidence suggests that its diffusion in the community settings is growing. To date, nearly 25%-35% of CDI cases are acquired in community, probably due to several fecal-oral transmission pathways (*e.g.*, zoonosis and food).

Nosocomial CDI, a community-acquired CDI, appear to differ for several characteristics. First, nosocomial patients are more likely to present with a severe clinical picture, while community patients can even be asymptomatic carriers, increasing the risk of CDI spreading. Moreover, community-based CDI is known to spread also among patients without standard risk factors.

RISK FACTORS FOR *C. DIFFICILE* INFECTION

Although the exact pathogenic pathways of CDI are not yet clarified, several risk factors have been identified over time [4]. Their knowledge is relevant as the management of modifiable risk factors is a prevention measure against CDI. Most relevant risk factors include older age, use of antibiotics, proton pump inhibitors, and others (**Figure 1**).

ANTIBIOTICS

If antibiotics remain today essential molecules in the therapeutic arsenal, it is also necessary to take into account their undesirable effects on the gut microbiota, as a considerable body of evidence supports the association between their use and many dysbiosis-associated diseases, including CDI [5].

> The use of systemic antibiotics is the most relevant modifiable risk factor for the development of CDI. Healthy gut microbiota can determine the successful colonisation of the large bowel by C. difficile or not, by direct and indirect pathways. In principle, the imbalance of healthy gut microbiota by broad-spectrum antibiotics may bring several consequences that drive to CDI.

First, antibiotics may kill commensal bacteria that may have a direct action against *C. difficile* (by secreting a number of bacteriocins) and also compete with the pathogen for nutrients (*e.g.*, sialic acid and succinate). Moreover, there is also an indirect protective role of commensal bacteria through the regulation of bile acids.

Recently, *Clostridium scindens* was associated with resistance to *C. difficile* colonisation. It has a bile acid inducible operon

which is able to encode dehydroxylating enzymes that convert primary bile acids into secondary bile acids. Primary bile acids promote the germination of *C. difficile* spores, while secondary bile acids are able to inhibit this process [6].

As a corollary of this evidence, patients with recurrent CDI are known to have an imbalanced microbial profile, with higher relative abundance of detrimental bacterial families as *Enterobacteriaceae* and *Veillonellaceae* and lower relative abundance of beneficial families, including *Ruminococcaceae, Bacteroidaceae* and *Lachnospiraceae*.

A number of systematic reviews, alone or with meta-analysis, have assessed the relevance of different antibiotic classes in CDI development. In the earliest meta-analysis (1998), antibiotics use was associated with a 6-fold increase in the risk of developing CDI, and the highest risk was observed for fluoroquinolones, clindamycin, cephalosporins. Moreover, the use of antibiotics was found to be an independent predictor of CDI recurrence (relative risk 1.76). One of the key factors to prevent CDI is represented by the antibiotic stewardship approach, so the knowledge of the CDI risk for different antibiotic classes is of paramount importance (**Table 1**).

The use of the following antibiotics is associated with a 2-fold higher risk of CDI among inpatients: clindamycin, cephalosporins, carbapenems, fluoroquinolones, trimethoprim, sulphonamides. In the community setting, respectively, antibiotics were found to have different risk levels for CDI development or recurrence, in-

▼ FIGURE ❶

A microbiota-centric view of *C. difficile* infection
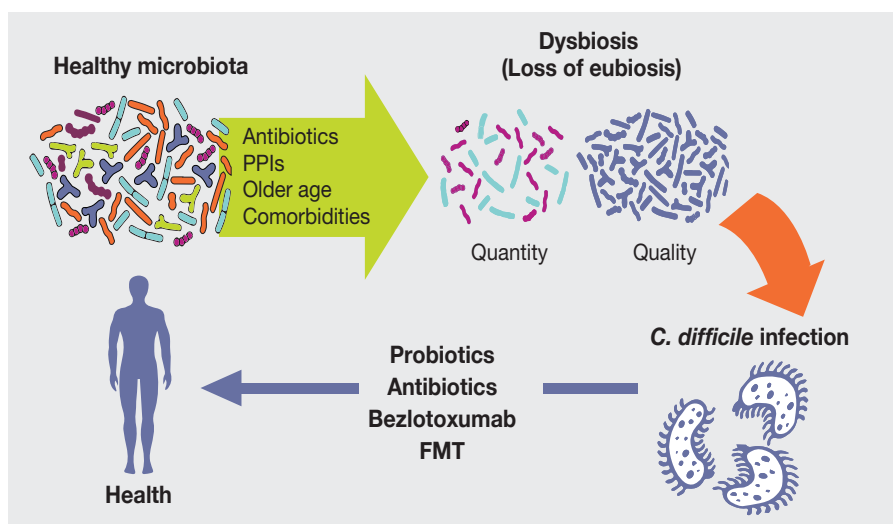
▼ TABLE 1

Antibiotics predisposing to CDI

Frequently	Infrequently	Rarely
Ampicillin and amoxicillin Cephalosporins Clindamycin	Tetracyclines Sulfonamides Erythromycin Chloramphenicol Trimethoprim Quinolones	Parenteral aminoglycosides Bacitracin Metronidazole Vancomycin

cluding: clindamycin (risk increased of 8 to 20 times), cephalosporins and fluoroquinolones (3-5 times increase), macrolides (2-3 times increase) [5].

GASTRIC ACID SUPPRESSION

Proton pump inhibitors (PPIs) are largely used worldwide for several upper gastrointestinal disorders, including gastroesophageal reflux disease, hiatal ernia, gastritis, *H. pylori* infection (together with antibiotic eradication therapy), peptic ulcer disease.

Overall PPIs are considered safe drugs. However, a large body of evidence shows that the use of PPIs is significantly associated with the development of CDI.

In principle, PPIs can increase the risk of *C. difficile* colonization by several pathways, including reduced acid production that can lead to small intestinal bacterial overgrowth and dysbiosis, and increase of bile salts that can promote the germination of *C. difficile* spores. Finally, there is no clear evidence if increased gastric pH is a safer environment for spores [6].

The clinical evidence of a significant association between PPIs and CDI comes from several systematic reviews and meta-analyses, with odds ratios ranging from 1.26 to 2.34, based on different reports (from 3 to 67, according to different meta-analyses).

Most evidence is heterogeneous and comes from observational cohorts, so potentially confounding factors, including other drugs and co-morbidities, could reduce the quality of this finding. However, the association between PPIs and CDI kept significant even after stratification for antibiotic use, both in cohort studies and in case-control reports.

The detrimental role of PPI was found to be stronger toward community-associated CDI, suggesting that there is a chronic overuse in communities rather then in hospitals.

Specifically, PPIs have been associated not only with CDI overall, but also with recurrent CDI by several meta-analyses (including from 3 to 16 studies), with odd ratios ranging from 1.52 to 2.51, although definitions of recurrence varied significantly among studies.

ADVANCED AGE

Advanced age is one of the best known risk factors for primary CDI and recurrent CDI.

Established evidence shows that CDI rates are much higher in adults over 65 years than in younger population. In a meta-analysis of 33 studies, age older than 65 years was identified as an independent predictor of recurrent CDI (relative risk 1.63).

However, age is a considerable confounder, as the use of several drugs that promote CDI, such as antibiotics or PPIs, is more common in older age. Increasing evidence suggests that the microbiota of elderly patients is less healthy (in terms of reduced microbial diversity and increase of opportunistic species) than normal, supporting again the role for microbiota imbalance in CDI [7].

OTHER DISORDERS

The association between CDI and selected comorbidities has also been explored systematically. In a systematic review, significantly higher risk of CDI was found for inflammatory bowel disease (OR 3.72), kidney insufficiency (OR 2.64), hematologic malignancies (OR 1.75), and diabetes mellitus (OR 1.15). This was especially true for community-acquired CDI [7].

THERAPEUTIC MANAGEMENT OF CDI

CONVENTIONAL TREATMENT OF CDI

Traditionally, metronidazole and vancomycin have been the most common treatment options for CDI, being used both as first line options, while only vancomycin was recommended, as tapered or pulsed regimen, to treat recurrent disease [8].

However, in recent years CDI has become more cumbersome to treat. In particular,

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metronidazole was shown to achieve lower cure rates than vancomycin, so that vancomycin has been preferred to metronidazole also in primary infection. Overall, also vancomycin is losing its efficacy, and the rates of recurrent disease have grown. Morover, hypervirulent strains of *C. difficile* have emerged, specifically the ribotype 027, which is less responsive to standard antibiotic therapy and is associated with more severe clinical pictures [8].

In recent years fidaxomicin, a narrow spectrum antibiotic, was shown to be superior than vancomycin in treating CDI recurrences. However, its high costs and the recent evidence of its inferiority compared with fecal microbiota transplantation (FMT) in treating recurrent CDI are potential limitations to its widespread use [9].

THERAPEUTIC MICROBIOTA MODULATORS: PROBIOTICS AND FECAL MICROBIOTA TRANSPLANTATION

Generally, probiotics are considered a reliable option to restore healthy gut microbiota after a dysbiotic event, *e.g.*, antibiotic treatments. Overall, some probiotics are known to be effective against antibiotic-associated diarrhea (AAD), which is a common adverse event of antibiotic regimens [10-12]. In a metanalysis of 21 randomized trials, *Saccharomyces boulardii* decreased significantly the risk of AAD (risk ratio: 0.47) [11].

As CDI is basically a subgroup of AAD, the efficacy of probiotics in preventing CDI was then investigated. Recently, a Cochrane review has shown, in a meta-analysis of 23 trials, that probiotics are both safe and effective for preventing CDI [13]. However, only specific probiotics, including *Saccharomyces boulardii, Lactobacillus casei, a mixture of L. acidophilus* and *Bifidobacterium bifidum*, and a mixture of *L. acidophilus, L. casei* and *L. rhamnosus*, have been found to be effective in preventing primary CDI after antibiotic therapies. In particular, S. boulardii was effective in preventing CDI in a cohort of elderly hospitalized patients, with likely saving of money. Indeed,

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a Canadian study showed that the use of preventative probiotics was able to save $ 518/patient than usual care, and to reduce the risk of CDI [11]. However, further, larger studies are needed to confirm the role of specific probiotics in CDI prevention.

> FMT is the infusion of stools from healthy donors in the gut of a recipient to cure a dysbiosis-related disorders. To date, several systematic review and meta-analyses have shown that FMT is highly effetive in curing recurrent CDI (up to 90% cure rates).

Based on this outstanding evidence, scientific societies have included FMT among the treatment options for recurrent CDI [14, 15]. FMT is also known to increase overall survival and decrease hospitalization length in patients with recurrent CDI [16]. Although FMT has been increasingly standardized over years, is still underdiffused worldwide. Future microbiota-based approaches that will guarantee a widespread diffusion of FMT include capsulized FMT and microbiota-based drugs.

∴ CONCLUSION

CDI is a burdensome disease that occurs mainly in patients with several risk factors, most of which are associated with gut microbiota imbalance, including antibiotic overuse, proton pump inhibitors, and older age. Also from a microbiological point of view, the microbial profile of patients with CDI is characterized by a deep imbalance of gut microbiota. Therapeutic microbiota modulators have been shown to be effective in preventing (specific probiotics, some *Lactobacillus* strains and *S. boulardii*) or curing (FMT) recurrent CDI, paving the way for a microbiota-based approach for the management of this disorder.

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THE MEDITERRANEAN DIET ALTERS THE GUT MICROBIOTA IN THE ELDERLY, REDUCES FRAILTY AND IMPROVES HEALTH: STUDY NU-AGE

Commentary on the article of Ghosh et al. Gut 2020 [1]

By Prof. Harry Sokol
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Ageing is accompanied by a deterioration of many bodily functions and inflammation, which collectively contribute to frailty. It has already been shown by the authors and other teams that frailty is associated with changes in the gut microbiota, and more especially in the context of a poorly diversified diet. The Mediterranean diet is associated with good health. In this study the authors sought to determine if 12 months of Mediterranean diet, known to be associated with good health, could modify the gut microbiota, reduce frailty and improve cognitive function.

The gut microbiota was profiled in non-frail or pre-frail subjects in five European countries before and after the adoption for 1 year of a Mediterranean diet tailored for elderly subjects (NU-AGE diet).

The results showed that it is feasible to improve the usual dietary regime in order to modulate the gut microbiota, and thus promote healthier ageing.

WHAT DO WE ALREADY KNOW ABOUT THIS SUBJECT?

Frailty which accompanies ageing involves the failure of several physiological systems and constant activation of the innate inflammatory immune response. Frailty can include the development of chronic low-grade inflammation, impaired cognitive function, sarcopenia and the development of chronic diseases such as diabetes and atherosclerosis. The modification of dietary regimens such as the adoption of a Mediterranean diet has been suggested as a key therapeutic strategy to combat frailty [2]. The Mediterranean diet is characterised by the consumption of larger amounts of vegetables, pulses, fruits, nuts, olive oil, fish and the consumption of smaller amounts of red meat, dairy products and saturated fats. The adhesion to this type of diet is associated with reduced mortality and increased anti-oxidant activity, as well as a reduction in the incidence of several diseases and inflammation. Several studies have shown that the adoption of this diet is related to a reduction in frailty. Beyond the inverse relationship with disease, closer adhesion to a Mediterranean diet was associated with beneficial changes in the composition of the gut microbiota (reduction in proteobacterial abundance, increased production of short chain fatty acids [SCFAs]). As a general rule however, few elderly subjects follow this type of diet and a large number suffer because of a restricted diet associated with a low-diversity gut microbiota. Changing this is a major challenge, in particular concerning persons in care homes. In previous studies the authors used bioinformatic analysis to identify specific microbial taxa which are gradually lost in the transition from a high-diversity microbiota of healthy subjects to a low-diversity microbiota of frail subjects. In a recent 6-month dietary intervention study in elderly individuals given supplementation with 5 prebiotics (up to 20 g/day), several microbial taxa were modified, but no change was noted in the overall diversity of the microbiota nor in the inflammatory markers. The authors therefore concluded that a more drastic dietary intervention was necessary. The dietary intervention NU-AGE project aimed to study the effect of administration of a personalised Mediterranean diet for 12 months in a large cohort of over 1,200 persons
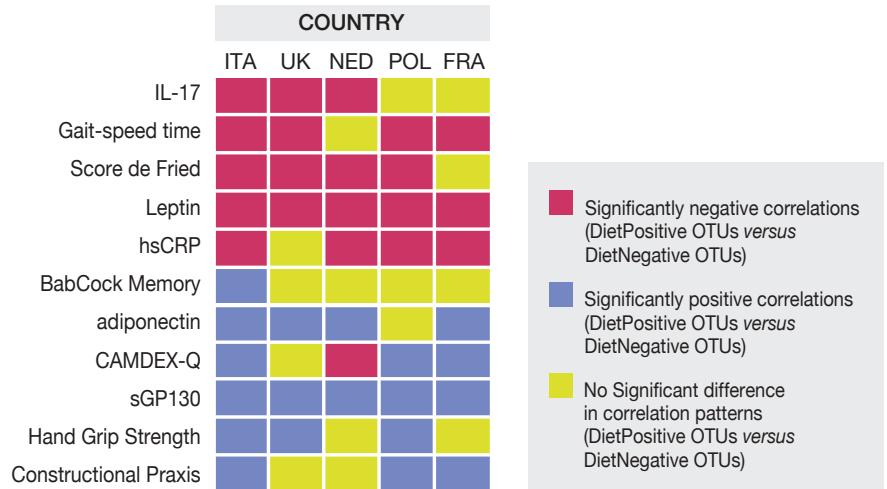
▼ FIGURE 1

Relationship between the abundance of the taxa sensitive to diet and the various tests measuring frailty, cognitive function and inflammation.

	COUNTRY				
	ITA	UK	NED	POL	FRA
IL-17					
Gait-speed time					
Score de Fried					
Leptin					
hsCRP					
BabCock Memory					
adiponectin					
CAMDEX-Q					
sGP130					
Hand Grip Strength					
Constructional Praxis					

■ Significantly negative correlations (DietPositive OTUs *versus* DietNegative OTUs)

■ Significantly positive correlations (DietPositive OTUs *versus* DietNegative OTUs)

■ No Significant difference in correlation patterns (DietPositive OTUs *versus* DietNegative OTUs)

aged 65 to 79 years, distributed across five European countries. A significant relationship was observed between increased adherence to the Mediterranean diet and global cognitive capacity and improved episodic memory [3]. Moreover, it was shown that greater adherence reduced the rate of bone loss in individuals with osteoporosis and improved innate immune function, blood pressure and arterial stiffness [4-6]. In the study described here, the authors analysed the gut microbiota of a sub-group of study subjects.

WHAT ARE THE MAIN INSIGHTS FROM THIS STUDY?

A total of 612 subjects were analysed (289 controls: 145 males, 144 females and 323 on a Mediterranean diet: 141 males, 182 females). At baseline, differences in terms of diet and microbiota were observed between the various countries. Relationships between the Mediterranean diet and the gut microbiota were revealed. Among the taxa associated with good adherence with the Mediterranean diet (DietPositive), we find an over-representation of species such as *Faecalibacterium prausnitzii*, *Eubacterium* and *Roseburia*, a

majority of which are associated with good health (including the production of SCFAs and anti-inflammatory effects). Inversely, certain taxa are depleted in case of good adherence to this diet, some of which have been linked to type 2 diabetes, colorectal cancer, cirrhosis or chronic inflammatory bowel disease. Taken together, these results suggest that adherence to a Mediterranean diet can modulate the microbiota in a direction positively associated with health.

Lastly, the authors observed that the abundance of DietPositive taxa were negatively correlated with some inflammatory markers (high-sensitivity CRP (hsCRP) and IL-17), and with clinical scores associated with increased frailty (Fried scores, gait speed time). In contrast, the abundance of these taxa was positively correlated with the improvement in cognitive function (Constructional Praxis score, Babcock memory score) and reduced frailty (hand grip strength) and two anti-inflammatory markers (adiponectin and sGP130). The opposite trend was observed with DietNegative taxa (**Figure 1**). Analysis of the inferred microbial metabolite profiles indicated that the diet-modulated change in microbiota was

associated with an increase in production of short/branched chain fatty acids and a lower production of secondary bile acids, p-cresols, ethanol and carbon dioxide.

WHAT ARE THE CONSEQUENCES IN PRACTICE?

These results confirm that dietary intervention is an effective means of improving health, at least partially, via a modulation of the gut microbiota. Of course we can recommend that elderly subjects adopt a Mediterranean diet, but the feasibility of this type of dietary intervention is questionable in the long-term. As this study has identified bacteria associated with the beneficial effects of the Mediterranean diet, it lays the groundwork for their use in the form of next-generation probiotics. This type of approach based on bacteria from the gut microbiota should be tested in this indication.

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COMMENTED ARTICLE
CHILDREN'S SECTION

:: METAGENOMICS OF THE FAECAL VIROME: CUMULATIVE EFFECT OF THE QUANTITY OF GLUTEN AND ENTEROVIRUSES RISK OF CŒLIAC DISEASE AUTOIMMUNITY IN PREDISPOSED CHILDREN – THE TEDDY STUDY

Commentary on the original article of Lindfors et al. (Gut 2019) [1]

By Prof. Emmanuel Mas
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A higher gluten intake, frequent gastrointestinal infections and adenovirus, enterovirus, rotavirus and reovirus have all been proposed as environmental triggers of cœliac disease (CD). However it is not known whether an interaction exists between the quantity of gluten ingested and exposure to viruses in the development of CD. This study sought to determine whether distinct viral exposures, alone or associated with gluten, increased the risk of CD autoimmunity in genetically predisposed children. It was concluded that frequent exposure to enteroviruses between the ages of 1 and 2 years was indeed associated with increased risk of CD autoimmunity, indicating a cumulative effect of the interaction between enteroviruses and higher gluten intake.

WHAT DO WE ALREADY KNOW ABOUT THIS SUBJECT?

Cœliac disease is an autoimmune pathology which occurs in genetically predisposed individuals of genotype HLA DQ2 and/or DQ8-positive. It is characterised by the presence of villous atrophy and lymphocyte infiltration of the epithelium of the small intestine. Gluten present in the diet induces an autoimmune response directed against tissue transglutaminase. The appearance of anti-transglutaminase antibodies (ATA) indicates the presence of cœliac disease autoimmunity.

The rise in the incidence of autoimmune diseases has led to suspect that environmental factors may have a role in their pathogenesis. Observational studies suggest that viral infections could cause a loss of oral tolerance to gluten and the development of cœliac disease.

WHAT ARE THE MAIN INSIGHTS FROM THIS STUDY?

It is a nested case-control study in the TEDDY (The Environmental Determinants of Diabetes in the Young) birth cohort which included 8,676 children before the age of 4 and a half months followed to the age of 15 years. The principal objective of this cohort was to identify the genetic and environmental factors associated with type 1 diabetes and cœliac disease. After forming pairs matched for family history of type 1 diabetes, sex and study inclusion site, 83 pairs (child with predisposition (case) and control) were retained in the final analysis for whom faecal virome data were available after introduction of gluten. Of these pairs, 16 had a family history of type 1 diabetes. During follow-up, 28 of the cœliac disease autoimmunity cases developed cœliac disease.

Stool samples were collected every month from the age of 3 months to 2 years; tests for enterovirus, adenovirus, astrovirus, norovirus, reovirus and rotavirus were performed. Every 3 months, a food questionnaire was used to collect information on breast-feeding and the age of introduction of gluten-containing foods. A 3-day record of food intake enabled a calculation of the quantities of gluten ingested at 6, 9, 12, 18 and 24 months.

Cumulative viral detections in stool samples between the ages of 1 and 2 years.

Stool samples were available for 72 pairs. The numbers of cases and controls correspond to the numbers of children having at least 1 viral sequence in the stools.

Virus/serotype	OR (95% CI)	p value	Positive cases (n)	Positive controls (n)
All viruses	1.60 (1.12 – 2.29)	0.01	59	58
HEV	2.56 (1.19 – 5.51)	0.02	31	16
HEV A	2.10 (0.67 – 6.60)	0.2	13	8
CVA	2.53 (0.73 – 8.78)	0.15	7	5
HEV B	2.64 (0.84 -8.36)	0.1	15	7
CVB	6.00 (1.27 – 28.46)	0.02	16	6
Echovirus	2.27 (0.68 – 7.61)	0.18	11	6
HAdV	1.41 (0.99 – 2.02)	0.05	52	55
HAdV A	5.11 (0.71 – 36.63)	0.1	9	1
HAdV B	6.12 (0.55 – 67.70)	0.14	4	1
HAdV C	1.74 (0.82 – 3.70)	0.15	19	17
HAdV F	1.09 (0.23 – 5.11)	0.91	7	7
Astrovirus	0.70 (0.29 – 1.68)	0.42	13	18
Norovirus	1.04 (0.42 – 2.62)	0.93	10	11
Reovirus	NA	NA	1	0
Rotavirus	NA	NA	0	0

CVA, coxsackievirus A; CVB, coxsackievirus B; HAdV, human adenovirus; HEV, human enterovirus; NA, not applicable

KEY POINTS

- Environmental factors play a role in cœliac disease.

- Enterovirus exposure is a risk factor for the development of anti-transglutaminase antibodies in HLA DQ2 and/or DQ8-positive children.

- This risk is potentiated by the intake of large amounts of gluten in the diet.

The percentage of stool samples positive for any virus fluctuated from 22 to 50%, without any age-related peak and for the enteroviruses this ranged from 0 to 21% after 6 months. Between 1 and 2 years, enteroviruses were detected in 31 cases versus 16 controls (**Table 1**). The cumulative number of stool samples positive for any virus was associated with an increased risk for cœliac disease autoimmunity (OR 1.60; $p = 0.01$), with a stronger association conferred by the enteroviruses (OR 2.56; $p = 0.02$).

The risk of cœliac disease autoimmunity was not increased by viral infections occurring after the age of gluten introduction while breast-feeding was still continuing. In contrast, after weaning, in stool samples collected between the ages of 1 and 2 years after gluten introduction, both the cumulative number of viruses detected (OR 1.41; $p = 0.05$) and also the numbers of enteroviruses (OR 2.47; $p = 0.03$) were associated with the risk of cœliac disease autoimmunity. There was a significant interaction between the presence of enterovirus detected between 1 and 2 years and the quantity of gluten ingested up to the age of 2 years in the risk of cœliac disease autoimmunity ($p = 0.03$). It is suggested that this increases with the amounts of gluten ingested: high (OR 8.3), middle (OR 2.9) and low (OR 1.0) (**Figure 1**).

WHAT ARE THE CONSEQUENCES IN PRACTICE?

The results of this study indicate the value of preventing the appearance of cœliac disease auto-antibodies in children at risk. This could be done by carefully monitoring the amounts of gluten ingested, in particular in case of exposure to enteroviruses, especially when the child is no longer being breast fed.

∴ CONCLUSION

This study demonstrated an association between gastro-intestinal exposure to enteroviruses and the risk of cœliac disease autoimmunity in genetically at-risk children. This risk increases as greater amounts of gluten are ingested.

▼ FIGURE ①

Effect of enterovirus exposure between the ages of 1 and 2 years and the risk of cœliac disease autoimmunity, stratified by cumulative gluten consumption up to the age of 2 years.
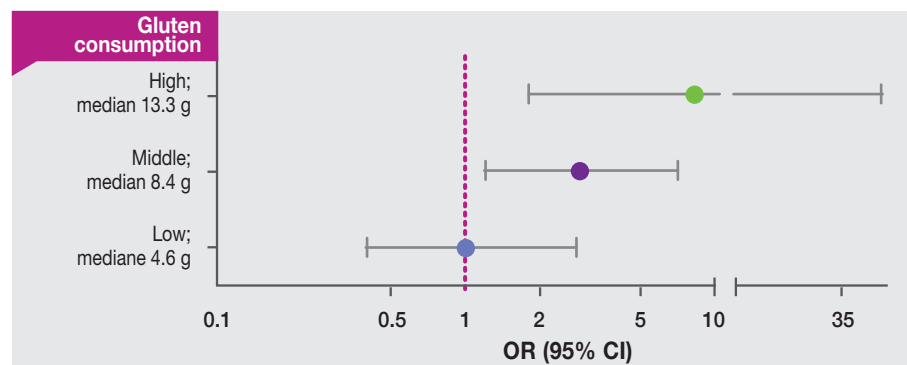
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GUT MICROBIOTA FOR HEALTH WORLD SUMMIT 2020

MARCH 2020

MADRID, SPAIN

This year's Gut Microbiota For Health World Summit was held in Madrid under rather exceptional conditions due to the rising Covid-19 epidemic. As many participants could not physically attend the meeting, this report is somewhat more extensive than the standard congress reviews in this Newsletter.

The 9th edition of this symposium addressed various aspects of the microbiota including dietary and non-dietary factors shaping the gut microbiota and the role of microbiota on brain function and in modulating the immune system.

In an introductory keynote lecture, Colin Hill (Cork, Ireland) underlined some roadblocks in the clinical translation of micro-

Photo: 123rf.

biome research. He made an appeal to adopt consensus definitions and to use precise language. The goal is to make microbiome science more numerate and use actual numbers of bacteria rather than only relative abundances and proportions. It tends to address the complexity related to microbiome individuality and to monitor the complexity of figures in order not to overwhelm readers.

Furthermore, he pointed out that the choice of methodology, such as biological vs. *in silico* methods, significantly affects the results.

For example, the faecal microbiome is only a very blurred approximation of the intestinal microbiome, intestinal transit times affect the composition of the microbiome and the conversion of *in silico* to in vivo methods is not always straightforward. Microbiome research should be a science where the highest standards are applied rather than a belief system.

DIETARY FACTORS SHAPING THE GUT MICROBIOTA

Previous cross-sectional studies already indicated that the composition of the faecal microbiota depends on dietary patterns. In particular, people that consume a plant-based diet have a more diverse microbiota with a higher proportion of short-chain fatty acids (SCFA) producing bacteria compared to people on a western-type diet high in refined carbohydrates and fat. Changing diet from a standard American

diet to a plant-based diet modified the microbiota and markedly improved the metabolic outcome of obese subjects as reported by Hana Kahleova (Washington DC, USA). Participants that switched to a plant-based diet for 16 weeks lost 5.8 kg of body weight, of which about two thirds was fat, and had improved insulin sensitivity compared to the control group that did not adapt its dietary pattern. Faecal Bacteroidetes and *Faecalibacterium prausnit-*

zii increased on the vegan diet, while *Bacteroides fragilis* decreased on both diets but less on the vegan diet. Furthermore, changes in bacterial composition correlated to changes in metabolic parameters.

MICROBIOTA-DRUG INTERACTIONS

As highlighted by Rinse K. Weersma (Groningen, The Netherlands), drugs interact with the intestinal microbiota according to different scenarios. Some drugs like proton pump inhibitors (PPI) affect the microbiota composition and functionality. The higher pH locally in the gut due to PPI intake results in "oralisation" of the gut microbiota as oral bacteria manage to penetrate deeper in the gastrointestinal tract. The oral antidiabetic metformin also affects the gut microbiota composition by increased numbers of *Akkermansia muciniphila* and SCFA production which contributes to its antihyperglycemic effect. Immune therapeutics do not directly affect the microbiome but as the microbiome is involved in immune homeostasis, it indirectly determines the response to those anticancer drugs. Furthermore, the microbiota also modifies the activity of drugs by activating or inactivating them or by influencing their toxicity. For example, the conversion of levodopa by the gut microbiota makes it less bioavailable to the brain which may explain part of the variable responses of patients to this drug. To be active, the prodrug sulfasalazine needs to be split by

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bacterial azo-reduction in the colon into 5-ASA and sulphapyridine whereas the cardiac glycoside digoxin is inactivated by microbial metabolism. Finally, the gut microbial conversion of the oral antiviral drug brivudine into bromovinyluracil is involved in its toxicity.

Athanasios Typas (Heidelberg, Germany) highlighted that the impact of non-antibiotic drugs on the microbiota is extensive. By screening 1,200 marketed drugs *in vitro* against 40 representative gut bacterial strains, at least a quarter of non-antibiotic drugs with human targets was found to inhibit at least one strain [1]. This *in vitro* inhibition was reflected in the side effects of the drugs in humans and was concordant with existing clinical trials indicating the relevance of the screening strategy. Remarkably, there was a substantial overlap in susceptibility of gut bacterial strains for human-targeted drugs and antibiotics which was attributed to the fact that the same pumps, transporters and detoxification mechanisms are used for both groups of drugs. These results imply that polypharmacy may be a strong driver for antibiotic resistance.

THE ROLE OF THE GUT MICROBIOTA IN THE GUT-BRAIN AXIS

In the last 30 years, very little progress has been achieved in therapy for mental health. John Cryan (Cork, Ireland) argued that the gut microbiota might provide a new target to improve brain health, despite it still being early days, as the gut microbiota affects brain health during different stages of life. The mode of birth that impacts the intestinal microbiota has been associated with neurodevelopmental disorders [2]. Mice born via caesarean section exhibit an increased stress response, higher anxiety and deficits in sociability. These effects can be reversed by targeting the gut microbiota. The fact that also germ-free mice show inappropriate brain development as evidenced by deficits in fear memory, increases in visceral pain and social deficits, corroborates a role for the gastrointestinal microbiota. Furthermore, during early adolescence, the brain is sensitive to microbial signals. Mice that received a high fat diet during the adolescent period had long-lasting differences in gut microbiota composition in adulthood,

together with differences in expression of genes related to neuroinflammation or neurotransmission, although no overt behavioural changes in adulthood were observed [3]. In aged male mice, shifts in the microbiota towards a profile previously associated with inflammatory diseases were associated with increased gut permeability, peripheral inflammation and behavioural changes including deficits in spatial memory and increased anxiety-like behaviour.

Well known as a "happiness" hormone, serotonin actually has a much more complex biological function. It is involved in bone density and in neural, platelet and gastrointestinal function which makes it an attractive intervention point to improve health. The vast majority of serotonin is located in gastrointestinal tissues. Jonathan Lynch (Los Angeles, USA) pointed out that the gut microbiota critically regulates serotonin production by the host. Especially indigenous spore-forming bacteria promote serotonin biosynthesis via production of soluble metabolites that directly signal to colonic cells. This bacterial mediated induction of serotonin regulates gastrointestinal motility and platelet function in mice [4]. Furthermore, luminal intestinal serotonin concentrations also modulate bacterial colonisation in the gut. The relative abundance of spore-forming bacteria, in particular *Turicibacter sanguinis*, increases when luminal intestinal serotonin levels are elevated. *T. sanguinis* expresses a receptor homologous to the mammalian serotonin transporter SERT that allows importing of serotonin resulting
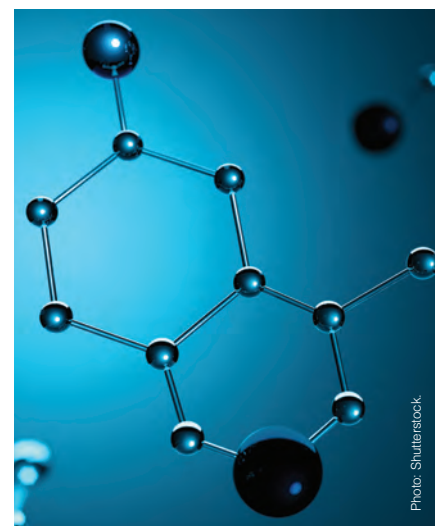

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in expression of sporulation factors and membrane transporters. These effects are reversed by exposure to fluoxetine, a serotonin reuptake inhibitor.

THE GUT MICROBIOME AND THE IMMUNE SYSTEM

Newborn babies acquire microbes at birth through vertical transmission from their mothers. This postnatal colonisation is assumed to be the main stimulus to the development and maturation of the immune system. Using a model of transiently colonising pregnant female mice, Kathy McCoy (Calgary, Canada) demonstrated that already during pregnancy the maternal gut microbiota shapes the function of the immune system of the offspring. Germ-free pups born to dams that were transiently colonised had increased levels of innate immune cells in the gut and increased expression of genes encoding epithelial antibacterial peptides and metabolism of microbial molecules compared to pups born to germ-free dams [5]. This maternal microbiota-mediated education of the immune system requires maternal antibodies that are transmitted to the offspring during pregnancy and in milk. Furthermore, the maternal gut microbiota protects pups from excessive inflammation. LPS-administration elicited a huge inflammatory response in pups born to germ-free dams whereas this response was blunted in pups born to colonised dams.

The period between birth and weaning, i.e. induction of a more diverse diet, is important for the ontogeny of the immune system, as highlighted by Gérard Eberl (Paris, France). The expansion of the gut microbiota that occurs at weaning induces a strong immune response that is associated with the induction of regulatory T cells [6]. Exposure of germ-free mice to microbes before weaning results in such (normal) immune reaction whereas no reaction occurs when the mice are exposed to microbes only after weaning, indicating that the immune system needs to be exposed to microbes in a specific time window. Pathological imprinting resulted in increased susceptibility to immune pathologies later in life. How the immune system remembers needs further clarification. Hints in the literature suggest towards epigenetic regulation of immunes genes and imprinting of the expression of genes in myeloid cells or stromal cells. Most likely, many different cells are imprinted, the significance of which needs to be investigated.

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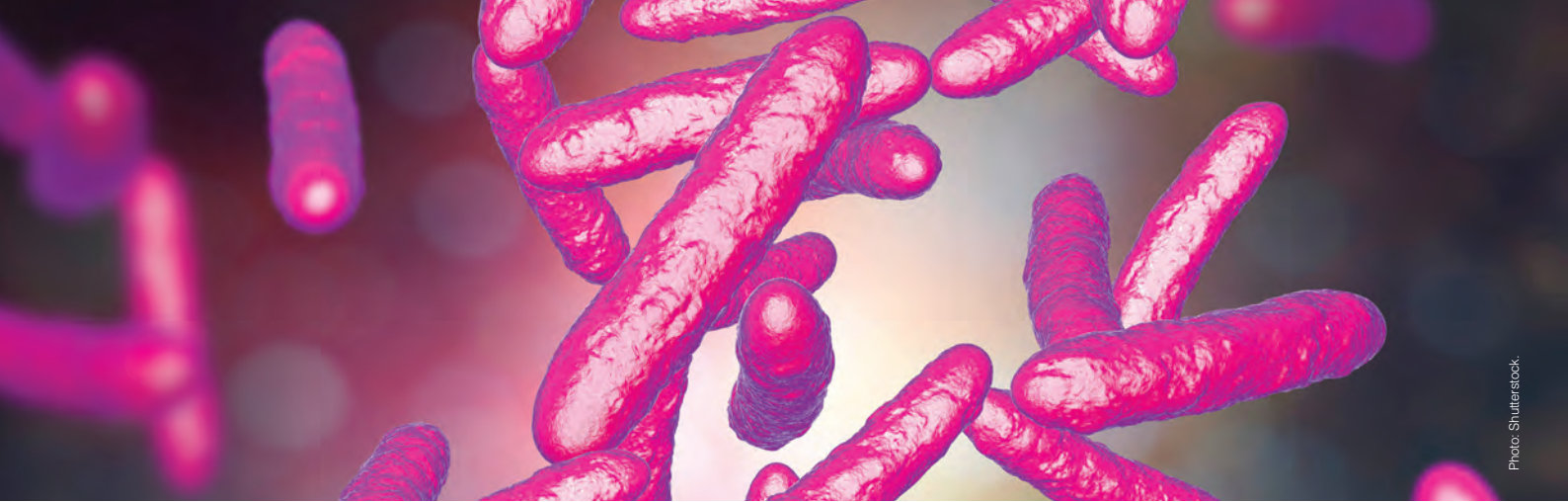
LITERATURE SELECTION

By **Prof. Markku Voutilainen**
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VAGINAL MICROBIOTA

⠿ VAGINAL MICRO-BIOME MAY PREDICT THE SEVERITY OF ENDOMETRIOSIS

Perrotta AR, Borrelli GM, Martins CO, *et al*. The vaginal microbiome as a tool to predict rASRM stage of disease in endometriosis: a pilot study. *Reprod Sci*. 2020.

Could gut and vaginal microbiomes predict the stage of endometriosis? According to this study, no differences were detected between samples from patients and healthy control subjects for follicular and menstrual phases of the menstrual cycle. At an individual level, the distribution of community state types differed significantly between the two menstrual phases. Among patients, Operational Taxonomic Units (OTU) of genus *Anaerococcus* (notably *A. lactolyticus* and *A. degenerii*) significantly differed between disease stages 1-2 and stages 3-4 (rASRN - revised American Society for Reproductive Medicine - classification). Authors conclude that vaginal microbiota may predict the stage of the disease.

⠿ VAGINAL MICROBIOTA AND CERVICAL CANCER

Pourmollaei S, Barzegari A, Farshbaf-Khalili A, *et al*. Anticancer effect of bacteria on cervical cancer: molecular aspects and therapeutic implications. *Life Sci* 2020.

Vaginal dysbiosis may be linked to the development, progression and stability of cervical cancer (CC), but it is unclear whether the relationship between the two is causal or correlative. To find out if cervicovaginal bacteria also have an anticancer effect, the use of microorganisms in the treatment of cancer (especially CC) was discussed in this article: probiotics, bacteria-based immunotherapy, bacterial toxins and spores, vectors in gene therapy, and inhibitors of tumor angiogenesis. Some bacteria inhibit CC by activating NK cells and dendritic cell maturation. Other mechanisms are production of cytotoxic compounds, regulating immune cell differentiation, and inhibiting cancer cell migration. In conclusion, genetically engineered bacteria may be an effective treatment for CC in the future. Larger sample size studies are needed to assess this option.

## ⁑ THE MICROBIOTA OF THE MEIBUM

Suzuki T, Sutani T, Nakai H, *et al.* **The microbiome of the meibum and ocular surface in healthy subjects.** *Invest Ophtalmol Vis Sci* **2020.**

The meibum prevents the evaporation of the eye's tear film, allows homeostasis of the ocular surface and has its own microbiota. Does this microbiota, and those of ocular surfaces, change with ageing?

This study shows that eyelid-skin samples from young subjects had low α-diversity (Shannon index) and that *Probionibacterium acnes* and *Staphylococcus epidermidis* were the dominant species. Meibum and conjunctival-sac microbiota were different from that of the skin and characterized by a high α-diversity index consisting in a large number of bacterial species.

In elderly subjects, *Corynebacterium* sp. and *Neisseriaceae* were the predominant taxa on eyelid skin and α-diversity Shannon index was significantly reduced at their meibum and conjunctival sac.

Authors conclude that meibum's microbiome is indeed altered with ageing, equally in men and in women.

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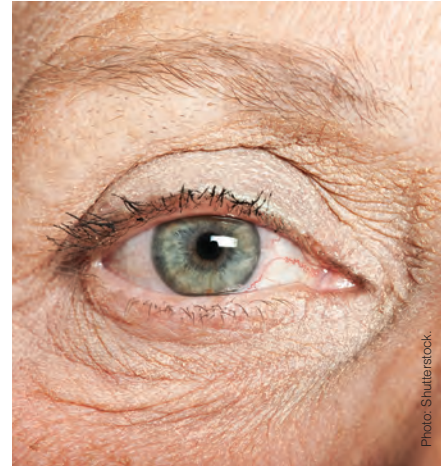
⁑ AGEING AND MICROBIOME CHANGES

Huang S, Haiminen N, Carrieri AP, *et al.* **Human skin, oral, and gut microbiomes predict chronological age.** *mSystems* **2020.**

We know that gut microbiome changes with age, as well as oral and skin ones. Which of them is best to predict ageing?

The authors evaluated the microbiome diversity from almost 9 000 skin, saliva and intestinal samples from healthy people across 10 studies.

Taxa enriched in young individuals (18 to 30 years) tended to be more abundant and prevalent than taxa enriched in elderly persons (> 60 years); and ageing may be linked to a loss of key taxa. Compared with gut and oral microbiome, the skin one was the best predictor of age (mean 3.8 yrs ± 0.45 SD). Authors identified genera and families including anaerobic bacteria (*Mycoplasma, Enterobacteriaceae, Pasteurellaceae*) that negatively correlated with age. Age-related changes in skin physiology (decreased sebum production, increased dryness) and host immune reactions may cause these microbiome changes.

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GUT MICROBIOTA
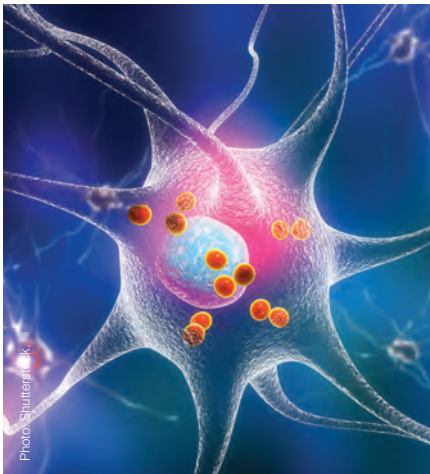
⫶ *BACILLUS SUBTILIS* AND PARKINSON'S DISEASE

Goya ME, Xue F, Sampedro-Torres-Quevedo C, *et al.* Probiotic Bacillus subtilis protects against α-synuclein aggregation in *C. Elegans. Cell Rep.* 2020.

To better understand the impact of gut microbiota on the progression / severity of Parkinson's disease (PD) and on α-synuclein (α-syn) aggregates in Lewy bodies, the authors used a nematode *Caenorhabditis elegans* model. They noted that both spores and vegetative cells of a *Bacillus subtilis* strain induced a biofilm formation in the worm gut and the release of bacterial metabolites. Thus, protective pathways such as sphingolipid metabolism were differentially regulated; preformed α-syn aggregates removal and α-syn aggregation inhibition were observed in young and old animals. From authors' point of view, the effects of this strain as a food supplement should be considered in PD treatment.

⫶ THE IMPACT OF ANTIBIOTICS ON CANCER IMMUNOTHERAPY

Iglesias-Santamaria A. Impact of antibiotic use and other concomitant medications on the efficacy of immune checkpoint inhibitors in patients with advanced cancer. *Clin Transl Oncol* 2020.

Gut microbiota may influence the efficacy and toxicity of anticancer therapy. According to present results, it significantly impacts the response to an Immune Checkpoint Inhibitors (ICI) treatment. The study evaluated the impact of antibiotics, proton pump inhibitors (PPI), steroids, and opioids use on the therapeutic response to ICI treatment defined by iRECIST[1] criteria. It showed that antibiotic use *per se* was not associated with any reduced efficacy of ICI treatment, but multiple or prolonged antibiotic courses impaired the outcome of immune therapy. This is the first study showing that concomitant use of opioids, but not PPIs or steroids, is associated with poorer outcomes of ICI therapy.

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[1] Developed for the use of modified Response Evaluation Criteria in Solid Tumours by the RECIST working group

CALL FOR RESEARCH PROPOSALS

⠿ BIOCODEX MICROBIOTA FOUNDATION

As we publish this, our summer issue, we have the pleasure and honour to announce that Professor Premysl BERCIK (Hamilton, Canada*) is the winner of the 2020 international scholarship for his research on the subject of "*Clostridium difficile*-induced post-infectious Irritable Bowel Syndrome: Study of the mechanisms and treatments".

Distribution of applicants for the 2020 international scholarship.
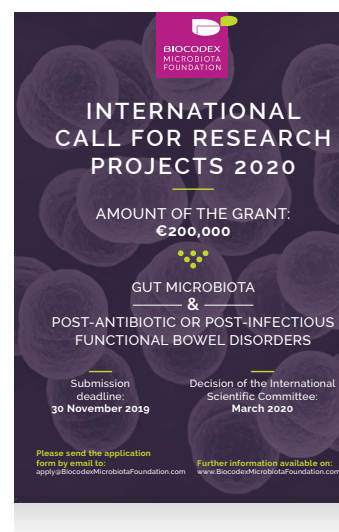
We also announce the theme of the 2021 call for projects, related to mental health: "Functional effects of the human gut microbiome on autism".

· **Visit www.biocodexmicrobiotafoundation.com to learn more.**

*Professor of medicine (Dpt of gastroenterology, Mc Master University) and Gastroenterologist (Hamilton Health Sciences)

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